

## Poster Sessions

### Antimicrobial susceptibility testing – EUCAST and beyond

#### P671 Adjusting EUCAST zone diameter breakpoints for *Haemophilus influenzae* on the Mueller-Hinton Fastidious media

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**Objectives:** The European Committee on Antimicrobial Susceptibility Testing (EUCAST) has published clinical MIC and zone diameter breakpoints for *Haemophilus influenzae* (HI). Zone diameter breakpoints were developed for the new Mueller-Hinton Fastidious media (MH-F) and have been tentative since 2010. The objective of this study was to optimise zone diameter breakpoints for HI using broth microdilution (BMD) as reference.

**Methods:** A total of 150 clinical isolates of HI were selected from the SENTRY collection (JMI Laboratories, USA). The collection was biased towards beta-lactam-resistant strains, including beta-lactamase positive and negative strains as well as strains with PBP changes (i.e. BLNAR). Disk diffusion was performed at the EUCAST Laboratory according to EUCAST methodology on Mueller-Hinton agar with 5% defibrinated horse blood and 20 mg/L beta-NAD (MH-F), using agar from two manufacturers. MIC values were determined by BMD, in Mueller-Hinton broth with 5% lysed horse blood and 20 mg/L beta-NAD (MH-F broth), at JMI Laboratories. BMD was performed on custom panels (TREK Diagnostics/Thermo Fisher Scientific) and the horse blood was lysed by repeated freezing and thawing. Data were analysed by EUCAST for antibiotic agents with both MIC and zone diameter breakpoints in EUCAST tables. Very major, major and minor errors (VME, ME and mE) were calculated.

**Results:** Correlation of inhibition zones and MIC values resulted in an adjustment of zone diameter breakpoints for HI as presented in Table 1. Several zone diameter breakpoints were increased by 1–5 mm to reduce

VMEs. New zone diameter breakpoints resulted in error rates (%) as follows (rates for old breakpoints in parenthesis): VME 1.9 (2.7), ME 1.4 (0.8) and mE 0.9 (1.4). Amoxicillin-clavulanate was not analysed due to suggested changes in both MIC breakpoints and disk potency. EUCAST has proposed revised MIC breakpoints for chloramphenicol and rifampicin, which would lower the total VME to 1.5%. The selection towards BLNAR isolates in this collection resulted in high error rates for some beta-lactam antibiotics, but the occurrence of such strains is rare among clinical isolates.

**Conclusions:** An analysis of the MIC/zone diameter correlates for *H. influenzae* resulted in adjusted zone diameter breakpoints for several antibiotics. Revised zone diameter breakpoints for HI will be published in the EUCAST Clinical Breakpoint Table v. 2.0 (January 2012).

#### P672 A comparison of MIC-based screening tests for betalactamase-negative ampicillin-resistant *Haemophilus influenzae*

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**Objectives:** Most B-lactamase negative ampicillin (AMP) resistant *H. influenzae* (BLNAR) have an N526K substitution in penicillin binding protein 3 (PBP3). Their detection is problematic because MICs cluster near the breakpoints (BPs), there is no consensus on BPs and there is poor correlation between MIC and disc diffusion (DD) zones. By strict CLSI criteria, BLNAR strains have AMP MICs  $\geq 4$  mg/L although most use the non-susceptible BP of  $\geq 2$ , consistent with the EUCAST resistant BP of  $>1$ . CLSI DD uses a 10  $\mu$ g disc even though Karpanoja (2004) showed poor correlation of zones sizes and MIC near the BP with these discs and recommended using a 2  $\mu$ g disc with better correlation, which is the disc strength used by EUCAST. Many of these problems are compounded in strains with both altered PBP3 and B-lactamase. Given that strains with altered PBP3 have reduced susceptibility to AMP, amoxicillin-clavulanate (AMC) and cephalosporins, a paradigm shift away from AMP MIC as a basis for detection is warranted. Here we compare the use of a cefotaxime (CTX) screen against current methods for the detection of strains with altered PBP3.

**Methods:** A collection of strains with and without N526K PBP3 substitutions and B-lactamase were tested by broth dilution and DD using CLSI and EUCAST methods to determine the performance of various substrates and BPs. Performance was determined using susceptibility or resistance from MIC and DD methods against the presence of N526 or N526K as the reference for genotypically defined susceptibility or resistance respectively.

Antimicrobial agent	MIC breakpoint (mg/L)		Disk content ( $\mu$ g)	Zone diameter breakpoint (mm)	
	S $\leq$	R $>$		S $\geq$	R $<$
Ampicillin	1	1	2	16	16
Cefepime	0.25	0.25	30	27 (25)	27 (25)
Cefixime	0.12	0.12	5	25 (22)	25 (22)
Cefotaxime	0.12	0.12	5	26 (22)	26 (22)
Cefpodoxime	0.25	0.5	10	26 (24)	23 (21)
Ceftibuten	1	1	30	25 (24)	25 (24)
Ceftriaxone	0.12	0.12	30	30 (27)	30 (27)
Cefuroxime	1	2	30	26 (25)	25 (22)
Cefuroxime axetil	0.12	1	30	50	26 (25)
Doripenem	1	1	10	20	20
Ertapenem	0.5	0.5	10	20	20
Imipenem	2	2	10	20 (16)	20 (16)
Meropenem (non-meningitis)	2	2	10	20	20
Ciprofloxacin	0.5	0.5	5	26 (23)	26 (23)
Levofloxacin	1	1	5	26 (21)	26 (21)
Moxifloxacin	0.5	0.5	5	25 (23)	25 (23)
Ofloxacin	0.5	0.5	5	23 (21)	23 (21)
Erythromycin	0.5	16	15	50	10 (12)
Telithromycin	0.12	8	15	50	12 (14)
Minocycline	1	2	30	24 (25)	21 (22)
Tetracycline	1	2	30	25 (24)	22 (21)
Chloramphenicol	1*	2	30	28	25
Rifampicin	0.5*	0.5*	5	18	18
Trimethoprim-sulfamethoxazole	0.5	1	1.25-23.75	23	20

\* Currently under consultation. Suggested new MIC breakpoints:  
Chloramphenicol S  $\leq 2$  mg/L  
Rifampicin S  $\leq 1$ , R  $> 1$  mg/L

Organisms	Antibiotic	Breakpoint	SN	SP	FN	FP
N526K (n=57)	AMP	CLSI / EUCAST $\geq 2 / > 1$ mg/L	32	97	42	1
	CTX	Proposed $\geq 0.06$ mg/L	67	86	20	5
versus	AMP 2ug	CLSI Karpanoja $< 16$ mm	30	97	43	1
	AMP 2ug	EUCAST $< 16$ mm	21	97	48	1
N526 (n=36)	AMP 10ug	CLSI $< 21$ mm	11	100	55	0
	CTX 0.5ug	Proposed $< 26$ mm	88	75	8	10
N526K (n=57)	AMC	CLSI $\geq 8$ mg/L	13	100	46	0
N526K# (n=10)	AMC	EUCAST $> 1$ mg/L	81	63	10	17
	CTX	Proposed $\geq 0.06$ mg/L	63	92	20	4
versus	AMC 30ug	CLSI / EUCAST $\leq 19 / < 20$ mm	0	100	53	0
	AMC 3ug	CLSI Karpanoja $< 16$ mm	51	100	26	0
N526 (n=36)	CTX 0.5ug	Proposed $< 26$ mm	90	76	6	11
N526# (n=23)						

SN = sensitivity: % of N526K strains showing resistance on test over all N526K strains.  
SP = specificity: % of N526 strains showing susceptibility on test over all N526 strains.  
FN = false negative: % of N526K strains showing susceptibility over all strains tested.  
FP = false positive: % of N526 strains showing resistance over all strains tested.  
# = strains also B-lactamase positive.

**Results:** In strains without B-lactamase, AMP MICs performed poorly in detecting N526K strains and as expected the AMP 10 µg disc performed significantly worse than the 2 µg disc even though they are calibrated to the same MIC BP. When strains with B-lactamase were included, there was a significant difference between the performance of EUCAST and CLSI AMC susceptibility because they use different BPs, and again the use of a low potency disc as recommended by Karpanoja improved performance. The proposed CTX based methods showed superior performance.

**Conclusions:** A screen using CTX as a substrate and BPs of  $\geq 0.06$  mg/L or  $\leq 26$  mm with a 0.5 µg disc, gives the best overall performance in detecting strains with N526K and is not influenced by the presence of B-lactamase. Strains with a positive screen could be reported as having “reduced B-lactam susceptibility” or further tested, depending on the requirements of individual laboratories.

#### P673 PCR screening for beta-lactamase-negative ampicillin-resistant strains of *Haemophilus influenzae*

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**Objectives:** Problems with MIC based methods and breakpoints, has prompted a move to genotypic definition and detection of B-lactamase negative ampicillin resistant (BLNAR) *H. influenzae*. Almost all BLNAR strains have an N526K substitution in penicillin binding protein 3 (PBP3) and single nucleotide specific (snp) PCR methods have been developed to interrogate the *ftsI* gene relevant to that substitution. In published methods, the design of specific reverse primers was based on the AAT (N) to AAG (K) mutation at the 1576–1578 codon seen in BLNAR strains sequenced at that time. There is relatively little published data on the evaluation of these primers, and their performance is in question as it is now recognised that in many BLNAR strains the N526K may be associated with AAA. This study aims to evaluate existing and new snp PCR primers for detecting N526K BLNAR *H. influenzae*.

**Methods:** Nineteen B-lactamase negative ampicillin susceptible (BLNAS-AAT) and 25 BLNAR (11-AAA, 14-AAG) strains were used to evaluate various primer sets on a real time PCR platform to differentiate BLNAR from BLNAS strains. Performance calculations are based on designation of an isolate as BLNAR (either by amplification or non-amplification depending on the assay) being considered a positive, with true positive status based on known genotype.

**Results:** Hasagawa’s PBP3-S PCR, designed to amplify normal BLNAS (AAT) was 100% sensitive and specific in BLNAR detection (Table 1), as all strains with AAT at position 1576–1578 of the *ftsI* gene amplified, and all those with the N526K substitution irrespective of how it was coded did not amplify. By comparison, Hasagawa’s PBP3-BLN and Nakamura’s PBP3-INT PCR assays, designed to amplify BLNAR strains, were relatively non-specific and amplified many BLNAS strains. When different Ct gates were applied, specificity was improved with corresponding loss of sensitivity due to an inability of the primers to differentiate AAT and AAA strains. However, the PBP3-BLNAR PCR of this study, used a degenerate reverse primer targeting both AAA and AAG BLNAR strains and was 100% sensitive and specific.

Author <sup>a</sup>	Primer Set	TP (n)	FN (n)	TN (n)	FP (n)	Sensitivity (%)	Specificity (%)
Hasegawa et al.	PBP3-S <sup>b</sup>	25	0	19	0	100	100
	PBP3-BLN <sup>b</sup>	21	4	5	14	84	26
Nakamura et al.	PBP3-INT <sup>b</sup>	24	1	5	14	96	26
Designed this study	PBP3-BLNAR <sup>c</sup>	25	0	19	0	100	100

<sup>a</sup> Hasegawa et al., 2003; Nakamura et al., 2009.

<sup>b</sup> These assays gated at 30 cycles consistent with original publications.

<sup>c</sup> PBP3-BLNAR assay set is gated at 20 cycles. All BLNARs amplify before 20 cycles.

TP; True positives, FN; false negatives, TN; true negatives, FP, false positives

**Conclusion:** Hasegawa’s PBP3-S or the PBP3-BLNAR PCR of this study can be reliably used to screen for N526K BLNAR strains (AAG or AAA) depending on whether amplification or non-amplification is seen as a better indicator. The other assays evaluated are unreliable due to difficulty in differentiating BLNAS strains from AAA type BLNAR strains resulting from poor primer design.

#### P674 Phenotypic detection of clinical isolates of *Haemophilus influenzae* with altered penicillin-binding protein

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**Objectives:** Resistance due to penicillin-binding protein (PBP) three alterations are difficult to demonstrate, because sequencing of the *ftsI* gene is usually impractical. We have evaluated several combinations of culture media, testing methodologies and antibiotics for detection of *Haemophilus influenzae* (H.i) with mutations in the *ftsI* gene.

**Methods:** We have studied 77 H.i in which the *ftsI* gene has been amplified by PCR sequenced: 12 strains with wild-type *ftsI* (six producing and six not producing beta-lactamase) and 65 with different mutation profiles [54 B-lactamase negative ampicillin-resistant (BLNAR) and 11 B-lactamase positive amoxicillin-clavulanate resistant (BLPACR)]. Twenty mutation patterns were observed [the two most prevalent: D350N+A502T+N526K (22%) and 350N+M377I+A502V+ N526K (18%)]. Ampicillin (AMP; 2 µg), amoxicillin-clavulanate (AMC; 20/10 µg), cefaclor (CC; 30 µg) and cephalotin (CF; 30 µg) were tested by disk-diffusion on Mueller-Hinton agar with lysed horse blood and NAD (MHF), *Haemophilus* test medium agar (HTM), and Chocolate agar (CHO). MICs of AMP, amoxicillin (AMX) and AMC were determined by E-test. Plates were incubated in 5% CO<sub>2</sub> and read at 20 and 44 hour. H.i ATCC 49766, 1021 (BLNAS) and 49247 (BLNAR) were used as control. Results were interpreted according to EUCAST or (for CF) the CA-SFM.

**Results:** MHF, HTM and CHO allowed growth at 20 hour of 76/77 (99%), 53/77 (69%) and 77/77 (100%) strains, respectively; for another 7 (9%) isolates HTM could be read at 44 hour. Similar inhibition zones and MICs were observed in MHF and CHO at 20 and 44 hour. Clinical categories for strains with wild-type *ftsI* were correct by both disk diffusion and Etest in the three media. For BLPACR with mutation *ftsI* resistance to AMC was detected by Etest for 10/11 strains in all media, and by disk diffusion in 2/11 (CHO), 1/11 (MHF) and 0/11 (HTM). For the remaining 54 strains with mutated *ftsI* the highest percentages of resistance at 20 hour were for CF-disc/CHO (81%) and CC-disc/CHO (80%). Higher resistance rates were obtained at 44 hour for CC-disc/CHO (91%), CF-disc/CHO (83%), AMX-Etest/CHO (82%), and AMX-Etest/MHF (81%). A CC-disc/CHO plus an AMX-Etest/CHO at 48 identified 93% of the isolates.

**Conclusions:** HTM does not allow adequate growth at 20 hour of a relevant number of *H. influenzae*. Detection of isolates with a mutated *ftsI* after a 20 hour incubation is best achieved using chocolate agar and CF or CC discs. Testing of AMX Etest plus CC discs in CHO agar with reading at 44 hour allows optimal detection of these isolates.

#### P675 Contemporary doxycycline and tetracycline susceptibility testing using CLSI and EUCAST criteria for Gram-positive pathogens: results from SENTRY programme

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**Objectives:** To assess the potency and intermethod agreement for doxycycline (DOXY) and tetracycline (TETRA) susceptibility (S) testing breakpoints when tested against SENTRY Antimicrobial Surveillance Program isolates of Gram-positive species (13 188 isolates) collected worldwide.

**Methods:** All organisms were cultured in 2010 with S testing by CLSI M07-A9 (2009) methods and results interpreted by CLSI M100-S11 (2011) and EUCAST (2011) criteria for TETRA and DOXY. A total of 9012 *S. aureus* (SA; 44.9% MRSA); 2325 *S. pneumoniae* (SPN); and

1851 beta-haemolytic streptococci (BHS; 42.8% *S. pyogenes* [SPYO]) were analyzed for S and cross-S rates by the two international breakpoint sets. The tetracycline's S breakpoint MIC (mg/L) criteria differ (CLSI/EUCAST, respectively) as follows: for SA ( $\leq 4/\leq 1$ ), for SPN ( $\leq 2/\leq 1$ ) and for BHS ( $\leq 2/\leq 1$ ). All quality control tests were within published CLSI ranges.

**Results:** S rates for DOXY were consistently greater than TETRA for each interpretive criteria used and for each pathogen group analyzed. The CLSI DOXY/TETRA S rates (EUCAST rates) were as follows: 99.2/94.2 (96.7/93.2)% for MSSA; 96.2/91.2 (93.5/88.1)% for MRSA; 75.3/73.2 (73.8/73.0)% for SPN; 81.2/80.3 (80.2/79.6)% for SPYO; and 15.7/14.6 (15.4/14.6)% for *S. agalactiae* (SAGA). DOXY (MIC90, 0.5 mg/L) was generally two- to four-fold more potent than TETRA (MIC90, 2 mg/L) vs. MRSA. Use of TETRA-S results to predict DOXY-S was excellent (>99.9–100.0%) for SA regardless of breakpoints used, as were predicted for SPN (99.8–100.0%), SPYO (99.6–99.9%) and SAGA (100.0%); errors usually higher applying the lower EUCAST breakpoints. Concerns persist that strains of staphylococci and streptococci having TET-R mechanisms could be categorized by CLSI as S (MICs at 2 or 4 mg/L) by current breakpoints e.g. 2.7% of MRSA tested against DOXY.

Organism/Antimicrobial (no. tested)	Tetracycline (no. isolates)					
	CLSI category <sup>a</sup>			EUCAST category <sup>b</sup>		
	Susceptible	Intermediate	Resistant	Susceptible	Intermediate	Resistant
<b>Doxycycline</b>						
MSSA (4,966)						
Susceptible	4,677	41	208	4,630	18	155
Intermediate	0	0	33	1	1	84
Resistant	0	0	7	0	0	77
<b>MRSA (4,046)</b>						
Susceptible	3,689	32	172	3,564	117	103
Intermediate	0	0	128	0	1	38
Resistant	0	0	25	1	0	222

a. CLSI (2011) criteria for tetracyclines: susceptible at  $\leq 4$  mg/L, and resistant at  $\geq 16$  mg/L. b. EUCAST (2011) criteria for tetracyclines: susceptible at  $\leq 1$  mg/L, and resistant at  $> 2$  ( $\geq 4$ ) mg/L.

**Conclusions:** CLSI and EUCAST interpretive criteria for tetracyclines (TETRA and DOXY) remain discordant, but each determines DOXY to have wider spectrum against four Gram-positive pathogen species and that TETRA-S can accurately predict DOXY-S (99.93–99.86% across 13 188 isolates). Moreover, molecular test-confirmed mechanisms appear highly probably among CLSI-susceptible (MICs, 2 or 4 mg/L) strains requiring international harmonization, to also include other tetracycline or-like agents and systematically applying pharmacodynamic principles.

#### P676 Antimicrobial susceptibility testing of *Listeria monocytogenes* with EUCAST breakpoints: a multi-laboratory study

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**Objectives:** The European Committee on Antimicrobial Susceptibility Testing (EUCAST) has recently proposed clinical MIC breakpoints based on epidemiological cut-off (ECOFF) values for *Listeria monocytogenes*. The objective of this study was to establish corresponding zone diameter breakpoints based on the EUCAST Mueller-Hinton Fastidious medium (MH-F) in a multi-laboratory study and to validate the use of a gradient test for MIC determination using broth microdilution (BMD) as reference.

**Methods:** A total of 129 clinical isolates of *L. monocytogenes* were collected from five sites (Denmark, Israel, Norway, Sweden and UK) and antimicrobial susceptibility testing was performed against ampicillin, benzylpenicillin, erythromycin, meropenem and trimethoprim-sulfamethoxazole. Disk diffusion was performed at all sites on Mueller-Hinton agar with 5% defibrinated horse blood and 20 mg/L beta-NAD (MH-F) according to EUCAST methodology. Media and disks from different manufacturers were used at the various sites, and both commercial and in-house prepared MH-F plates were tested. MIC determination was performed with BMD (TREK

Diagnostics/Thermo Fischer Scientific) and Etest (bioMérieux) at Southmead Hospital, Bristol, UK. BMD was performed on custom panels containing MH broth with 5% lysed horse blood (by repeated freezing and thawing) and 20 mg/L beta-NAD (MH-F broth) and Etest was performed on MH-F media. All data were analysed by EUCAST.

**Results:** All *L. monocytogenes* isolates grew well on the MH-F medium. The correlation between BMD and Etest MICs was excellent, with 99.4% of Etest values within  $\pm 1$  dilution of the BMD values. Zone diameter breakpoints were established (Table 1) in such a way that the MIC ECOFF corresponded to the zone diameter distribution ECOFF. Slightly different inhibition zones were obtained at the sites, depending on differences between manufacturers of media and disks.

#### EUCAST Clinical MIC breakpoints for *Listeria monocytogenes* and corresponding zone diameter breakpoints.

Antibiotic agent	MIC breakpoint (mg/L)		Disk content ( $\mu$ g)	Zone diameter breakpoint (mm)	
	S $\leq$	R $>$		S $\geq$	R $<$
Ampicillin	1	1	2	16	16
Benzylpenicillin	1	1	1 unit	13	13
Erythromycin	1	1	15	25	25
Meropenem	0.25	0.25	10	26	26
Trimethoprim-sulfamethoxazole	0.03	0.03	1.25-23.75	29	29

**Conclusion:** MH-F is a suitable medium for antimicrobial susceptibility testing of *L. monocytogenes* for both gradient tests and disk diffusion. As a result of a multi-laboratory study, we present zone diameter correlates to currently proposed EUCAST clinical MIC breakpoints.

#### P677 Susceptibility testing of ten antibiotics against *Corynebacterium* spp. determined by broth microdilution, Etest and EUCAST disc diffusion methods

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**Objective:** EUCAST has not yet defined breakpoints for clinical categories of clinically relevant antibiotics for *Corynebacterium*. In this study, the performance of three methods for susceptibility testing of five *Corynebacterium* species has been evaluated.

**Methods:** The activity of Penicillin G (PNG), ciprofloxacin (CIP), moxifloxacin (MOX), gentamicin (GEN), vancomycin (VAN), clindamycin (CLI), tetracycline (TET), linezolid (LIN), rifampin (RIF) and cotrimoxazole (SXT) against 60 *Corynebacterium* spp. (12 isolates from each of the following species: *C. striatum*, *C. amycolatum*, *C. jeikeium*, *C. urealyticum* and *C. pseudodiphtheriticum*) were determined using BMD (broth microdilution with cation-adjusted Mueller-Hinton broth with 3% laked horse blood), or on solid medium (Mueller-Hinton agar with 5% defibrinated horse blood and 20 mg  $\beta$ -NAD/L) by Etest and disk diffusion (disk contents as defined by EUCAST). BMD was performed in one lab in Spain, Etest in one lab in Sweden and disk diffusion in three labs (one in Spain, two in Sweden). Plates were incubated at 35°C in air (microdilution) or 5% CO<sub>2</sub> (agar media) and read after 16–20 hour or, in case of insufficient growth, after another 24 hour.

**Results:** Microdilution results could be read after 16–20 hour incubation for all species but *C. pseudodiphtheriticum*. Etest MICs and inhibition zones could be determined at 16–20 hour for all 12 *C. striatum*, but incubation for 40–44 hour was needed to a varying degree for the other species.

Essential agreement (EA, that in  $\pm 1$  dilution) between microdilution and Etest was: PNG and VAN (96.7%), GEN (91.7%), RIF (88.3%), LINZ (81.7%), CIP (76.7%), MOX (71.7%), CLI (66.7%); TET (65%), and SXT (55.0%). For species, EA were: *C. urealyticum* (95.8%), *C. jeikeium* (85.8%), *C. striatum* (80.8%), *C. jeikeium* (73.3%) and *C. pseudodiphtheriticum* (66.6%).

Correlation between BMD MICs and inhibition zones was good, i.e. isolates with high MICs had small inhibition zones and vice versa. Correlation between inhibition zones from three labs was over all good, even when zones had to be read after another 24 hour.

**Conclusions:** Growth of *Corynebacterium* spp. on MH-F differs between species and it is sometimes necessary to incubate plates for longer than the standard 16–20 hour. There is a good correlation between BMD and Etest for penicillin G, vancomycin and gentamicin. More studies on MIC/zone correlation on species-identified *Corynebacterium* must be performed before breakpoints (or ECOFFs) can be established.

#### **P678** Antimicrobial resistance of *Corynebacterium ulcerans* and *C. pseudotuberculosis*

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**Background:** Zoonotic *Corynebacterium ulcerans* and *C. pseudotuberculosis* strains are increasingly recognized as causative agents for human infections presenting as classical or cutaneous diphtheria. Besides prompt diphtheria antitoxin therapy in cases showing classical signs of pharyngeal diphtheria, they require specific antibiotic treatment both to eradicate the pathogens in patients and to prevent infection among contacts. Benzyl penicillin and erythromycin are recommended for specific antibiotic treatment, although only very few antimicrobial susceptibility data are available.

**Methods:** In this study, 74 isolates of toxigenic and non-toxicogenic clinical isolates of *C. pseudotuberculosis* (n = 49) and *C. ulcerans* (n = 25) from humans and animals collected in the National Consiliary Laboratory for Diphtheria from 2001 to 2011 were tested. Minimal inhibitory concentration (MIC) of benzyl penicillin, ceftriaxone, erythromycin, tetracycline, clindamycin, and ciprofloxacin were determined using the Etest system (McFarland standard 0.5; Mueller Hinton blood agar supplemented with 5% sheep blood). In the absence of standardized breakpoints, antibiotic susceptibility was determined by using the CLSI criteria for broth microbouillon dilution susceptibility testing for *Corynebacterium* species.

**Results:** Benzyl penicillin, erythromycin and ciprofloxacin seem to be highly active substances in both potentially toxigenic *Corynebacterium* species. All *C. pseudotuberculosis* strains were completely susceptible to all examined antibiotics. All tested *C. ulcerans* strains were susceptible to penicillin and ceftriaxone, while 80% of the *C. ulcerans* strains were resistant against clindamycin. Moreover, one tox-positive *C. ulcerans* strain isolated from a 56-year-old patient with diphtheria like disease was multi-resistant to erythromycin, clindamycin and tetracycline, but susceptible to penicillin.

**Conclusions:** In Germany penicillin and erythromycin can still be recommended for specific antibiotic treatment of diphtheria caused by *C. ulcerans* or *C. pseudotuberculosis*. Antibiotic resistance against first line drugs may occur, but multi-drug resistant strains are rarely observed. Although clindamycin is not a first-line drug for diphtheria therapy, it should be noted that *C. ulcerans* is not surely covered by this antibiotic. Our data emphasize the necessity of accurate species identification and susceptibility testing of potentially toxigenic *Corynebacterium*.

#### **P679** Comparison of the *Clostridium difficile* agar and Brucella agar for the antimicrobial susceptibility testing of *Clostridium difficile*

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**Background:** As the name *Clostridium difficile* implies, culture of this pathogen is not easy, and the isolation rate could be affected by specimen treatment method, culture media, or quality of anaerobic condition, etc. Active growth is especially critical when rapid and abundant growth of the microorganism is highly needed, such as antimicrobial susceptibility testing. At a preliminary study, the growth

speed and size of colonies were better in *C. difficile* (CD) agar than on the Brucella agar, so we compared the two media for the antimicrobial susceptibility testing (AST) of *C. difficile* isolates.

**Methods:** The two media used were supplemented (Hemin + VitK1) Brucella agar containing 5% sheep blood, and CD agar (*Clostridium difficile* Agar Base, Oxoid, UK) containing taurocholate (0.1%) and 7% horse blood. The six antibiotics (clindamycin, metronidazole, moxifloxacin, piperacillin-tazobactam, rifaximin, and vancomycin) were added to CD and Brucella media to make AST agar plates containing various concentrations of antibiotics. We used inoculums of 1 McFarland turbidity standard instead of 0.5 standard for the ordinary AST. A total of 171 isolates of *C. difficile* were inoculated with 36-pin replicator to the AST agar plates and incubated in anaerobic chamber for 48 hours.

**Results:** Table 1.

Antimicrobials	Media & number	MIC (ug/ml)			% Resistance		
		Range	MIC50	MIC90	S	I	R
Clindamycin	CD(138)	1.5–256	≥256	≥256	11.6	21.0	67.4
	B(139)	1–256	≥256	≥256	10.8	20.9	68.3
Metronidazole	CD(117)	0.06–8	0.5	4	100.0	0.0	0.0
	B(117)	0.25–4	1	2	100.0	0.0	0.0
Moxifloxacin	CD(164)	0.5–128	16	32	42.1	0.6	57.3
	B(160)	0.5–128	16	64	42.5	0.6	56.9
Piperacillin-Tazobactam	CD(169)	4–64	16	32	99.4	0.6	0
	B(165)	1–64	16	16	99.4	0.6	0
Rifaximin	CD(159)	0.0015–8	0.015	> 8	No standardized breakpoint		
	B(150)	0.0007–8	0.007	> 8	No standardized breakpoint		
Vancomycin	CD(171)	1–8	4	8	100	0	0
	B(165)	0.125–4	1	2	100	0	0

**Conclusion:** Though the growth speed was faster and size of the *C. difficile* colonies was bigger on the enriched *C. difficile* media than the Brucella media, the antimicrobial susceptibility testing results by % resistance were not significantly different by the media themselves. Also the MIC distributions of the six antibiotics were not significantly affected by the media, but MIC50 and MIC90 of the vancomycin was two fold higher in the CD media.

#### **P680** The Brucella blood agar for disc diffusion antimicrobial susceptibility testing – reproducibility results for *Clostridium difficile* ATCC 700057

U.S. Justesen, L.T. Erikstrup\*, T.K. Danielsen, E. Matuschek, G. Kahlmeter (Odense, Aarhus, DK; Växjö, SE)

**Objectives:** The EUCAST disk diffusion antimicrobial susceptibility testing method for fastidious organisms is based on the Mueller-Hinton fastidious agar (MH-F). In a pilot study, most anaerobic bacteria did not grow well enough on MH-F to permit antimicrobial susceptibility testing. We decided to investigate whether or not the Brucella Blood Agar supplemented with hemin and vitamin K (BBA), recommended for antimicrobial susceptibility testing of anaerobic bacteria with gradient strips, might also be suited for disk diffusion testing of *C. difficile*.

**Methods:** *C. difficile* ATCC 700057 was tested with Etest (bioMérieux, Craaponne, France) gradient strips (piperacillin/tazobactam, meropenem, metronidazole, clindamycin, penicillin G, vancomycin, moxifloxacin and tigecycline) on BBA (Becton Dickinson, Heidelberg, Germany) according to the manufacturer's instructions. The corresponding disk (EUCAST disk strength) was included on each plate (Oxoid, Basingstoke, UK). All tests were repeated twelve times. I.e. twelve plates were incubated day 1 at 37°C in an anaerobe environment (10% CO<sub>2</sub>, 10% H<sub>2</sub> and 80% N<sub>2</sub>) for 24 hours with each antimicrobial agent to assess intra-day variability. This was repeated day 2 to assess inter-day variability. Etest results were compared with the acceptable ranges for ATCC 700057 (reference

Antimicrobial agent	Inhibition zone diameters (mm) for <i>Clostridium difficile</i> ATCC 70007		
	Day 1 and 2 at 37°C		35°C
	(10% CO <sub>2</sub> , 10% H <sub>2</sub> and 80% N <sub>2</sub> )		(10% CO <sub>2</sub> , 10% H <sub>2</sub> and 80% N <sub>2</sub> )
Piperacillin-tazobactam (30.6 microg)	27.5 (25.7-28.3)	26.9 (25.2-28.3)	26.5 (25.7-27.3)
Meropenem (10 microg)	29.7 (28.7-30.1)	31.4 (30.5-32.1)	31.5 (30.9-31.8)
Metronidazole (5 microg)	38.7 (37.4-40.0)	38.6 (37.6-39.7)	38.9 (38.2-40.0)
Clindamycin (2 microg)	10.3 (9.6-10.8)	10.8 (10.4-11.1)	11.8 (10.8-12.3)
Penicillin G (1 U)	6.0 (5.0) NO ZONE	8.3 (5.9-9.0)	6.4 (5.0-8.2)
Vancomycin (5 microg)	23.1 (22.5-23.3)	23.0 (22.6-23.7)	23.5 (23.1-24.3)
Moxifloxacin (5 microg)	25.5 (24.5-26.3)	25.4 (24.9-25.8)	25.1 (24.4-25.9)
Tigecycline (15 microg)	34.5 (34.0-34.9)	34.5 (33.4-35.1)	35.5 (34.7-36.2)

agar dilution testing, CLSI guideline M11-A7). Twelve plates with disks only were also incubated at 5% CO<sub>2</sub> (10% H<sub>2</sub> and 85% N<sub>2</sub>) at 37°C and another twelve plates at 35°C with 10% CO<sub>2</sub>, 10% H<sub>2</sub> and 80% N<sub>2</sub>.

**Results:** All Etest results were within acceptable ranges and the intra- and inter-day variability was ≤1 dilution step. Zone diameter mean values and ranges are shown in the table.

The greatest difference between two mean values was 3.2 mm and the greatest range was 3.3 mm. There were small effects of the changes in CO<sub>2</sub> levels and temperature on the inhibition zone diameters.

**Conclusion:** Both intra- and inter-day reproducibility was excellent with disk diffusion on BBA with the eight tested antimicrobial agents. Studies to decide whether this can be repeated with clinical isolates and whether disk diffusion can distinguish resistant isolates from wild type *C. difficile* are in progress.

#### P681 Disc diffusion antimicrobial susceptibility testing of *Clostridium difficile*

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**Objectives:** The clinical significance of *Clostridium difficile* and the emergence of decreased susceptibility, especially towards metronidazole, have highlighted the importance of antimicrobial susceptibility testing of *C. difficile* and the importance of having a simple testing method for the routine laboratory. The aim of our study was to evaluate disk diffusion for antimicrobial susceptibility testing of *C. difficile* by comparing disk diffusion results with Etest gradient strips.

**Methods:** Consecutive clinical and toxigenic isolates of *C. difficile* (n = 211) were collected from patients with diarrhoea hospitalised at Aarhus University Hospital (n = 110) in 2008 and from Odense University Hospital (n = 101) in 2010. Furthermore, ten clinical isolates of *C. difficile* from the University Hospital of Wales with reduced susceptibility to either metronidazole or vancomycin, were included. The isolates were tested with Etests (metronidazole, vancomycin, clindamycin, and moxifloxacin) (bioMérieux, Craponne, France) and the following corresponding disks (microgram) were used: metronidazole (5), vancomycin (5), clindamycin (2), and moxifloxacin (5) (Oxoid, Basingstoke, UK). All tests were performed on Brucella Blood Agar supplemented with hemin and vitamin K (Becton Dickinson, Heidelberg, Germany). The plates were incubated at 37°C

Antimicrobial agent	Disk diffusion zone diameter (mm)		Etest MIC (mg/L)		ECOFF ≤
	Median (range) Danish isolates	Median (range) Welsh isolates	Median (range) Danish isolates	Median (range) Welsh isolates	
Metronidazole	37 (23-45)	12 (9-14) <sup>1</sup>	0.094 (0.023-1)	2 (1.5-3) <sup>1</sup>	2
Vancomycin	23 (19-28)	18 (15-19) <sup>1</sup>	0.5 (0.25-1.5)	2 (1.5-3) <sup>1</sup>	2
Clindamycin	11 (6-17)		3 (0.38-256)		16
Moxifloxacin	24 (6-28)		0.75 (0.38-32)		4

1. n=4 for isolates with reduced susceptibility towards metronidazole 2. n=6 for isolates with reduced susceptibility towards vancomycin.

in an anaerobe environment (10% CO<sub>2</sub>, 10% H<sub>2</sub> and 80% N<sub>2</sub>) for 24 hours. The zone diameter was read at 100% inhibition.

**Results:** Zone diameter (median and range) and MIC (median and range) for the Danish and the Welsh isolates are shown in the table. The majority of the Danish isolates had MICs below the ECOFF (i.e. MICs representative of the wild type), whereas the Welsh isolates were selected because of known reduced susceptibility to metronidazole or vancomycin. For each MIC value, the inhibition zones varied from 1 to 9 mm (the majority exhibiting 5–6 mm variation).

**Conclusion:** There was a good agreement between MIC values and zone diameters for the antimicrobial agents tested. Disk diffusion was able to detect reduced susceptibility towards metronidazole and vancomycin. Disk diffusion is an option for antimicrobial susceptibility testing of *C. difficile*. Zone diameter breakpoints can now be established by EUCAST.

#### P682 EUCAST standardised disc diffusion methodology for *Campylobacter jejuni* and *C. coli*

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**Objectives:** Antimicrobial susceptibility of *Campylobacter* spp. is often performed with disk diffusion at clinical microbiological laboratories, even though standardised methodologies and interpretive criteria are lacking to various extents. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) is in the process of establishing clinical MIC breakpoints for *Campylobacter* spp. The objective of this study was to investigate if EUCAST methodology can be used as a reproducible disk diffusion test for *C. jejuni* and *C. coli*. **Methods:** Disk diffusion according to EUCAST methodology was performed at three sites (FI, NL and SE). Preliminary testing was performed on 15 *C. jejuni* and 15 *C. coli* from NL with known MIC values (broth microdilution, ISO 20776-1:2006), including isolates with and without phenotypic resistances. Additional testing was performed on 17 *C. jejuni* and 12 *C. coli* from FI. All isolates were tested in triplicate for ciprofloxacin 5 µg, erythromycin 15 µg and tetracycline 30 µg. Preliminary testing included different media (unsupplemented MH agar vs. MH agar with 5% defibrinated horse blood and 20 mg/L beta-NAD, MH-F), incubation temperatures (35, 37, 39 vs. 41°C) and length of incubation (24 vs. 44 hour). A McFarland 0.5 inoculum and incubation in plastic containers (microaerobic environment) were used. Because of the inhibition zone halo effect obtained when tilting plates, inhibition zones were recorded as the smallest and largest appearing zone.

**Results:** The proposed disk diffusion methodology is presented in Table 1. Both *C. jejuni* and *C. coli* grew best on MH-F plates at 41°C. Swarming was avoided by drying plates before inoculation. *C. jejuni* grew well after 24 hour of incubation whereas some *C. coli* required 44 hour before inhibition zones could be read. All isolates with MICs above the epidemiological cut off (ECOFF) were identified as non-wild type also with the disk diffusion test, irrespective of whether the smallest or largest inhibition zone was used. The results were reproducible within and between the sites.

#### EUCAST proposed disk diffusion methodology for *Campylobacter jejuni* and *Campylobacter coli*.

Medium	Mueller-Hinton agar with 5% defibrinated horse blood and 20 mg/L β-NAD (MH-F) (Plates should be dried before incubation to avoid swarming, either at room temperature (20-22°C) over night or at 35°C for 1-2 hours.)
Inoculum	McFarland 0.5
Incubation	Microaerobic environment 41°C 24 hours ± 30 minutes ( <i>C. coli</i> with non-sufficient growth after 24 h incubation are re-incubated immediately and read after totally 40-48 h incubation.)
Reading of zones	EUCAST standard reading instructions: Read MH-F plates from the front with the lid removed illuminated with reflected light. Zone edges should be read at the point of complete inhibition as judged by the unaided eye.

**Conclusion:** Disk diffusion testing of *C. jejuni* and *C. coli* on the EUCAST MH-F media at three sites resulted in reproducible inhibition

zones, separating wild-type isolates from those with MICs above ECOFF. Further evaluation of the methodology is currently performed at several laboratories in Europe.

### P683 Development of an agar dilution susceptibility testing method for *Actinomyces* species

M. Wootton\*, V. Hall, V.E. Daniel, R. A. Howe (Cardiff, UK)

**Objectives:** Actinomycosis is a chronic disease characterized by abscess formation, tissue fibrosis and draining sinuses, caused by *Actinomyces* species. These bacteria are normal colonising organisms of the oropharynx, gastrointestinal tract and female genital tract, requiring a break in the mucous membranes to invade deeper body structures and cause illness. Infections often develop in tissue adjacent to mucous membranes; oral and cervicofacial infections are most common but any body-site can be infected and, rarely, disseminated spread can occur. Susceptibility testing of anaerobes in general, and in actinomycetes in particular, is problematic due to growth requirements and slow growth. Currently, only CLSI have described an anaerobe susceptibility testing method. This study aims to compare combinations of media, inoculum size, blood and additives on growth & quality of MIC cut off to inform the development of a EUCAST method.

**Methods:** Forty-five *Actinomyces* species of varied morphology were used; *A. israelii*, *A. gerencseriae*, *A. graevenitzi*, *A. meyeri*, *A. naeslundii*, *A. odontolyticus*, *A. urogenitalis*, *A. turicensis*, *A. cardiffensis*, *A. funkei*, *A. europaeus*. Agar dilution (AD) (CLSI) with Penicillin was performed using McFarland 1, 2 and 4 inoculum densities. Combinations of media, blood and additives used were: Brucella agar (BA) + 5% laked sheep blood (LSB) + haemin (h) + vitamin K (vk) (CLSI), Mueller Hinton agar (MHA) + 5% defibrinated horse blood (DHB) + NAD (EUCAST-F), plus CLSI and EUCAST method variants; 1- BA + DHB + h + vk, 2- BA + 5% LSB + NAD + h + vk, 3- BA + 5% DHB + h + vk, 4- MHA + LSB + NAD + H + vk, 5- MHA + DHB + NAD + h + vk. Quality of growth and cut off were compared. MICs for each combination were compared to CLSI method.

**Results:** Quality of growth and cut offs were compared (Table 1). Growth and cut off quality was good to poor when using MHA but good to excellent for BA. It was considered that CLSI method resulted in accurate MICs. Percentage agreement to CLSI MICs was best (98%) in combination 3 and worst in EUCAST-F (66%).

	Quality of growth	Quality of cut off	% MIC agreement with CLSI	% MIC disagreement with CLSI
CLSI	Excellent	Excellent	N/A	N/A
EUCAST	Good	Fair	66	34
1	Good	Good	96	4
2	Good	Good	97.8	2.2
3	Very good	Very good	98	2
4	Poor	Very poor	90.1	9.1
5	Poor	Poor	74	26

**Conclusions:** The CLSI method using Brucella agar and 5% laked sheep blood gives the best quality growth and cut offs for *Actinomyces* species using penicillin. However DHB can be substituted with little loss to growth and cut off quality or MIC accuracy.

### P684 Comparison of neo-sensitabs (ROSCO) tablets with paper discs (OXOID) for antimicrobial susceptibility testing of Gram-negative clinical isolates according to the EUCAST recommendations

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**Objectives:** The EUCAST disk diffusion method was developed using 6 mm paper disc but Neo-sensitabs (ROSCO) have 9 mm diameter: Are

Neo-sensitabs tablets equivalent for the proposed by the EUCAST? The aim of this study was to validate the complete exchangeability between neo-sensitabs and paper discs among clinical Gram-negative microorganisms.

**Methods:** One hundred and seventy-five clinical Gram-negative isolates were included: 150 Enterobacteriaceae (including ESBLs and AmpC isolates) and 25 Gram negative non fermenters (including multi-resistant *P. aeruginosa*). Strains were tested in parallel for 18 antimicrobial drugs (paper discs and neo-sensitabs) According to the EUCAST disk diffusion method for antimicrobial susceptibility testing (V 1.1 2010). MICs were determined by microdilution (BD Phoenix System). Four quality control test were included: *P. aeruginosa* (ATCC27853), *E. coli* (ATCC25922), *E. coli* (ATCC35218), *K. pneumoniae* (ATCC 700603). To minimize the inoculum effect, paper discs and tables were tested into the same plate. Plates were read by the SIRscan system (i2a). To allow comparison, zones around OXOID disks with diameters below 9 mm were read as 9 mm. Results were analysed by Passing-Bablok regression and Pearson's correlation.

**Result:** No differences were observed in the inhibition diameter zone between neo-sensitabs and oxoid paper discs (see table 1). Overall percentage of 0.14% of minor error and 0.14% of major error was observed. Good correlation between the diameter of inhibition zones and MIC values were observed with both discs (<3% of discrepancies).

antimicrobial	PB (95% CI)		PC	discrepancies (%)
	intercept	slope		
ampicillin 10 µg	0,999 (0,999 to 0,999)	1,000 (1,000 to 1,000)	0,999	0
amoxicillin-clavulanic acid 20-10 µg	0,999 (0,999 to 0,999)	1,000 (1,000 to 1,000)	0,999	0
giperacillin-hydrocloridam 30-6µg	0,999 (0,999 to 0,999)	1,000 (1,000 to 1,000)	0,999	0
cefazolin 30 µg	0,999 (0,999 to 0,999)	1,000 (1,000 to 1,000)	0,999	0
Cefazone 30µg	0,999 (0,999 to 0,999)	1,000 (1,000 to 1,000)	0,992	0
Cefazone 15µg	0,999 (0,999 to 0,999)	1,000 (1,000 to 1,000)	0,999	0
Ceftriaxone 30 µg	0,999 (0,999 to 0,999)	1,000 (1,000 to 1,000)	0,992	0
cefepime 30µg	0,999 (0,999 to 0,999)	1,000 (1,000 to 1,000)	0,999	0
Achroman 30 µg	0,999 (0,999 to 0,999)	1,000 (1,000 to 1,000)	0,992	0
Moxiparam 15µg	3,299 (0,999 to 5,393)	1,000 (0,999 to 1,000)	0,997	0
ertapenem 10 µg	0,999 (0,999 to 0,999)	1,000 (1,000 to 1,000)	0,979	0
gentamicin 10 µg	0,999 (0,999 to 0,999)	1,000 (1,000 to 1,000)	0,979	0
Tobramycin 10 µg	0,999 (0,999 to 0,999)	1,000 (1,000 to 1,000)	0,991	3 minor, 1 major
Amikacin 30 µg	0,999 (0,999 to 0,999)	1,000 (1,000 to 1,000)	0,962	0
retifurankan 150µg	0,999 (0,999 to 0,999)	1,000 (1,000 to 1,000)	0,993	1 very major
co-trimoxazole 1:15-23:75	0,999 (0,999 to 0,999)	1,000 (1,000 to 1,000)	0,995	0
Levofloxacin 5µg	0,999 (0,999 to 0,999)	1,000 (1,000 to 1,000)	0,992	0
Ciprofloxacin 5µg	0,999 (0,999 to 0,999)	1,000 (1,000 to 1,000)	0,995	0

PB: Passing-Bablok test; PC: Pearson's correlation.

**Conclusion:** Our results showed excellent correlation between ROSCO neosensitabs and OXOID paper disk for Enterobacteriaceae antimicrobial sensitivity testing (EUCAST method) for all antibiotics tested. Overall percentage of 0.29% of error was observed. The majority of discrepancies concerning tobramycin.

### P685 Effect of manganese in test media on in vitro tigecycline susceptibility of *Enterobacteriaceae* and *Acinetobacter baumannii*

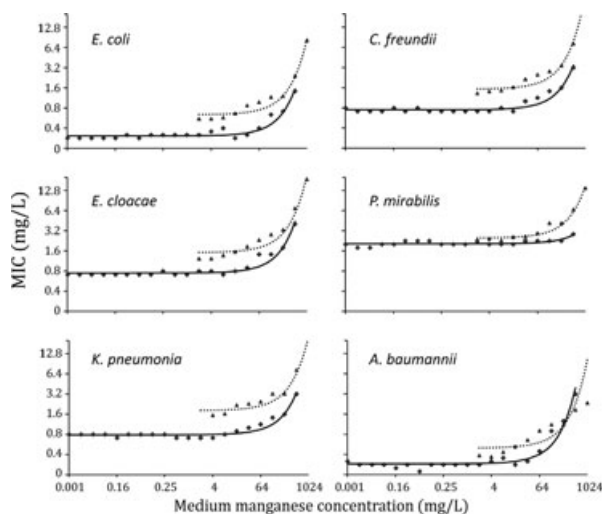
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**Objectives:** Several studies reported that in vitro bacterial susceptibility to tigecycline varies by test media and test conditions. This variability may be due to differences in manganese (Mn) concentration between test media, resulting in falsely elevated MICs when using standard media. We assessed the effect of Mn concentration in test media on MICs of tigecycline, using of five species of Enterobacteriaceae and *Acinetobacter baumannii*.

**Methods:** For each species, antimicrobial susceptibility was tested for five strains and an ATCC control strain, and MICs were determined by broth microdilution (BMD) and by Etest on freshly prepared media. We used two commercial test media with standardised low Mn concentration (Synthetic Amino Acid Medium (0 mg/L Mn) or Isosensitest agar (2 mg/L Mn), supplemented with increasing quantities of MnCl<sub>2</sub>, resulting in medium Mn concentrations ranging

(with two-fold incremental steps) between 0.001 and 1.024 mg/L and between 2 and 1.024 mg/L, for broth BMD and Etests, respectively.

**Results:** For both BMD and Etests, the effect of manganese on MICs was negligible for medium concentrations below 8 mg/L. MIC values increased substantially, however, when medium manganese concentrations exceeded 64 mg/L (figure). Compared to values measured on a Mn free medium, MICs were doubled at medium Mn concentrations of 220, 240, 200, 230 and 136 mg/L for *E. coli*, *K. pneumoniae*, *C. freundii*, *E. cloacae* and *A. baumannii*, respectively (BMD). For *P. mirabilis*, this effect seemed less pronounced (figure). Independent of Mn concentrations, MICs determined by Etest were higher than those determined by BMD (geometric mean difference 0.8 doubling dilution [95% CI: 0.7–0.9]).



**Conclusions:** In vitro bacterial susceptibility to tigecycline is influenced by manganese concentrations in the test medium. Susceptibility testing should be performed on a medium with standardised low manganese content (<8 mg/L). Due to poor reproducibility of its mineral content, the Mueller-Hinton medium may be unsuitable for this purpose. Because Mn concentrations in human serum (0.8–1.3 µg/L) are substantially lower than those found in standard susceptibility test media, Mn is unlikely to interfere with tigecycline antibacterial activity under physiological conditions. Lastly, the effect of medium Mn concentration could not explain the differences in MICs observed with Etest and broth microdilution, and further studies should clarify the causal factors involved.

**P686** New EUCAST breakpoint for detection of antimicrobial susceptibility in *Helicobacter pylori*

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**Background:** CLSI had breakpoints for *H. pylori* since 1999 but only for clarithromycin. Other breakpoints proposed by previous studies, by BSAC or breakpoint for other bacteria are used for studying antimicrobial activity of other antimicrobial agents. EUCAST group from ESCMID (European Committee on Antimicrobial Susceptibility Testing) has proposed in March–April 2011 breakpoint for all antimicrobials used to treat *H. pylori*.

The aim of this study was to determine the resistance percentage in *H. pylori* clinical isolates using EUCAST breakpoint.

**Methods:** Susceptibility to amoxicillin (Amx), tetracycline (Te), metronidazole (Met), clarithromycin (Cla), rifampicin (Rif) and levofloxacin (Lev) in 300 *H. pylori* clinical isolates from 1 March 2009 to 30 October 2011. Biopsies were cultured in selective and non

selective media. Strains identified by colony and Gram stain morphology, and urease, oxidase and catalase positive test. Antimicrobial susceptibility was performed by E-test and EUCAST breakpoint. According to previously used Breakpoints resistance to Amx was considered when >2 mg/L, to Met when ≥8 mg/L, to Cla when >0.5 mg/L, to Rif when >32 mg/L, and to Te and Lev when >4 mg/L. According to EUCAST Breakpoint resistance to Amx was considered when >0.12 mg/L, to Met when >8 mg/L, to Cla when >0.5 mg/L and to Te, Rif and Lev when >1 mg/L.

**Results:** The percentage of strains susceptible, intermediate or resistant with each breakpoint used is in the table.

		Amx	Cla	Rif	Lev	Te	Met
With old breakpoint	S %	93,6	45,5	97,6	93,3	100	63,9
	I %	0,7	1,3	0,4			
	R %	5,7	53,2	2	6,7		36,1
With EUCAST breakpoint	S %	87	45,5	70	93,3	99,7	64,6
	I %		0,7				
	R %	13	53,8	30	6,7	0,3	35,4

**Conclusions:** Levofloxacin resistance did not change with the use of the new breakpoint. Clarithromycin, metronidazole and tetracycline susceptibility changed only in a few strains. However, new breakpoint affected seriously amoxicillin and rifampicin resistance, with 13.6% of the strains being resistant to amoxicillin and 30.3% to rifampicin.

**P687** Susceptibility of *Actinomyces* species to potential therapeutic antimicrobials

M. Wootton\*, V. Hall, V.E. Daniel, R.A. Howe (Cardiff, UK)

**Objectives:** Actinomycosis is a chronic disease characterized by abscess formation, tissue fibrosis and draining sinuses, caused by *Actinomyces* species. These bacteria are normal colonising organisms of the oropharynx, gastrointestinal tract and female genital tract, requiring a break in the mucous membranes to invade deeper body structures and cause illness. Infections often develop in tissue adjacent to mucous membranes; oral and cervicofacial infections are most common but any body-site can be infected and, rarely, disseminated spread can occur. Current treatment of actinomycosis is penicillin or amoxicillin. However, these infections are usually polymicrobial requiring treatment which covers all organisms. This study aims to determine the susceptibilities of *Actinomyces* species to 11 antimicrobials thought to offer alternative therapies.

**Methods:** Two hundred and eighty-seven *Actinomyces* species of varied morphology were used; *A. israelii*, *A. gerencseriae*, *A. graevenitzi*, *A. meyeri*, *A. naeslundii*, *A. odontolyticus*, *A. urogenitalis*, *A. turicensis*, *A. cardiffensis*, *A. funkei*, *A. europaeus*, *A. georgiae*, *A. neuii*, *A. timonensis*, *A. oricola*, *A. hominis*, *A. johnsonii*, *A. oris*, *A. radingae*, *A. honkongensis*. MICs were determined for penicillin (P), amoxicillin (A), ceftriaxone (CX), erythromycin (E), clindamycin (CD), clarithromycin (CM), meropenem (M), rifampicin (R), tetracycline (T), doxycycline (D) and ciprofloxacin (CP) using agar dilution (CLSI).

**Results:** MIC50, MIC90, MIC range, percentage resistance, percentage susceptibility are shown in table 1. R was most active with MIC50 and MIC90 of 0.004 and 0.008 respectively. Only a few isolates of *A. neuii*, *A. radingae*, *A. turicensis* and *A. funkei* had MICs

	MIC Range	MIC50	MIC90	% R
P	0.015-1	0.06	0.25	6.6%
A	0.008-2	0.25	0.5	0%
CX	0.015-4	0.12	0.5	N/A
E	0.008->64	0.06	0.25	N/A
CD	0.008->64	0.12	0.5	4.2%
CM	0.004->64	0.03	>64	N/A
M	<0.002-0.5	0.008	0.12	0%
R	<0.002-0.12	0.004	0.008	N/A
D	0.03-32	0.5	1	N/A
T	0.12-64	0.5	2	N/A
CP	0.25-128	16	32	N/A

>0.03. M was very active against all species, exhibiting MIC50/MIC90 of 0.008/0.12. P, A, CX, E, CD, D, T were all active against most species. All isolates of *A. urogenitalis* exhibited  $\geq$ MIC90 MICs to all antimicrobials except R, M and CX. All 10 *A. funkei* showed A MICs  $\geq$ 0.25. CP & CM were least active against all species. Using EUCAST BPs, no resistance was found to occur with A & M, whilst 6.6% and 4.2% resistance was found in penicillin & clindamycin respectively.

**Conclusions:** Rifampicin and meropenem were most active, with meropenem showing efficacy against all species. Some species showed association with resistance to certain antimicrobials therefore species determination is important when choosing therapy.

## Community-acquired infections

### P688 Time trends in length of hospital stay, post-discharge mortality, and risk of readmission among patients hospitalised for community-acquired bacteraemia in Denmark, 1995–2006

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**Objective:** During the past decades the length of hospital stay for patients with bacteraemia has declined substantially. The continuing decline raises concerns that increasing numbers of patients may be discharged prematurely before their condition has been fully stabilized, leading to more adverse events in the period early after discharge. We examined changing trends over calendar time in length of stay, post-discharge mortality, and hospital readmission among patients discharged alive after a hospitalization with community-acquired bacteraemia.

**Methods:** Population-based cohort study of patients who were discharged alive after hospitalization with community-acquired bacteraemia, 1995–2006. Outcome measures were length of stay, 30-day post-discharge mortality and all-cause 30-day readmission. We used Cox regression analysis to examine changes in hazard ratios (HRs) for 30-day post-discharge mortality and readmission over four 3-year calendar periods, adjusting for age, sex, and comorbidity.

**Results:** Of the 4492 patients who were hospitalized with community-acquired bacteremia between 1995 and 2006, 597 (13.3%) died during hospitalization and 3895 (86.7%) were discharged alive. The mean length of hospital stay with community-acquired bacteremia decreased from 18.5 days in 1995 to 15.5 days in 2006, corresponding to a 16% relative reduction over this period. There was a slight decline in 30-day post-discharge mortality from 7.1% in the reference period 1995–1997 to 6.0% in 2004–2006. Adjusted 30-day post-discharge mortality HRs were 0.71 (95% CI 0.49–1.04) in 1998–2000, 0.88 (95% CI 0.63–1.25) in 2001–2003, and 0.84 (95% CI 0.59–1.19) in 2004–2006, when compared with 1995–1997. The proportion of patients who were readmitted to hospital within 30 days of discharge was 16.4% in 1995–1997, 17.9% in 1998–2000, 19.8% in 2001–2003, and 19.1% in 2004–2006, corresponding to adjusted HRs of readmission of 1.10 (95% CI 0.89–1.38) in 1998–2000, 1.21 (95% CI 0.98–1.50) in 2001–2003, and 1.17 (95% CI 0.95–1.45) in 2004–2006, compared with 1995–1997.

**Conclusion:** We found a decrease in length of stay for patients hospitalized with community-acquired bacteraemia between 1995 and 2006, and an increase over time in the proportion of patients who were

readmitted within 30 days after discharge. However, 30-day post-discharge mortality tended to decrease over time

### P689 Current management of patients hospitalised with complicated skin and soft tissue infections across Europe (2010–2011): assessment of clinical practice patterns and real-life effectiveness of antibiotics (REACH study)

J. Garau\*, F. Blasi, J. Medina, M. Ávila, K. McBride, H. Ostermann on behalf of the REACH study group

**Objectives:** Data on the management of hospitalised complicated skin and soft tissue infections (cSSTIs) are limited. REACH (NCT01293435) was a retrospective, observational cohort study aiming to provide data on current clinical management of moderate to severe cSSTIs in European hospitals.

**Methods:** Data were collected from 129 sites in ten European countries. The population comprised patients  $\geq$ 18 years, hospitalised between March 2010 and February 2011 with a cSSTI requiring intravenous antibiotic treatment. Variables were collected via an electronic Case Report Form.

**Results:** The total population included 1996 patients; mean age 60.6 years; 57.8% male, 1154 from university hospitals, 842 from non-university hospitals. Based on information in the patient records, 78.0% (n = 1557) of patients reported  $\geq$ 1 co-morbidity or receiving relevant medications in the 3 months prior to hospitalisation (64.3%; n = 1284); 29.9% (n = 596) had received antibacterials/antivirals. One-quarter of infections (25.6%; n = 510) were recurrences and 10% (n = 199) were nosocomial. Microbiological diagnosis was available for 51.1% (n = 1020) of patients, revealing Gram-positive cocci in 68.9% (n = 703) (9.9% [n = 101] methicillin-resistant *Staphylococcus aureus*, 26.9% [n = 274] methicillin-sensitive *S. aureus*) and Gram-negative bacilli in 33.6% (n = 343) of patients. Once hospitalised, patients were usually treated on Day 1 (81.6% [n = 1629] empirically and 17.3% [n = 346] with a specific therapy). The most common antibiotic agent used, alone or in combination, was amoxicillin-clavulanate (29.9%; n = 596) followed by piperacillin-tazobactam (18.2%; n = 364). Treatment failure (defined as a need for antibiotic change) was reported in 46.6% (n = 930) of patients (mortality rate 3.4%). Failure of initial therapy was more common in patients with co-morbidities vs those without (49.3%; n = 767 vs 37.1%; n = 163), in university vs non-university hospitals (49.7%; n = 573 vs 42.4%; n = 357) and in patients with nosocomial vs non-nosocomial cSSTI (53.3%; n = 106 vs 45.9%; n = 824). Surgery was required by 37% (n = 739) of patients. Initial treatment with piperacillin-tazobactam was associated with a failure rate of 49.6% vs 31.7% for amoxicillin-clavulanate. Outcomes varied by country; treatment failure varied between 55.6% in Italy and 33.8% in the Netherlands.

**Conclusions:** These data give a current view of management of cSSTIs in European hospitals in 2011 and provide evidence of a high failure rate of initial antibiotic therapy.

### P690 Health economic analysis of current clinical management of patients hospitalised with complicated skin and soft tissue infections across Europe (2010–2011) (REACH study): use of resources and consequences of treatment failure

H. Ostermann\*, F. Blasi, J. Medina, E. Pascual, K. McBride, J. Garau on behalf of the REACH study group

**Objectives:** Data describing the economic burden of hospitalised complicated skin and soft tissue infections (cSSTIs) in Europe are limited. REACH (NCT01293435) was a large, retrospective, observational study collecting empirical data on current management strategies and economic burden of cSSTI in European hospitals.

**Methods:** Data collected from 129 sites in ten European countries between March 2010 and February 2011 were recorded using an



Resource	Treatment failure		
	cSSTI N=1996*	With n=930*	Without n=1066*
Length of hospitalisation, days, mean (SD; median)	19.3 (22.0; 13.0) (n=1944)	24.5 (24.6; 16.0) (n=910)	14.7 (18.3; 10.0) (n=1034)
Admitted to ICU at any time, n (%)	130 (6.5)	91 (9.8)	39 (3.7)
Time in ICU, days, mean (SD; median)	9.7 (13.5; 4.5) (n=118)	11.4 (15.2; 5.0) (n=79)	6.2 (8.6; 3.0) (n=39)
Surgical intervention after cSSTI diagnosis, n (%)	739 (37.0)	387 (41.6)	352 (33.0)
Blood pressure support during hospitalisation, n (%)			
Fluid resuscitation	217 (10.9)	152 (16.3)	65 (6.1)
Vasopressors	75 (3.8)	68 (7.3)	7 (0.7)
Invasive procedures	13 (0.7)	7 (0.8)	6 (0.6)
Isolation required, n (%)	106 (5.3)	71 (7.6)	35 (3.3)
Parenteral nutrition, n (%)	89 (4.5)	68 (7.3)	21 (2.0)
Duration of parenteral nutrition, days, mean (SD; median)	14.8 (33.6; 7.0) (n=88)	17.0 (38.2; 7.0) (n=67)	7.5 (4.7; 6.0) (n=21)
Acute renal failure necessitating renal replacement therapy, n (%)	41 (2.1)	34 (3.7)	7 (0.7)
Duration of renal failure, days, mean (SD; median)	12.1 (32.5; 4.0) (n=37)	13.4 (34.8; 4.5) (n=32)	3.6 (3.7; 2.0) (n=5)
Complications during hospitalisation, n (%)			
Septic shock	55 (2.8)	51 (5.5)	4 (0.4)
Home-based care, n (%)	282 (14.1)	162 (17.4)	120 (11.3)

\*Patient numbers refer to patients for whom data on the variable(s) in question were available.

electronic Case Report Form. Patients were aged  $\geq 18$  years and hospitalised with a cSSTI requiring IV antibiotics.

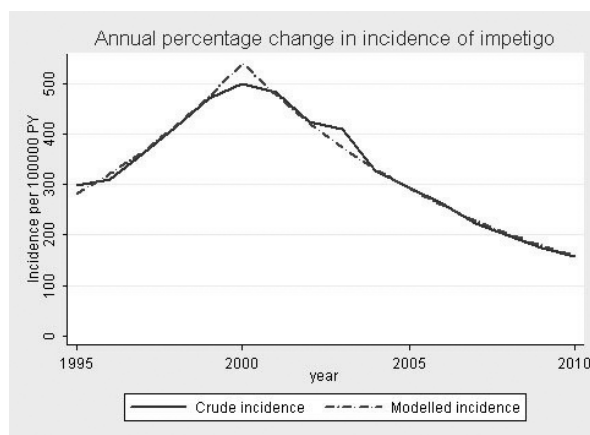
**Results:** The analysis population comprised 1996 patients (mean age: 60.6 years). Patients had a mean duration of initial hospital stay of 18.0 days. The most common types of lesion were abscess (23.1%) or fasciitis (21.6%), surgical intervention was required by 37% (n = 739) of patients and 6.5% (n = 130) were admitted to the intensive care unit (ICU), where the mean duration of stay was 9.7 days. Treatment failure, defined as a need for antibiotic change, occurred in 46.6% of patients (n = 930). An analysis of resource use in patients with vs without treatment failure is shown in Table 1. Notable differences between the two groups were: overall duration of hospitalisation (>9 days longer in patients with failure), admission to ICU (9.8% vs 3.7%), requirement for surgical intervention (41.6% vs 33.0%), parenteral nutrition (7.3% vs 2.0%) and home-based care after discharge (17.4% vs 11.3%). Patients hospitalised again after initial discharge (recurrence) (8.6%; n = 172) remained in hospital longer compared with patients with an initial infection (36.1 vs 17.3 days), and were more likely to be admitted to ICU (9.3% vs 5.1%), to require surgical intervention (52.9% vs 34.6%) and to require home-based care after discharge (24.4% vs 14.7%). Patients developing complications (septic shock) during treatment (2.8%; n = 55) had longer overall hospitalisation (37.9 vs 18.8 days), and were more likely to be admitted to ICU (63.6% vs 4.9%), require parenteral nutrition (36.4% vs 3.6%) and develop acute renal failure necessitating renal replacement therapy (23.6% vs 1.4%) than patients without complications. Overall mortality was 3.4% (n = 68).

**Conclusions:** The REACH study revealed that resource utilisation was increased in patients with cSSTIs who experienced treatment failure compared with those patients who did not.

#### P691 A hidden epidemic of impetigo – trends from the THIN database of primary care consultations

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**Objectives:** Outbreaks of impetigo have been described in Northern Europe but information on the incidence and natural history of disease in the general population is limited. Impetigo is primarily a disease of childhood caused by infection with either staphylococci or Group A streptococci. To investigate the burden of disease in primary care we examine time trends in the incidence of impetigo over the past two decades using data from the Health Improvement Network (THIN) primary care database.



**Methods:** We included patients registered with 419 General Practices between 1995 and 2010, equivalent to 30 400 person years of follow-up. Time trends in the incidence of impetigo were estimated adjusted for gender, age and deprivation score. We used segmented regression analysis (<http://surveillance.cancer.gov/joinpoint/>) to quantify change in the annual incidence of infection.

**Results:** Ninety five thousand six hundred and seventy-eight people had at least one recorded episode of impetigo. The annual incidence of infection increased from 299 (95% CI 284, 316) per 100 000 PY in 1995 to 498 (95% CI 488–509) per 100 000 PY in 2000 and then steadily decreased to 156 (95% CI 149, 163) per 100 000 PY in 2010. Two distinct time trends were identified with an annual percent change (APC) in incidence of +13.9% (95% CI: 9.9, 18.1) between 1995 and 2000, and –11.6% (95% CI: –10.8, –12.3) between 2000 and 2010.

**Conclusions:** We have identified a previously un-described epidemic of impetigo in the UK which peaked in 2000 and was followed by a rapid and unexplained decline over the following 10 years. Population based studies from Norway and Denmark report similar trends, suggesting an epidemic of impetigo may have spread across Europe between 2000 and 2004. Factors driving this surge and subsequent decline in impetigo are unclear and are likely to reflect changes in the pathogenicity of the causative organism, host factors or antibiotic prescribing practices.

Surveillance systems based on primary care patient records have potential to identify otherwise hidden epidemics. More routine use should be made of such data to facilitate timely investigation of epidemic trends and guide public health action.

#### P692 Multiplex PCR for the rapid diagnosis of skin/soft tissue and bloodstream infections

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**Objectives:** To assess the diagnostic performance of the SeptiFast multiplex PCR tool (Roche Diagnostics) for organism detection and identification in skin/soft tissue and bloodstream infections as compared to standard current diagnostic practice. In addition, qualitative factors such as the ease of use, throughput and hands-on time were assessed.

**Methods:** Patients with features of acute (<72 hours) skin/soft tissue infection (n = 8) or suspected sepsis (n = 9) were enrolled in this exploratory diagnostic study. Parallel samples (wound swabs or 1.5 mL whole blood) were taken for PCR and conventional culture. Samples for PCR were processed immediately after sampling while conventional cultures were run in parallel. The time from patient sampling to final result was recorded.

**Results:** There was good correlation of results between the SeptiFast kit and skin swab cultures for 7/9 patients but generally PCR detected more species compared to culture. In two other cases, the PCR and swab culture results disagreed. In one case, PCR results indicated

coagulase-negative *Staphylococcus* spp. while culture indicated *Staphylococcus aureus* and in the other case, PCR indicated *Enterococcus faecalis* while culture indicated mixed coliforms. The predominant bacterial species detected were coagulase-negative *Staphylococcus* spp. (4/9), *Proteus mirabilis* (3/9) and *Enterococcus faecalis* (3/9). In addition, the fungi *Candida albicans* (2/9), *C. glabrata* (1/9) and *Aspergillus fumigatus* (3/9) were detected. Anaerobes detected by culture in one sample were not detected by PCR due to kit limitations. Of the patients with suspected sepsis, all were found to be negative by both PCR and 48 hour blood culture. The time from the start of sample processing to final report was between 5–7 hour for PCR as compared to conventional blood/swab culture which was 24–48 hour.

**Conclusions:** The agreement between the PCR and the standard wound swab cultures was generally good. Despite the identification profile of the PCR technique being limited to 25 bacterial and fungal species, more organisms were identified in skin/soft tissue samples by PCR than by culture. The multiplex PCR and blood culture results agreed fully, with the added benefit that the PCR results were available within a few hours of sampling. This multiplex PCR technique is a more rapid approach to identification of sepsis than blood culture although some development is required to extend its use for the diagnosis of other infection

**P693** Assessment of two early-response outcome measures in a phase 2 clinical trial of the pleuromutilin BC-3781 in acute bacterial skin and skin structure infections

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**Background:** Clinical trials must use validated outcome measures that are relevant to patients and physicians but also to regulators worldwide. A Phase 2 trial of BC-3781, a novel IV and orally administered pleuromutilin, in acute bacterial skin and skin structure infections (ABSSSI) allowed an assessment of three different measures of clinical success: (i) FDA's proposed Early Response outcome measure (success: no increase in area, length or width of skin lesion erythema vs. baseline with a temperature  $\leq 37.6^{\circ}\text{C}$  at Study Day 3); (ii) the Foundation for the National Institutes of Health (FNIH) proposed Early Response outcome measure (success:  $\geq 20\%$  decrease in skin lesion erythema vs. baseline); and (iii) the traditional test-of-cure (TOC) clinical response (i.e., success being complete resolution of ABSSSI or improvement such that no additional therapy needed).

**Methods:** This randomized, double-blind trial compared 100 mg BC-3781 (BC-100) vs. 150 mg BC-3781 (BC-150) vs. 1 g vancomycin (VA), each given IV q12h for 5–14 days, for treatment of ABSSSI caused by a gram-positive pathogen. Eligibility required the presence of burn or surgical site/traumatic wound infection, cellulitis, or abscess with cellulitis. Also required were  $\geq 2$  signs of systemic inflammation (e.g., fever, elevated WBC count) or the presence of a significant

		Treatment Group		
		BC-100	BC-150	VA
Outcome Measure	Analysis Population			
Early Response - FDA (Day 3)	ITT	85.7% (60/70)	83.1% (59/71)	80.3% (53/66)
	CE	88.3% (53/60)	88.9% (48/54)	86.3% (44/51)
Early Response FNIH (Day 3)	ITT	74.3% (52/70)	70.4% (50/71)	71.2% (47/66)
	CE	76.7% (46/60)	75.9% (41/54)	76.5% (39/51)
Clinical Response (TOC)	ITT	85.7% (60/70)	83.1% (59/71)	81.8% (54/66)
	CE	90.0% (54/60)	88.9% (48/54)	92.2% (47/51)

systemic or local co-morbidity (e.g., diabetes mellitus). Early Response success or failure was determined programmatically using erythema length/width and temperature as recorded daily during the study. Clinical response was determined at TOC. Analyses were conducted in the ITT and Clinically Evaluable (CE) populations.

**Results:** The success rates for each outcome measure are shown in the Table. Early Response success rates were numerically lower than TOC success rates. Some patients who met Early Response criteria for "success" were a Clinical Response failure at TOC; notably, depending on the definition of Early Response, greater or lesser numbers of Early Response "failures" were a success at TOC (data not shown).

**Conclusions:** An Early Response analysis can be useful to clinicians and regulators in determining the rapidity of response of ABSSSI to antibacterial therapies; however, in this study a change in the definition of the Early Response measure had a substantial impact on the observed response rate with resultant implications for study sample size as well as for correlation with the outcome assessed at TOC.

**P694** Safety and efficacy of PTK 0796 (omadacycline) as treatment of complicated skin and soft tissue infection

G.J. Noel\*, M. Draper, H. Hait, S.K. Tanaka (Boston, US)

**Background:** Having completed phase 1 and 2 clinical programs, PTK 0796, an aminomethylcycline, broad-spectrum antibacterial agent active against all the leading causes of complicated skin and soft tissue infection (cSSTI) including methicillin-resistant *Staphylococcus aureus* (MRSA), has begun assessment in phase 3 trials.

**Methods:** A randomized (1:1), controlled, evaluator-blind, stratified by infection type, trial comparing PTK 0796 (100 mg iv; 300 mg po QD) to linezolid (LZD; 600 mg iv/po BID) was designed to establish non-inferiority between treatment arms regarding efficacy based on clinical assessment immediately after (EOT), and 10–17 days after (TOC) completing therapy in the intent-to-treat (ITT) and clinically evaluable (CE) populations. Subjects were given iv therapy initially with an option to transition to oral formulations of either PTK 0796 or LZD. Moxifloxacin (400 mg QD) was added to LZD treatment if infection due to Gram-negative bacteria was suspected.

**Results:** The trial was administratively stopped to address alignment with a new FDA guidance on ABSSSI, after 143 of the planned 790 subjects were enrolled. Subjects enrolled at six sites with 140 and 127 qualifying for the ITT and CE populations, respectively. A total of 44 (65%) PTK 0796 and 48 (67%) LZD subjects had cellulitis and 14 (21%) PTK 0796 subjects and 14 (19%) LZD subjects (19%) had wound infections. The mean and median duration of therapy were 10.1 and 10.0 days for PTK 0796 and 9.9 and 9.5 days for LZD subjects. Clinical success in each treatment arm is shown in the table.

MRSA was the most frequently isolated pathogen and success in the CE population occurred in 96.2% (25/26) with PTK 0796 and 93.5% (29/31) with LZD treatment. There were comparable numbers of treatment emergent adverse events (56;82.4% in PTK 0796 and 58;80.6% in LZD) and study-drug related adverse events (41;60.3% in PTK 0796 and 41;56.9% in LZD) across treatment arms. The most common adverse events reported involved the gastrointestinal system and the most common of these was nausea, reported by 18 PTK 0796 and 19 LZD treated subjects.

	PTK 0796	LZD	Difference (95% CI)
	success/total (%)	success/total (%)	
Intent-to-treat (ITT) population			
EOT	61/68 (89.7%)	66/72 (91.7%)	-2.0% (-12.4, 8.5)
TOC	58/68 (85.3%)	64/72 (88.9%)	-3.6% (-15.5, 8.3)
Clinically Evaluable (CE) population			
EOT	59/60 (98.3%)	64/67 (95.5%)	2.8% (-4.0, 9.6)
TOC	58/60 (96.7%)	64/67 (95.5%)	1.1% (-6.5, 8.8)

**Conclusions:** Results of this phase 3 trial experience are consistent with those of the phase 2 clinical program that also involved patients with cSSTI and showed comparable efficacy and overall safety/tolerability between PTK 0796 and LZD. Although stopped before meeting planned enrolment goals, results in the CE population met the

protocol-defined criteria of a 10% margin to conclude non-inferiority between treatments.

**P695** **The burden of community-acquired gastroenteritis in hospitalised adults at a London teaching hospital: a retrospective analysis**

M. Mirfenderesky\*, H. Wilkin-Crowe, T. Planche, A. Breathnach (London, UK)

**Objectives:** Acute gastroenteritis is a common condition affecting approximately one in five adults over a year period. A small minority subsequently require hospitalisation however these cases are not well defined. We wish to characterise the burden of community acquired gastroenteritis (CAG) requiring hospitalisation at our institution.

**Methods:** We performed a retrospective analysis of all adult (16≥) positive stool samples received between February 2008 and February 2009 at a 950 bed teaching hospital in South London. Results of positive stool samples, patient demographics and admission details were gathered from electronic patient record systems. Samples received within 3 days of admission were classified as community acquired. All stools samples were processed for the presence of Salmonella, Shigella, Campylobacter and *E. coli* 0157 according to standard laboratory procedures. Samples from patients aged >65 were processed for *C. difficile* using an ELIZA toxin test (Premier®) and confirmed with a latex agglutination (Microgen®) and a cytotoxicity assay. Subsequent tests for Vibrios, Aeromonas, Yersinia, *C. difficile*, ova & parasites were performed where appropriate. All samples excluding those from GPs were processed for norovirus using RT-PCR (Cepheid®). Investigations for other viruses were not routinely performed.

**Results:** During the 13 month period 816 enteric pathogens were isolated of which 77% (631) were community acquired. Thirty percent of these community cases (190) were admitted. In decreasing order of frequency these were norovirus (32%), *C. difficile* (29%), *Campylobacter* (24%), *Salmonella* (9%), *Giardia lamblia* (3%) and *Shigella* (2%). The average age of admitted cases was 56 compared with 43 in those that were not admitted. The average length of stay was 9 days with a loss of 1612 bed days over the 13 month period. Individuals with *C. difficile* and norovirus comprised 1169 bed days between them.

**Conclusion:** One hundred and ninety patients were admitted with pathogen confirmed CAG. Patients with norovirus and *C. difficile* comprised 61% of all admitted CAG. These patients were older with more prolonged admissions than other causes of CAG. Between them they comprised a 72% share of the total number of bed days occupied by cases of CAG. This has important infection control implications as without sufficient isolation facilities and stringent infection control practices these infections can result in nosocomial outbreaks with devastating consequences for hospitals.

**P696** **Comparison of risk factors for Campylobacter jejuni, Campylobacter coli and Campylobacter foetus isolated in France**

E. Bessede\*, L. Labadie, S. Bakiri, P. Lehours, F. Megraud (Bordeaux, FR)

**Objective:** *Campylobacter* spp. are recognized as the leading cause of bacterial enteric infections worldwide but little information is known on the risk factors of the main two species found: *Campylobacter jejuni* and *Campylobacter coli*.

**Material and Methods:** Our laboratory is the National Reference Center for Campylobacters in France and receives isolates from a network of laboratories from all over the country. The epidemiological data obtained from 2003 to 2010 were used to compare the characteristics of these infections. During this period all isolates were identified by using both phenotypic methods and molecular methods

except in 2010 when MALDI-TOF mass spectrometry was used. A univariate and a multivariate analysis were performed on the characteristics of the main species found. All statistical analyses were performed using SPSS V 11.5.

**Results:** In total, 22 245 isolates were identified; 7138 isolated from hospital laboratories and 16 842 from private laboratories. The repartition for the main species was *C. jejuni* (17 575, 79.3%), *C. coli* (3382, 15.2%) and *Campylobacter fetus* (873, 3.9%). Eight other species represented only 1.5% of the cases. Globally, Campylobacter infection had a sex ratio of 1.2, was observed at all ages but predominated in children 0–10 years, and was more common during summertime. Concerning the thermotolerant Campylobacters, the results of the multivariate analysis showed that *C. jejuni* was associated with summertime (May–September) while *C. coli* was associated with an occurrence at higher age (mean age 7 year older) and with a trip abroad ( $p < 0.01$ ). In comparison to thermotolerant Campylobacters, *C. fetus* was associated with an occurrence at a higher age, with specimens other than stools, partly blood culture and with a more frequent hospitalization.

**Conclusion:** Comparison of the characteristics of *C. jejuni* and *C. coli* infection on a large number of strains allowed us to show that the epidemiology of these two closely related enteropathogens are not strictly superposed, while the well known characteristics of *C. fetus* infection were observed.

**P697** **Travel-related outbreak of multi-resistant Shigella sonnei among Dutch water polo players**

J.E. Stalenhoeef\*, D.W.L. Scoop, E.C.J. Claas, I. van Disseldorp, F.P. Kroon, E.J. Kuijper (Leiden, NL)

**Objectives:** *Shigella sonnei* is an uncommon cause of foodborne outbreak of gastro-enteritis in The Netherlands. Empirical treatment, if necessary, consists of trimethoprim-sulfamethoxazole or ciprofloxacin. We report a travel related outbreak of multiresistant *S. sonnei* in water polo players who returned from Tblisi, Georgia.

**Methods:** In September 2011 a dutch male water polo team joined a European tournament in Tblisi, Georgia. During their 5 day stay, 14 out of 15 team members and support staff (mean age 30, range 18–57) had acute onset of fever and gastrointestinal symptoms with watery diarrhea and abdominal cramps (attack rate 93%). Three players were hospitalized in Georgia, where they were empirically treated with ciprofloxacin and IV fluids for 2 days. The other patients did not receive antibiotics. Faecal specimens were submitted for conventional cultures of enteropathogens and for molecular testing of 15 common gastrointestinal pathogens by multiplex PCR (xTag GPP, Luminex Molecular Diagnostics). *S. sonnei* strains were biochemically identified using API 20E and agglutination (Remel Europe Ltd., Dartford, UK). The Georgian National Center for Disease Control investigated the source of the outbreak. No cases were reported among hotel staff or other guests and no food contamination could be documented as source. Further spread of disease was prevented by advice on personal hygiene. No secondary cases were found among household contacts.

**Results:** Stool samples from 11 patients revealed the presence of *S. sonnei*, which was also found by multiplex PCR performed on seven available stool samples. PCR also identified the presence of heat-labile enterotoxin producing (LT) *Escherichia coli* in three of seven samples investigated and *G. lamblia* in one sample. Three cases had negative stool cultures, but the specimens were obtained 3 weeks later, after recovery of symptoms. Antibiotic sensitivity testing revealed that all isolates were resistant to trimethoprim-sulfamethoxazole, nalidixic acid, tetracycline, norfloxacin, ciprofloxacin and erythromycin and sensitive to amoxicillin and 3rd and 4th generation cephalosporins.

**Conclusion:** We report an outbreak of severe gastroenteritis caused by multidrug resistant *S. sonnei*, concomitant with LT-producing *E. coli*. Secondary cases were not found and a source could not be identified.

**P698 National enteric pathogens laboratory-based surveillance network (UEPLA) in Turkey, July 2007–June 2011**

B. Levent\*, R. Gulesen, H. Kalaycioglu, F. Sezen, A. Gozalan on behalf of the UEPLA Participants

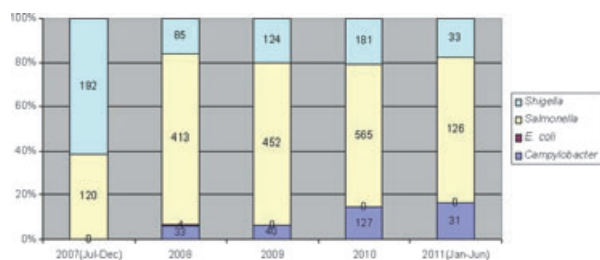
**Objectives:** The National Enteric Pathogens Laboratory-based Surveillance Network (UEPLA) was established in Turkey in 2007. The general aim of the network was to convey a laboratory based surveillance to promote sharing of information between participating laboratories and the reference laboratory, Refik Saydam National Public Health Agency, to facilitate typing of selected gastrointestinal pathogens and their antimicrobial resistance.

The aim of this study is to describe the isolates sent by the participating laboratories to national reference laboratory in the framework of UEPLA in Turkey between July 2007 and June 2011.

**Methods:** The UEPLA includes 34 laboratories nationwide. The selected microorganisms were *Salmonella*, *Shigella*, *Campylobacter* and verotoxigenic *Escherichia coli* (VTEC). Isolates were confirmed by using biochemical methods, serotyped, and screened for antimicrobial resistance using disc diffusion test. Data was collected by Open-Office 3.0 programme and analyzed by SPSS 15.0 software package, CHICAGO, IL, RSHMB 9887381.

**Results:** Totally 2526 isolates were analyzed between July 2007 and June 2011. The distribution of confirmed microorganisms by years are given in Figure 1.

Among the 1676 *Salmonella* isolates, 1101 (65.7%) were *Salmonella enteritidis*; 102 (6.1%) *S. typhimurium* and 96 (5.7%) *S. paratyphi* B. The most common *Shigella* serogroup was *Shigella sonnei* (n = 420, 68.3%). Of the 231 isolates of *Campylobacter* 160 (69.3%) were *C. jejuni* and 65 (28.1%) were *C. coli*. *Salmonella* isolates were resistant to nalidixic acid (n = 200, 11.9%) and ampicillin (n = 160, 9.6%). *Shigella* isolates were resistant to streptomycin (n = 436, 70.9%), tetracycline (n = 241, 39.2%), trimethoprim/sulfamethoxazole (n = 236, 38.4%), and ampicillin (n = 206, 33.5%). None of the *E. coli* strains were verotoxigenic.



**Conclusions:** UEPLA results demonstrate that the *S. enteritidis*, *S. sonnei* and *C. jejuni* are most prevalent gastrointestinal pathogens in Turkey. Highest resistance percentages were observed to nalidixic acid in *Salmonella*, and streptomycin in *Shigella*. The number of participating laboratories and the interested pathogens are expected to increase in years. The UEPLA will strengthen the collaboration between participating laboratories and the reference laboratory.

**P699 Detection of *Vibrio cholerae* and *Acanthamoeba* species from same natural water samples collected from different cholera endemic areas in Sudan**

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**Background:** *Vibrio cholerae* O1 and *V. cholerae* O139 infect humans, causing the diarrheal and waterborne disease cholera, which is a worldwide health problem. *V. cholerae* and the free-living amoebae *Acanthamoeba* species are present in aquatic environments, including drinking water and it has shown that *Acanthamoebae* support bacterial

growth and survival. Recently it has shown that *Acanthamoeba* species enhanced growth and survival of *V. cholerae* O1 and O139. Four hundred Water samples from different cholera endemic areas in Sudan were collected during cholera outbreak and 48 were collected during no cholera outbreak from the same areas with the aim to detect both *V. cholerae* and *Acanthamoeba* species from same natural water samples by polymerase chain reaction (PCR).

**Methods:** samples were examined by PCR to detect *Vibrio cholerae* toxin gene (toxA) and *Acanthamoeba* 18S RNA gene.

**Result:** For the first time both *V. cholerae* and *Acanthamoeba* species were detected in same natural water samples collected from different cholera endemic areas in Sudan. Eighty-nine percent of detected *V. cholerae* was found with *Acanthamoeba* in same water samples. Other samples during no cholera outbreak showed that 11 (13%) water samples contained *Acanthamoeba* only and no *V. cholerae* detected.

**Conclusions:** The current findings disclose *Acanthamoebae* as a biological factor enhancing survival of *V. cholerae* in nature.

**P700 Cross-sectional survey on seroprevalence of leptospirosis in Austria**

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**Study design:** Explorative nationwide cross-sectional seroprevalence survey.

**Objective:** To assess the prevalence of infections with *Leptospira* in Austria and the occupational exposure of military personnel. In addition, information on possible risk factors for exposure to *Leptospira* was obtained

**Methods: Setting:** Serum samples and demographical data were obtained from adults volunteering for international military employments between April and June 2009 at the Military Hospital Vienna.

**Participants:** Four hundred healthy adults from all federal states of Austria between 18 and 57 years were included.

**Variables:** Antibody titres against *Leptospira* were determined by microscopic agglutination test using a panel of cultures with 14 different serovars. Samples with titres  $\geq 1:100$  were considered positive. Variables surveyed included professional soldier or civilian, previous foreign military assignments, residential area, occupational animal contact and regular outdoor activities

**Statistical Methods:** Differences in proportions were tested using Fisher's exact test. A two-sided p value  $< 0.05$  was considered statistically significant.

**Results: Participants:** Eighteen participants were excluded from the statistical analysis because of impurities in the serum. The remaining 382 participants consisted of 166 professional soldiers and 216 civilians.

**Main results:** Overall, 88 (23%) individuals tested positive in serologic screening. The proportion of seropositive samples was

Serovars	Frequency	Percentage (%)
Canicola	63	71.6
Hardjo	45	51.1
Copenhageni	19	21.6
Bratislava	11	12.5
Tarassovi	10	11.4
Pyrogenes	4	4.5
Saxkoebing	2	2.3
Pomona	1	1.1
Grippotyphosa	1	1.1
Bataviae	1	1.1
Wolfii	0	0
Australis ballico	0	0
Hebdomadis	0	0
Autumnalis Aka	0	0

significantly lower among professional soldiers (15.7) than among civilians (28.7) ( $p = 0.003$ ). There were no significant differences between seroprevalence of *Leptospira* and any of the remaining variables surveyed. Subject sera were most commonly reacting with organisms of the serogroup Canicola (71%) and Hardjo (51%).

**Conclusions:** Our data demonstrate serologic evidence of *Leptospira* infection among the Austrian population. No increased risk of exposure to *Leptospira* could be detected for military personnel.

**P701 A mathematical model predicting the rate of early onset GBS disease**

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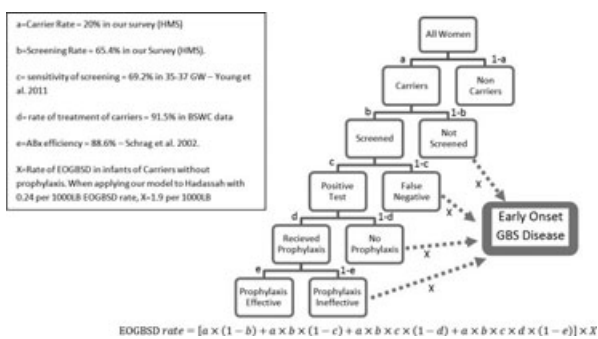
**Objectives:** Providing a tool for evaluating the effect of the pre-natal screening rate and method on the predicted morbidity in early onset group B streptococcal disease (EOGBSD), as universal GBS screening at 35–37 GW, formally adopted and applied in the USA, is still under dispute in other countries, including Israel, which has a low rate of EOGBSD (0.37 per 1000 live births [LB]).

**Methods:** A mathematical model was developed, describing the probability of EOGBSD as a product of the probabilities of predisposing events. Screening sensitivity and effectiveness of antibiotic prophylaxis were obtained from existing literature. Carriage during labor and pre-partum screening rates were surveyed in 80 women giving birth during January to April 2009 in Hadassah Hospital Mount Scopus (HMS) delivery unit. All women treated in Beit-Shemesh Women's Health Centre (BSWC) who gave birth at Hadassah hospitals (2006–2008) were reviewed for the rate of prophylaxis administration to known carriers of GBS.

**Results:** We found a 20% (95%CI: 11.9–30.4%) carriage rate and a 65.4% (95%CI: 53.8–75.8%) screening rate. 91.5% (43/47) of the BSWC GBS carriers who gave birth at Hadassah were treated during labour.

Given the rate of EOGBSD in Hadassah Hospitals (0.24 per 1000 LB), our model (Fig. 1) calculated the probability of neonatal disease from a GBS colonised mother without prophylaxis to be 1.9 per 1000 LB.

Using our model with the national EOGBSD rate and the screening rate as a variable, we conclude that there is a very low screening rate in Israel. Furthermore, 100% screening is expected to decrease morbidity to 0.17 per 1000 LB.



**Conclusion:** We developed a model which calculates the probability of GBS infection in a neonate considering screening, carriage and prophylaxis rates. Using our data we can calculate the effect of trends in prophylaxis on morbidity rate. Our model's main weakness is the lack of extensive national data regarding rates of screening, carriage and prophylaxis. Such data will enable our model to assess effects on EOGBSD rate, assisting policy makers to decide about the need for GBS prophylaxis.

**P702 Pneumococcal conjunctivitis in young children in Gipuzkoa, northern Spain, 2001–2010: non-encapsulated genotypes and influence of conjugate vaccines**

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**Background:** Acute conjunctivitis (AC) is the most frequent ophthalmic infection being *Streptococcus pneumoniae* a significant aetiological agent. Non-typeable pneumococci have been associated to AC but little is known about the serotype prevalence of other pneumococci causing AC. The 7-valent pneumococcal conjugate vaccine (PCV7) was introduced in Spain in June 2001.

**Aim:** Analyze the serotype distribution of pneumococci causing AC in children aged <5 years old after PCV7 (2001–2010) in the University Hospital Donostia, north Spain and characterise non-encapsulated isolates.

**Methods:** Serotypes were determined using the Pneumoarray or multiplex PCR and confirmed by the Quellung reaction. Capsule absence in non-typeable isolates was confirmed by a negative result in a specific PCR of the capsular *cpsA* gene. Molecular characterization was done by PFGE and MLST and antimicrobial susceptibility testing by the broth microdilution method. Statistical analysis was done by the Chi square test.

**Results:** During the study period 519 episodes of pneumococcal AC in 496 children were included (mean age 1.05 years). Non-encapsulated isolates were responsible for 169 (32.6%) episodes. Of encapsulated isolates, the most prevalent serotypes were 19A (10%), 15B/C (4.6%), 6A y 23F (3.9% each) and 19F (3.5%). A decreasing trend for PCV7 serotypes causing conjunctivitis was observed ( $p < 0.01$ ). The rate of PCV7 serotypes fall from 37.5% in 2001 to 2% in 2010 ( $p < 0.01$ ). Conjunctivitis caused by serotypes 19F and 23F showed a decreasing trend ( $p < 0.05$ ). Contrarily, rates of serotypes 15B/C increased ( $p < 0.05$ ).

Non-encapsulated isolates were more resistant than encapsulated to oral-penicillin and co-trimoxazole: 47.3% vs 18.6% ( $p < 0.01$ ) and 58.6% vs 28.6% ( $p < 0.05$ ), respectively.

95/169 non-encapsulated isolates characterized by PFGE gave 26 different patterns (similarity >80%). MLST of representative isolates of the most common PFGE patterns showed CC941 (32.6%) and ST448 (25.3%) to be the most prevalent among non-encapsulated isolates. This distribution was not homogeneous: between 2004 and 2006 more than 50% of the non-encapsulated conjunctivitis were caused by CC941 while in 2009–2010 more than 50% were due to ST448.

**Conclusions:** Non-encapsulated pneumococci are an important cause of conjunctivitis, but genotype distribution showed changes in the distribution of clones throughout time. The decrease in PCV7 serotypes causing conjunctivitis can be attributed to the herd effect of PCV7 on carriage.

**P703 Bartonella quintana in Ethiopian head lice**

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**Objectives:** To further characterise the head lice vectors of *Bartonella quintana* in Ethiopia.

We have recently reported the finding of *B. quintana* among both head lice and clothing lice collected from Ethiopia. We now report the characterisation of the lice vectors using amplification and sequencing of the mitochondrial gene cytochrome B (cytB).

**Methods:** Lice from Ethiopia were collected and pooled by patient (each pool <20 lice) from the inhabitants of Jimma, Ethiopia and its surrounds during summer 2010. A total of 98 pools were collected of which 65 were of head lice and 33 clothing lice. These were screened using a PCR specific for the intragenic transcribed spacer ITS. To further characterise the genetic background of the vector of those lice found to be positive for *Bartonella*, we sequenced the cytochrome b gene comparing this with available data from GenBank.

**Results:** Of the 98 lice pools screened, one of 33 of the clothing lice pools and 6 of 65 of head lice pools were infected with *B. quintana*. Sequence analysis of the louse vector was undertaken for both clothing and head lice, and compared with those found to be infected with *Bartonella*. Sequencing of *cytB* divided lice into two deeply rooted clades, both of which contained sequences of Ethiopian clothing lice. Interestingly, all head lice analysed fell into the second clade, whether infected with *B. quintana* or not.

**Conclusion:** The finding of *B. quintana* among head lice is important given the frequency of infestations seen among humans, especially children, around the globe. The perception among the general public is that head lice do not carry pathogens, a belief that should now be rectified. Whether all genetic lineages of head lice are equally competent for this pathogen under natural circumstances remains to be elucidated by comprehensive studies of head lice from diverse locations.

## Community-acquired respiratory tract infections

### P704 The viral aetiology of an influenza-like illness during the 2009 pandemic

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Many viruses can cause influenza-like illness; however, in nearly 50% of cases, the etiologic agent remains unknown. The distribution of viruses in patients with influenza-like illness was investigated during the 2009 A/H1N1 influenza pandemic (A/H1N1p). From June 2009 to January 2010, 660 patients with suspected influenza infection were questioned and examined, and nasal swabs were collected. All patient samples were tested for influenza virus, and 286 negative nasal swabs were further tested for 18 other respiratory viruses using real-time RT-PCR. Two waves of influenza-like illness were observed in the epidemic curve (weeks 35–42 and weeks 42–49). At least eight viruses co-circulated during this period: human rhinovirus (HRV) (58), parainfluenza 1–4 viruses (PIV) (9), human Coronavirus (hCoV) OC43 (9), enterovirus (EV) (5), adenovirus (AdV) (4) and human metapneumovirus (hMPV) (2); however, 204 samples remained negative for all tested viruses. Influenza-like illness symptoms, according to the CDC definition, were reported in 75% of the cases. These patients had positive swabs for A/H1N1p, HRV, hCoV-OC43, PIV, AdV and hMPV without significant difference with non-influenza-like illness patients. This study showed that many respiratory viruses circulated during this period and that the A/H1N1p did not impact on the kinetics of other respiratory viruses. The proportion of non-documented cases remains high. Influenza-like illness was not efficient to distinguish A/H1N1p infection from that due to other respiratory viruses. However, in multivariate analysis, cough, chills, conjunctive hyperemia and dyspnea were significantly associated with influenza virus vs. other respiratory viruses.

### P705 Epidemiology and clinical features of severe influenza in adults: 7 years of surveillance in Toronto, Canada

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**Background:** The epidemiology of influenza requiring ICU admission in adults has not been well studied.

**Methods:** Population-based surveillance for laboratory confirmed influenza in adults (>15 years) requiring ICU admission (LCI-ICU) in Toronto/Peel (pop 4M) was performed from 12/04 to 5/11; Consenting patients with a positive direct test (antigen or PCR) or culture for influenza were enrolled. From the 2006/7 to the 2010/11 season, active

surveillance for influenza was conducted in 6 of 19 ICUs in the surveillance area. Influenza A isolates were subtyped from 2007/8 season on.

**Results:** From 1/12/04 to 31/5/11, 576 adults with LCI-ICU were identified: 198 with 2009pH1N1 and 378 with seasonal influenza (158 AH3N2, 143 A-notsubtyped/able, 11 sH1N1, 77 B). Median annual incidence was 2.2/100000 (range 0.38–4.3). Patient median age was 68.5 years (range 17–101); 297 (52%) were male. Four hundred and seventy-two (92%) had at least one chronic condition qualifying them for seasonal influenza vaccine (but only 59% of those with seasonal influenza had been vaccinated); the median Charlson score was 4 (range 0–13); 57 (10%) were residents of long term care facilities (LTCF). The median admission APACHEII score was 15 (range 2–42). Forty-two (7%) patients had an admitting diagnosis of influenza; 189 (33%) had an admitting diagnosis of pneumonia, 134 (23%) another respiratory condition, 96 (17%) a cardiac condition; 115 (20%) another diagnosis. Thirty-four (5.9%) had a concomitant bacteremia (14 *S. aureus*, 14 *S. pneumoniae*, 3 *E. coli*, 1 each GAS, *H. influenzae*, *S. marcescens*). Four hundred and ninety-two (85%) patients received antibacterials at admission; 380 (66%) received antivirals (378 oseltamivir; 2 zanamivir). The median ICU LOS was 6 days (range 1–>100); 317 (55%) patients required mechanical ventilation for a median of 8 days (range 1–>100). One hundred and eleven patients (19.3%) died within 15 days of admission; these included 60/380 (16%) patients treated with antivirals and 50/1195 (26%) others ( $p = 0.005$ ). In multivariable analysis, APACHEII score (OR/10 points 3.5, 95%CI (2.5, 4.8) residence in long term care (OR 2.0, 95% CI 1.0, 4.0) and failure to treat with oseltamivir (OR 2.7 95%CI .1.6–14.9) were risk factors for mortality.

**Conclusions:** Influenza is an important cause of respiratory illness requiring ICU admission during the winter season, particularly in unvaccinated, at-risk adults. *S. aureus* is the most common complicating bacterial infection. Treatment with oseltamivir was associated with a significant reduction in mortality

### P706 Retrospective comparison of PCR confirmed influenza H1N1 infections and PCR-negative influenza-like illnesses in South East Austria: a case-control study

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**Objectives:** Pandemic 2009 influenza A (H1N1) virus has spread rapidly resulting in millions of laboratory confirmed cases and over 18 000 deaths worldwide. Compared to previous non-pandemic influenza seasons epidemiology of the H1N1 pandemic in 2009–2010 differed significantly. As sensitivity of currently available rapid antigen tests has shown to be low, PCR is the recommended test for diagnosis and confirmation of infection. PCR results may, however, are usually not rapidly available. Development of a clinical score for H1N1 influenza may facilitate clinical diagnosis of H1N1 infection and consecutively help to improve outcome and reduce health care related costs.

**Methods:** We retrospectively analyzed case files from patients in South East Austria with PCR confirmed H1N1 influenza in the 2009/2010 season regarding clinical, laboratory findings, and outcome. In a second step the same data collection was performed in a group of PCR-negative patients, which were tested during the influenza season 2009/2010 for influenza-like illness. The data from the PCR-positive group were compared to the data from the PCR-negative group.

PCR confirmed 624 cases of H1N1 influenza during the surveillance period from Oct 2009 until Jan 2010. Data were obtained from 222/624 (36%) of patients who presented to participating hospitals. No data was available of the other 402 patients as these patients presented to family doctors only. Results were compared to data obtained in 117 patients with negative PCR result.

**Results:** Clinical characteristics of patients collective with PCR confirmed Influenza H1N1 infection ( $n = 222$ ) and of patients

	PCR positive group (n=222)	PCR negative group (n=117)
Age (years)	33.1 ± 16.6*	34.7 ± 15.4*
Hospitalization (days)	4.58 ± 2.1*	6.34 ± 4.1*
Onset symptoms to presentation (days)	3.34 ± 1.1*	3.7 ± 2.1*
Prodrome before fever onset †	23/213 (10%)	32/109 (29%)
Fever/38°C at home †	19/198 (9%)	36/102 (35%)
Fever/38°C at presentation †	198/198 (100%)	28/97 (29%)
Temperature less than 38.2°C †	38.2 ± 0.3*	38.2 ± 0.3*
Cough †	155/216 (72%)	41/113 (36%)
Rhynorrhoea †	48/222 (22%)	28/113 (25%)
General weakness †	134/219 (61%)	39/113 (34%)
Headache †	47/222 (21%)	29/113 (26%)
Salivary count	186/186 (100%)	96/112 (86%)
Weight (kg)	68.28 ± 11.1*	68.45 ± 16.1*
Laboratory values †		
WBC count (10 <sup>9</sup> /day 1)	7.206.74	11.229.69
WBC count (10 <sup>9</sup> /day 2/3)	6.955.85	16.258.29
WBC count (10 <sup>9</sup> /day 4/5)	6.385.38	6.317.38
Thrombocyte count (10 <sup>9</sup> /day 1)	214.281	271.084
Thrombocyte count (10 <sup>9</sup> /day 2/3)	187.181	246.229
Neutrophil % day 1	68.73	72.73
Neutrophil % day 1	67.16	73.11
Neutrophil % day 2/3	67.66	73.11
Neutrophil % day 4/5	67.76.33	73.11
Eosinophil % day 1	0.86.1	7.267.8
Eosinophil % day 2/3	16.10	6.48.9
Lymphocyte % day 1	19.16	23.96
Creatinine protein (mg/dl) day 1	28.92	55.96
Creatinine (mg/dl) day 1	0.868.87	1.296.73
gST (10 <sup>9</sup> /day 1)	54.23	55.91
AST (10 <sup>9</sup> /day 1)	43.29	43.91
ALT (10 <sup>9</sup> /day 1)	27.19	38.22
Creatinikinas (10 <sup>9</sup> /day 1)	274.115	133.92
Lactate dehydrogenase (10 <sup>9</sup> /day 1)	246.223	246.213

† Mean ± median displayed.  
\* statistically significant difference, p<0.05  
Normal ranges, where applicable: Creatinine protein, 0-0.8 mg/dl; Creatinine, 0.7-1.3 mg/dl; gST 0-35 U/L; AST 0-35 U/L; ALT 0-40 U/L; Creatinikinas, 0-170 U/L; Lactate dehydrogenase 120-240 U/L

collective with clinical suspicion of H1N1 infection in which PCR turned out negative (n = 117) are depicted in table 1.

**Conclusion:** Occurrence of a prodrome before fever onset and body temperature below 38°C, measured at home and at presentation were significantly associated with negative H1N1 PCR result. Cough and general weakness, on the other hand, were predictors of positive H1N1 PCR result. Total white blood cell count, relative eosinophil count, and thrombocyte count seemed to be lower in patients with confirmed H1N1 infection, while creatinine kinase values seemed to be higher. For the first time we found an correlation between eosinopenia, elevated creatinine kinase values and Influenza H1N1 infection. Presented data may help to implement a clinical score for H1N1 infection.

### P707 A 2-year retrospective study of H1N1 influenza pandemic: experience from a teaching hospital in northwestern England

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**Background:** H1N1 virus, a subtype of influenza A was responsible for the global pandemic outbreak as termed by the World Health Organization (WHO) in 2009. This strain also called as swine flu affected more than 70% of young and middle aged with pre existing immunity. As per health protection agency (HPA) report more than 1000 patients succumbed to this illness in 2 years. Also the seasonal flu activity in 2010/11 was higher than last winter and that H1N1 "swine" flu was the dominant strain.

**Aim:** To compare the pandemic over 2 years, study the demographics, clinical characteristics, complications and mortality rate in patients who were screened for H1N1 influenza.

**Methods:** The medical records of all patients (n-91) who were prescribed with Oseltamivir between January 2009–December 2010 were retrospectively reviewed. Patient demographics, symptoms, mode of screening, clinical outcome, complications and mortality rate were analysed.

**Results:** Ninety-one patients (males-30, females-61) were included in the study. During 2009/2010 and 2010/11, 24 and 67 patients respectively were screened; male to female ratio was 1:2 and 1:3; The median age at presentation in males was 43 (range 0–91) and in

Total number of H1N1 Influenza cases	n-37	
Clinical Characteristics	Number	Percent
Fever >38°C	25/37	67.5%
Cough	30/37	81%
Headache	28/37	75.6%
Rhynorrhoea	24/37	64.8%
Myalgia	18/37	48.6%
Vomiting	5/37	13.5%
Diarrhea	4/37	10.8%
Contact with Confirmed Cases	4/37	10.8%
Chest x-ray abnormalities	15/37	40.5%
Hospital Admission	34/37	91.8%
ITU Admissions	2/37 (2009) 2/37 (2010)	5.4% 5.4%
Prior Vaccination	2/37	5.4%
Outcome (Mortality)	2/37 (2009-1, 2010-1)	5.4%
<b>Complications</b>	<b>n-12</b>	<b>32%</b>
Type-1 Respiratory Failure	5	
ARDS	1	
Pleural Effusion	1	
Pneumonia	2	
Multiorgan Failure	1	
Death	2	5.4%

females 38 (range 0–94). The predominant symptoms were fever, cough, headache, rhynorrhoea, myalgia, vomiting and diarrhoea (Table 1a). There was an increase in the number of patients who were screened during December 2010 (n-57). Eighty-nine patients were screened with a nasopharyngeal swab and 2 had a bronchoalveolar lavage. The reasons for screening included clinical suspicion of H1N1 influenza, clinical symptoms disproportionate to their diagnosis, severe disease process or recent exposure to H1N1 contacts.

37/91 patients (41%) were found positive for H1N1 with PCR technique and had the full course of Oseltamivir for 5 days. Six patients were positive for H1N1 in 2009 and 31 patients in 2010. Twenty-five patients had an uneventful recovery and 12 (32%) patients had complications (Table 1b). The mortality rate from our hospital was 5.4% (n-2).

**Conclusions:** Over a period of 2 years, we encountered 37 cases of H1N1 Influenza. Majority of the patients were females with no previous cardio respiratory illness and with no significant co-morbidities as reflected by the HPA data. Influenza is a preventable illness and vaccination is the best way to protect against any infection. A concerted effort must be made by the healthcare professionals to encourage vaccination.

### P708 Pandemic 2009 (H1N1) influenza A infection in the elderly requiring ICU admission: clinical characteristics and risk factors for mortality

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**Objective:** Little information is available regarding the clinical characteristics, presentation and prognosis of elderly patients infected with influenza virus A (H1N1) 2009 and admitted to the intensive care units (ICUs). Our aims are to examine epidemiological and clinical data of patients over 64 years old and to identify independent predictors of mortality in this cohort.

**Methods:** This was a prospective, observational, multicenter study conducted in 148 Spanish ICUs, with patients infected with influenza

virus A (H1N1) 2009 confirmed by RT-PCR (Real Time Polymerase Chain Reaction) from April 2009 to July 2011. Patients over 64 years were compared with the rest with a bivariate analysis. Logistic regression analysis was carried out to determine independent predictors of mortality in patients over 64 years.

**Results:** A total of 1485 patients were included, of whom 188 (12.7%) were over 64 years, with a higher proportion of men (68.6% vs. 31.4%,  $p < 0.005$ ). Severity of illness assessed by Acute Physiology and Chronic Health Evaluation (APACHE) II and Sequential Organ Failure Assessment (SOFA) were significantly higher in elderly patients. Prevalence of chronic diseases (diabetes, chronic renal disease, hematologic disease, chronic pulmonary obstructive disease and cardiac insufficiency) was more common in patients over 64 than in the rest. Viral pneumonitis was more frequent in patients younger than 65 years (72.4% vs. 55.9%,  $p < 0.001$ ) although incidence of co-infection was similar in both groups. Presence of leukocytosis and renal dysfunction at admission was higher in the elderly group. Non-invasive mechanical ventilation was more often used in patients over 64 years (37.5% vs. 28.6,  $p < 0.05$ ). ICU mortality was greater in patients over 64 years (29.1% vs. 19.3%;  $p < 0.005$ ). In these patients, APACHE II score (OR = 1.14; 95% CI, 1.13–1.24;  $p = 0.001$ ), oseltamivir therapy initiated after 48 hours (OR = 3.3; 95% CI, 1.02–10.5;  $p < 0.05$ ), and use of corticosteroids (OR = 1.60; 95% CI, 1.12–6.7;  $p < 0.05$ ) were identified as independent variables associated with mortality.

**Conclusions:** Patients over 64 years infected with influenza A (H1N1) 2009, admitted to the ICU have a higher incidence of underlying diseases and differences in clinical presentation compared to younger patients. Elderly patients should receive prompt treatment with oseltamivir and the use of corticosteroids is discouraged.

#### **P709** A retrospective cohort study: can we predict a diagnosis of H1N1 pneumonia?

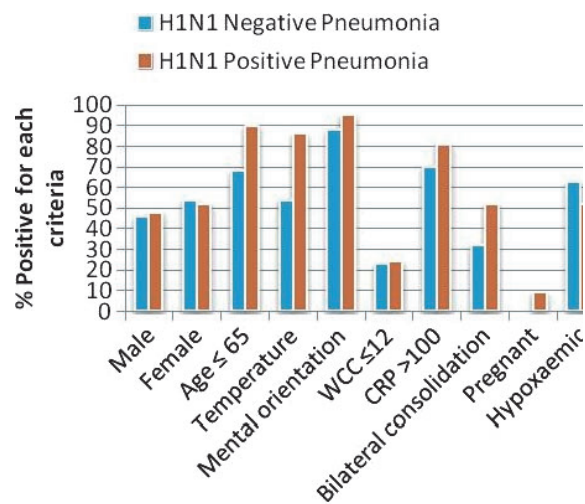
*J.E.K. Sherman\*, R. Thompson, C.M.E. Edwards (London, UK)*

**Objectives:** Prior to the h1n1 epidemic in the winter of 2010 a communication from the Department of Health suggested that five indicators could determine whether or not someone with pneumonia was likely to have h1n1. These were age  $\leq 65$ , temperature  $\geq 38^\circ$ , white cell count  $\leq 12$ , preserved mental orientation and chest x-ray showing bilateral consolidation. The aim of this study was to retrospectively evaluate the effectiveness of this clinical scoring system in diagnosis of H1N1 pneumonia.

**Methods:** The clinical details of all patients tested for H1N1 between November 2010 and February 2011 were obtained from the microbiology department. Age, sex, temperature on admission, WCC, CRP, CXR findings and hypoxaemia were for each patient. Each patient was assigned a score out of five based on the five clinical variables; age  $\leq 65$ , temperature  $\geq 38^\circ$ , WCC  $\leq 12$ , preserved mental orientation and CXR showing bilateral consolidation. The distribution of scores and of the five clinical variables within H1N1 pneumonia and community acquired pneumonia cohorts were compared. Sensitivity, specificity, negative predictive value (NPV) and positive predictive values (PPV) of the clinical scoring system were calculated.

**Results:** One hundred and seventy-nine patients were analysed. Sixty-two patients tested positive for h1n1, 21 were also treated for pneumonia. One hundred and seventeen patients tested negative for h1n1, 57 were also treated CAP. As illustrated in the figure there was little difference in any criterion between the two groups. The greatest difference between H1N1 positive and negative was with a score of 4/5 (38% of the H1N1 positive group, 16% H1N1 negative). For a score of 4/5 the PPV was 47%, NPV 78%, sensitivity 38% and specificity 34%, P was not significant.

Age  $\leq 65$ , temperature  $\geq 38^\circ\text{C}$  and bilateral consolidation on CXR showed the biggest difference in distribution between the H1N1 pneumonia and CAP cohorts. PPV and NPV for a score of  $\geq 3/5$  was calculated, where the three variables had to include age, temperature and bilateral consolidation. There was no significant difference between



the groups, PPV 45%, NPV 75%, sensitivity 24% and specificity 89%,  $p$  was not significant.

**Conclusion:** The use of the five clinical criteria identified as being predictive of h1n1 pneumonia is unhelpful as an aid in the diagnosis of h1n1 pneumonia. We would contest that a thorough clinical history and examination and appropriate investigations are paramount when determining a diagnosis of h1n1 pneumonia.

#### **P710** Did the 2009 influenza pandemic change influenza prevention, diagnosis and management? Evidence from surveillance for influenza-associated hospitalisation in Canada

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**Background:** The 2009 influenza (flu) pandemic changed attitudes and practice. In Ontario, recommendations were written for testing and treatment, but seasonal flu vaccination rates decreased post-pandemic.

**Objectives:** To compare epidemiology, clinical features and management of adults with laboratory-confirmed seasonal flu requiring hospitalization (LCSI-H) pre- and post-pandemic in Toronto.

**Methods:** Population-based surveillance for LCSI-H has been performed in Toronto (popn~4M) since 2004. LCSI-H is defined as illness requiring admission with positive direct test (antigen or PCR) or culture for flu. From 2006/7 season onward, ICUS in 6/19 hosps conducted active surveillance for LCSI-H. FluA subtyping started in 2006. This analysis compares LCSI-H in the 2004/5–2007/8 seasons (pre-pandemic) and the 2010/11 season (post). Subgroup analysis was performed for flu sub/types.

**Results:** The incidence of LCSI-H was 7.7/100 000 pop pre- and 26.4/100 000 post-pandemic. Flu B comprised 336 (20%) infections pre v 96 (8.7%) post; among subtyped flu A, (H3N2) comprised 78% (222/284) pre v 89% (832/934) post. Comparing pre and post cases, there was no difference in median age (78 year pre vs 76.5 year post), percent male (566.49% vs 531.48% post), proportion with chronic conditions creating a risk of complicated flu (906.79% vs 881.80%), Charlson score  $> 2$  (895.77% vs 860.78%) median admission APACHEII score (12 vs. 12), proportion with admitting diagnosis of pneumonia/flu (453.39% vs. 406.37%), or proportion receiving antibiotics (82% vs. 84%) or admitted to ICU (213.19% vs. 209.19%). Post-pandemic cases were less likely to be nursing home residents (137.12% vs. 185.16%,  $p = 0.02$ ), to be nosocomial (97.9% vs. 161, 14%,  $p = 0.001$ ), and to be vaccinated against flu (633.64% vs. 495.56%,  $p < 0.001$ ), but more likely to be treated with antivirals (468.41% vs. 792.73%,  $p < 0.001$ ). Pre-pandemic



cases were more likely to be diagnosed in hospitals with active ICU surveillance (577.50% vs. 420.41%,  $p < 0.001$ ), and somewhat more likely to die (7.8% at 15 days vs. 6.4%,  $p = 0.22$ ). Analysis by subtype did not change results. In multivariable analysis, infection pre vs post pandemic was not associated with mortality, but antiviral use was associated with decreased mortality (OR 0.51, 95% CI 0.35, 0.71).

**Conclusion:** The post-pandemic increase in LCSI-H post-pandemic appears associated with increased testing and reduced pop'n vaccination rates, but clinical and epi features are unchanged. Increasing use of antivirals may have improved outcomes.

#### **P711** Influenza outbreak 2010/2011 – significant burden on hospital resources

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**Objectives:** To describe the clinical and epidemiological features of influenza in patients hospitalized at Galway University Hospital (GUH) during the 2010/2011 influenza season, 1 year after the H1N1 pandemic.

**Methods:** All adult patients (>15 years) admitted to GUH with a positive Influenza RNA PCR between 01/09/2010 and 01/05/2011 were included. Medical records were reviewed.

**Results:** Sixty patients were admitted to GUH with laboratory confirmed Influenza from 21/12/2010 to 04/03/2011. The mean age of patients was  $49.6 \pm 18.8$  (range 16–88). Twelve (20%) were <25 years old, 49 (82%) <65 years. Seventy-seven percent were confirmed Influenza A (H1N1); 23% Influenza B.

The average duration of symptoms on admission was 4.3 days. Forty-two (70%) had at least one co-morbidity; chronic lung disease in 43%. Sixty-eight percent had a normal chest X-ray.

Ten patients (17%) required ICU admission. Compared to non ICU admissions, those admitted to ICU had a longer mean duration of symptoms at presentation (+3.1 days; CI 6.2, 0.1;  $p = 0.0467$ ) and a longer mean number of days from onset of symptoms to commencement of Oseltamivir (+3.8 days; CI 7.0, 0.5;  $p = 0.0236$ ). Seven patients (12%) required mechanical ventilation. Total ICU bed days was 130 with mean length of stay of 13 days (range 1–40).

The mean length of stay for community acquired Influenza was 8.6 days. Mean length of stay was longer in those with co-morbid lung disease, by 9.8 days ( $p = 0.0487$ ), but not with any other co-morbidity. Three patients (5%) died from Influenza, all of whom had significant co-morbidities. The date of onset of symptoms peaked on 02/01/2011. Nine influenza admissions (15% of total) occurred on 1 day (07/01/2011). Between 4th and 14th January, 52% of all admissions and 70% of ICU admissions occurred. For 7 days in a row in January, 7 ICU beds were occupied by patients with influenza, which equates to 78% of ICU capacity.

**Discussion:** A high number of Influenza H1N1 cases required admission to hospital over a short period of time, with those affected being relatively young. There was a high ICU utilisation rate and this accounted for 78% of ICU capacity for a 7 days period. The EpiCurve is consistent with an epidemic, with the peak of onset of symptoms and peak of admissions illustrating a very short warning period for the hospital to prepare. It is likely that this pattern will be repeated in the coming influenza season.

#### **P712** Clinical features of adult community-acquired pneumonia caused by Gram-negative bacilli: comparison with pneumococcal pneumonia

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**Objectives:** Despite *Streptococcus pneumoniae* is the most common pathogen causing community-acquired pneumonia (CAP), and gram-

negative bacilli (GNB) have also been reported as an important pathogen in Korea. The aim of this study is to evaluate clinical features and risk factors of adult community-acquired pneumonia by GNB requiring admission by comparison with those of pneumococcal pneumonia (PP).

**Methods:** We have conducted nationwide multicenter prospective observational study of adult CAP in 14 teaching hospitals in Korea since October 2009. During the study period from October 2009 to September 2011, eighty nine cases were enrolled in the group of CAP by GNB, which were diagnosed by sputum and blood culture and receiving inpatient treatment, and clinical features of them were compared with those of eighty cases of PP, which were diagnosed by serologic test and culture methods, and also receiving inpatient treatment.

**Results:** In the group of CAP by GNB, *K. pneumoniae* was the most common etiologic microorganism (37 cases, 41.6%), followed by *P. aeruginosa* (17, 19.1%), *E. coli* (7, 7.9%), *Enterobacter cloacae* (6, 6.7%), *H. influenzae* (5, 5.6%), etc. No difference was detected among the baseline characteristics such as age, gender distribution, status of residence, site of admission, functional status of patients between the groups of CAP by GNB and PP. Diabetes mellitus (31.5% vs. 10.0%,  $p = 0.002$ ) and a recent exposure to immunosuppressants (3.4% vs. 0%,  $p = 0.002$ ) were observed as the significant risk factor of the CAP by GNB. Among the clinical manifestations of CAP, arthralgia (3.4% vs. 0%,  $p = 0.002$ ), skin rash (2.2% vs. 1.3%,  $p = 0.019$ ), crackle (76.4% vs. 52.5%,  $p = 0.002$ ) and purulent sputum (68.5% vs. 56.3%,  $p = 0.038$ ) were more frequently observed in the group of CAP by GNB. But the pneumonia severity scores such as PSI and CURB-65 and other laboratory findings were not different between the two groups. There was no difference observed in the incidence of acute respiratory distress syndrome, the need and duration of mechanical ventilation, the duration of antimicrobial treatment, and clinical outcomes between the two groups.

**Conclusion:** Data from this study showed that diabetes mellitus and the recent exposure to immunosuppressant treatment were the significant risk factors of the CAP by GNB, and some clinical manifestations were more frequent in the CAP by GNB, comparing with PP. But the analysis of clinical outcomes didn't show any significant difference.

#### **P713** Burden of community-acquired pneumonia in older adults in the new EU countries of the Central Europe

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**Objectives:** Older adults are in an increased risk of respiratory infections including community acquired pneumonia (CAP). The former socialistic countries of the Central Europe form a unique region with specific health care and epidemiology characteristics, yet with rather scarce local underlying epidemiology figures. The objective was to estimate incidence and case fatality rate (CFR) of CAP in adults 50+ years of age in the Czech Republic (CR), Hungary (HU), Poland (PL) and Slovakia (SK).

**Methods:** The incidence and the CFR for hospitalised CAP were estimated using the national surveillance systems (PL, CR, SK) and national insurance records (HU). National retrospective patient chart reviews (CZ, SK) were used to estimate the non-hospitalised CAP incidence as a portion of the hospitalised CAP. In PL we used national surveillance data and in HU the national insurance fund records to estimate outpatient CAP incidence.

**Results:** Hospitalised CAP incidence per 100 000 person years was 457 in CR, 879 in HU, 879, 364 in PL, and 524 in SK. The CFR per 100 cases of hospitalised CAP was estimated as 21 in CR; 20 in SK; and 18 both in PL and HU. Non-hospitalised CAP incidence per 100 000 person years was 710 in CR, 3551 in HU, 316 in PL, and 598 in SK. Compared with adults 50–64 years of age, the incidence of hospitalised CAP were 2.3-fold higher in those 65–74, 5.2-fold higher in 75–84 and 10.8-fold higher in those 85+, manifesting an exponential trend seen

also in mortality. By contrast, the incidence of non-hospitalised CAP was generally flat or declining with age, reflecting a higher likelihood of hospitalisation with increasing age. The total number of hospitalisations in the 65+ group and deaths in CR, HU, PL, and SK were 17 473 and 3686; 23 652 and 4796; 35 895 and 7325; 6321 and 1497, respectively) In PL, for instance, adults over 65 represent 14% of the study population, while accounting for 80% of deaths from CAP.

**Conclusion:** The morbidity and mortality of hospitalised CAP increases sharply with advancing age, signalling a likely increasing public health problem as the population ages over time. In HU, where the primary incidence data were provided by the national insurance fund, substantially larger incidence was calculated in both types of CAP with strikingly high number of outpatient CAP cases. This may be mainly due to different coding practice. Nevertheless, CAP poses a significant burden in all four countries among adults 50+ years of age.

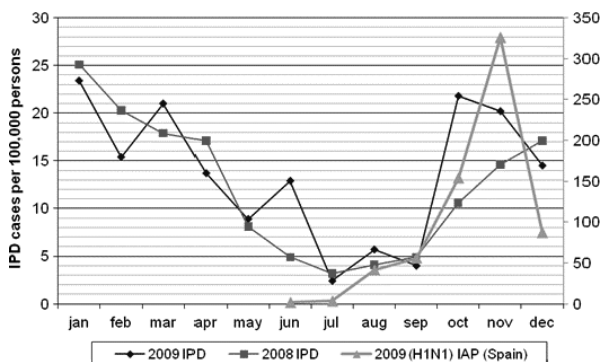
**P714** **Has the incidence of invasive pneumococcal disease been influenced by the 2009 influenza A (H1N1) pandemic? Prospective multicentre study in Barcelona, Spain**

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**Background:** The incidence of Invasive Pneumococcal Disease (IPD) seems to be associated with viral respiratory illnesses, such as influenza. We therefore analyzed: (i) changes in the incidence of IPD in adults during the Influenza A (H1N1) Pandemic (IAP), (ii) changes in clinical characteristics of IPD episodes and, (iii) trends in serotype (ST) distribution.

**Methods:** A prospective multicenter study on IPD from January to December 2009 in an area of Barcelona of 1 483 781 adult inhabitants was performed. Serotyping was made by Quellung. The incidence of IPD and ST distribution in 2009 were compared with 2008 data.

**Results:** Two hundred and three cases of IPD were detected, 62% being males with a mean age of 61.2 year (18–98). 15.8% were healthcare or hospital-related, 69.9% had comorbidity. 76.3% had pneumonia. Mortality was 16.2%. IAP was confirmed in 4/53 (7.5%) cases. The most frequent serotypes in 2008 were 1 (20.4%), 7F (10.2%), 3 (9.6%), 19A (9%) and 14 (7.2%) whereas the most frequent in 2009 were 3 (9.1%), 7F (7.4%), 16F (7.4%), 9V (6.8%), and 13 (5.7%), 19A (5.7%). An increase (85.5% vs. 89.7%) in non-7pneumococcal conjugate vaccine ST (PCV7ST) was noted in 2009 compared to 2008. The annual incidence of IPD in 2008 and 2009 was 147.2 and 164.2, respectively per 100 000 inhabitants and year. Monthly incidence of IPD in adults of this series according to 2008 vs. 2009 and monthly incidence of IAP in Spain is shown in the graph.



**Conclusions:** Compared to 2008, the annual rate of IPD increased by 10% in 2009. IAP was associated with peaks in the incidence of IPD in adults in June, October and November 2009. Non-PCV7ST tended to increase in 2009 and some changes in demographic and clinical data were noted in patients with IPD during the 2009 IAP.

**P715** **Incidence and distribution of *Streptococcus pneumoniae* serotypes in adults in Catalonia, Spain**

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**Objectives:** *Streptococcus pneumoniae* is an important public health problem because of its high morbidity and mortality in children and adults. In Spain, the 23 valent pneumococcal polysaccharide vaccine (PCV23) was introduced in 1999 in adults  $\geq 65$  years. The effectiveness of PCV23 is controversial while conjugate vaccines have been proven to be safe, immunogenic and effective in preventing pneumococcal disease caused by vaccine included serotypes in children. In Catalonia, 7-valent conjugate vaccine (PCV7) has not been introduced into the routine vaccination schedule. The main goal of this study is to know the incidence of invasive pneumococcal disease (IPD), clinical syndrome and serotype distribution in adults  $\geq 50$  years after the licensing of PCV7 in Catalonia.

**Methods:** We conducted a prospective study based on notifications to the Microbiological Reporting System of Catalonia with an active surveillance. Patients studied were adults  $\geq 50$  years old with confirmed IPD by culture during 2005 and 2009. Age groups analyzed were: 50–54, 55–59, 60–64, 65–69, 70–74, 75–79 and  $\geq 80$  years. The incidence rate was calculated from data obtained of Statistical Institute of Catalonia. To assess association of variables, Chi-square test was used. Significance level was set at 0.05.

**Results:** During the study period, 3085 IPD cases were identified: 3078 (99.8%) were confirmed by culture. Annual incidence was 25.6/100 000 persons-year. Incidence rate in 2009 (28.7/100 000 hour) was higher than 2005 (17.2/100 000 hour) (OR = 44.8;  $p < 0.01$ ). Annual incidence (100 000 hour persons-year) per age groups was: 11.5, 15.7, 21.2, 26.7, 26.9, 36.3 and 51.1 for 50–54, 55–59, 60–64, 65–69, 70–74, 75–79 and  $\geq 80$  years, respectively. Clinical presentation was: 72% bacteremic pneumonia (5.6% empyema), 15.2% bacteremia without focus, 6% meningitis, 1.9% peritonitis, 0.6% arthritis and 0.03% cellulitis. A total of 2125 (68.9%) cases were serotyped. There were identified 64 different serotypes. The most prevalent serotypes were: serotype 3 (7.4%), serotype 1 (6.8%), serotype 19A (6.3%), serotype 14 (5.4%) and serotype 7F (5.2%). Percentage cases due to PCV7, PCV10, PCV13 and PCV23 serotypes were: 19.4%, 39.5%, 63.3% and 73.7%, respectively.

**Conclusion:** The incidence of IPD in adults is high mainly in  $\geq 80$  years old. Great variability of serotypes was observed in adults. Serotype 3 and serotype 1 were the most prevalent serotypes. PCV13 and PCV23 offers a good coverage against IPD in adults.

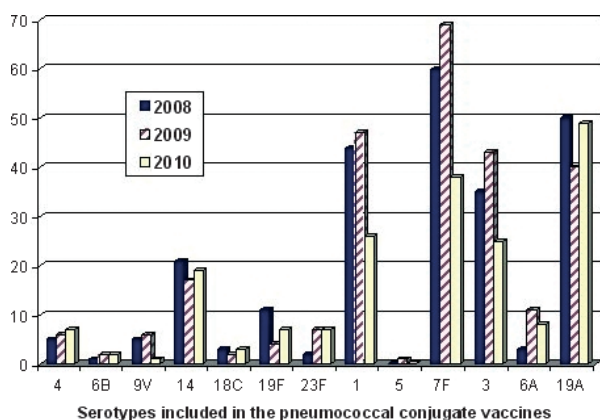
**P716** **Invasive pneumococcal disease in the Comunidad Valenciana, Spain, 2008–2010**

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The emergence of complicated pneumococcal disease is a fact in many geographical areas. In the Comunidad Valenciana (CV), Spain, invasive pneumococcal disease (IPD) is a mandatory reportable disease since 2007. In this community, vaccination with the pneumococcal polysaccharide vaccine (PPV-23) is recommended in adults more than 65 years old. In children, the pneumococcal conjugate vaccine (PCV) is also recommended, but not included in the childhood immunization schedule (CIS), and subsidized by the Health Service only in case of high risk of IPD.

**Objectives:** To describe the epidemiology and serotype distribution of the *Streptococcus pneumoniae* isolates causing IPD in children and adults in the CV, between the years 2008 and 2010.

**Methods:** Retrospective study including all reported cases of patients with culture-proved IPD admitted to 24 medical centers in the CV, between 2008 and 2010. Epidemiological data were retrieved from the microbiology surveillance network of the CV (RedMIVA). Serotyping



of the strains of *S. pneumoniae* received at the Microbiology Department of the University Hospital La Fe was done through slide agglutination test (Denka Seiken, Tokyo, Japan). Some pneumococci were also sent to the National Reference Laboratory for serotyping and confirmation.

**Results:** In the period of study, 1577 cases of IPD were reported in the CV – 591 in 2008, 566 in 2009 and 420 in 2010. Regarding sex and age of the patients, 58.1% were men, 12.4% were children <5 years old and 40.9% were adults 65 years and older. Pneumonia was the clinical presentation in 59.4% of the patients, followed in frequency by sepsis (16.2%), meningitis (9.6%) and empyema (7%). A total of 967 (61.3%) strains of *S. pneumoniae* were serotyped – 339 (57.3%) in 2008, 354 (62.5%) in 2009 and 274 (65.2%) in 2010- and 47 different serotypes were found. The distribution of the serotypes included in the PCVs is shown in Chart 1. These accounted to more than 70% of the serotyped strains. Frequent serotypes not included in this chart were 22, 23F, 11A and 8, all in the PPV-23.

**Conclusions:** The incidence of IPD has diminished in the CV. Young children and elderly were the most affected groups of population. Pneumonia was the most frequent clinical presentation. Serotypes 7F, 19A, 1, 3 and 14 were predominant, with data similar to that observed in the rest of Spain. The introduction of the PCV in the CIS could reduce the incidence of IPD in our community.

#### P717 Serotypes causing invasive pneumococcal disease in adults (≥18 years) in Spain, 2010–2011: a multicentre hospital-based study

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**Objectives:** To explore serotypes (Sts) causing invasive pneumococcal disease (IPD) in adults in order to determine coverage by PCV13.

**Methods:** A prospective, active, hospital-based surveillance of all culture-confirmed IPDs in adults (≥18 years) was performed in seven Spanish hospitals (August 2010-June 2011). IPD was considered isolation of *S. pneumoniae* in normally sterile fluids (blood, cerebrospinal fluid, pleural fluid...). Clinical presentations were classified as complicated pneumonia -CP- (pneumonia with pleural effusion, and/or empyema and/or multilobar presentation), non-complicated pneumonia (N-CP), meningitis (M), primary bacteremia (PB), sepsis (S), peritonitis (P) and others (O). Serotyping was performed by the Quellung reaction or dot blot assay. Sts 6A, 6B, 19A and 19F were identified by real-time PCR.

**Results:** One hundred and ninety-one cases (mean age 62.2 ± 17.8 years) were identified. The table shows by clinical presentation the number of cases and percentages of Sts accounting for >5% in total population.

	CP	N-CP	M	PB	S	P	O	Total
n	70	66	19	14	9	8	5	191
<b>PCV13 Sts (%):</b>								
3	15.7	13.6	5.3	0.0	0.0	12.5	0.0	11.5
19A	8.6	10.6	10.5	0.0	22.2	12.5	0.0	9.4
7F	8.6	13.6	0.0	0.0	0.0	12.5	0.0	8.4
1	10.0	6.1	5.3	0.0	0.0	0.0	0.0	6.3
14	5.7	7.6	0.0	0.0	11.1	12.5	0.0	5.8
Others*	17.1	6.1	5.3	7.1	0.0	25.0	40.0	11.5
<b>Non-PCV13 Sts (%):</b>								
PPV23 non PCV13	18.6	16.7	5.3	7.1	33.3	25.0	60.0	18.3
6C	2.9	7.6	10.5	14.3	0.0	0.0	0.0	5.8
Others	12.9	18.2	57.9	71.4	33.3	0.0	0.0	23.0
<b>PCV13 coverage</b>	<b>65.7</b>	<b>57.6</b>	<b>26.4</b>	<b>7.1</b>	<b>33.3</b>	<b>75.0</b>	<b>40.0</b>	<b>52.9</b>

\*including Sts 4, 19F, 6A, 6B, 9V, 23F and 18C (no cases by St 5)

By age group, PCV13 serotypes accounted for 57.4% of cases in ≥18 – ≤49 year; 58% in ≥50–≤64 year; 57.7% in ≥65–≤74 year and 42.6% in ≥75 year; PPV23 non PCV13: 20.4%, 20%, 11.5% and 18%, respectively; and serotype 6C: 7.4%, 6.0%, 3.8%, 4.9%, respectively.

**Conclusion:** More than 20% of cases were due to 19A and 3 with a PCV13 coverage depending on the age group and clinical presentation. The highest coverage was for adults younger than 75 years of age. For pneumonia, that represented the most frequent presentation accounting for 71.2% IPDs, the PCV13 coverage was 61.7%.

#### P718 Early detection of community outbreaks of respiratory tract infections from house-call visits in the metropolitan area of Athens, Greece

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**Objectives:** The traditional Serfling-type approach for influenza-like illness surveillance requires long historical time-series. We retrospectively evaluated the use of recent, short, historical time-series for recognizing the onset of community outbreaks of respiratory tract infections (RTIs).

**Methods:** The data used referred to the ratio of diagnoses for upper or lower RTIs to total diagnoses for house-call visits, performed by a private network of medical specialists (SOS Doctors) in the metropolitan area of Athens, Greece, between 01/01/2000 and 10/12/2008. The reference standard classification of the observations was obtained by generating epidemic thresholds after analyzing the full 9-year period. We evaluated two different alert generating methods [simple regression and cumulative sum (CUSUM), respectively], under a range of input parameters, using data for the previous running 4–6 week period. These methods were applied if the previous weeks contained non-aberrant observations.

**Results:** We found that the CUSUM model with a specific set of parameters performed marginally better than simple regression for both groups. The best results (sensitivity, specificity) for simple regression and CUSUM models for upper RTIs were (1.00, 0.82) and (1.00, 0.90) respectively. Corresponding results for lower RTIs were (1.00, 0.80) and (1.00, 0.86) respectively.

**Conclusions:** Short-term data for house-call visits can be used rather reliably to identify respiratory tract outbreaks in the community using simple regression and CUSUM methods. Such surveillance models could be particularly useful when a large historical database is either unavailable or inaccurate and, thus, traditional methods are not optimal.

#### P719 A randomised, double-blind, multicentre study to evaluate the efficacy and safety of oral solithromycin (CEM-101) compared to oral levofloxacin in the treatment of patients with community-acquired bacterial pneumonia

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**Trial Design:** This was a multi-center, Phase 2, double-blind, randomized, efficacy and safety study to evaluate oral solithromycin

vs. oral levofloxacin in the treatment of adults with moderate to moderately severe community-acquired bacterial pneumonia (CABP). [Clinical Trials registration NCT01168713]

**Methods:** Patients with CABP were enrolled and randomized (1:1) to either: solithromycin 800 mg orally (PO) on Day 1 followed by 400 mg PO daily on Days 2–5 or levofloxacin 750 mg PO daily on Days 1–5. Randomization was stratified by age (<50 or ≥50 years) and pneumonia severity index (PORT) score. Co-primary efficacy outcome measures were Investigator's assessment of clinical success at test-of-cure (TOC; 4–11 days after the last dose of study drug) in the Intent-to-Treat (ITT) and Clinically Evaluable (CE) populations. Early response success (at Day 3) was defined as improvement in at least two cardinal symptoms (cough, sputum production, chest pain, or dyspnea) without worsening in any.

**Results:** The 132 patients randomized were primarily (95%) from US sites, 50.7% male, and 82.6% Caucasian, with a mean age of 55. Randomized patients had PORT scores of II (73%), III (20%), or IV (5%). Clinical success rates, presented in the table below, were comparable across the analysis populations, at both early response (Day 3) and TOC visits, as well as among subgroups with a baseline elevated procalcitonin (PCT) and with an identified pneumococcal infection. More levofloxacin recipients experienced one or more treatment-emergent adverse events (TEAEs) during the study (45.6%) than did solithromycin recipients (29.7%). The majority of TEAEs were gastrointestinal, were of mild or moderate intensity, and included nausea (1.6% solithromycin; 10.3% levofloxacin), diarrhea (7.8% solithromycin; 5.9% levofloxacin), and vomiting (0% solithromycin; 4.4% levofloxacin). Six patients, all receiving levofloxacin, discontinued study drug due to an AE. There was one death in the trial, attributed to a pulmonary embolism, in a levofloxacin recipient.

Clinical Success Rates Visit: Population	Solithromycin	Levofloxacin
	n/N (%)	n/N (%)
TOC: ITT (co-primary efficacy outcome)	55/65 (84.6)	58/67 (86.6)
CE (co-primary efficacy outcome)	46/55 (83.6)	54/58 (93.1)
microITT	14/18 (77.8)	10/14 (71.4)
ME	12/15 (80.0)	10/13 (76.9)
TOC: ITT, Baseline Plasma PCT ≥ 0.2 ng/mL	22/25 (88.0)	24/27 (88.9)
TOC: ITT, Baseline Plasma PCT < 0.5 ng/mL	14/15 (93.3)	12/13 (92.3)
TOC: <i>Streptococcus pneumoniae</i> diagnosis	5/7 (71.4)*	2/3 (66.7)
Day 3: ITT	47/65 (72.3)	48/67 (71.6)

\* includes one patient who missed EOT visit, but was considered a success when evaluated at TOC.

Notes: n/N (%): n=number of patients in the specific category; N=number of patients with an assessment of the specified parameter; percentages=100 x (n/N). PCT=procalcitonin. *S. pneumoniae* diagnoses were established by blood or sputum culture, or by detection of streptococcal capsular antigen in urine (BINAX assay). Success at Day 3 was defined as improvement in at least two cardinal symptoms (cough, sputum production, chest pain, or dyspnea) without worsening in any.

**Conclusions:** Solithromycin demonstrated efficacy comparable to levofloxacin and a favorable safety and tolerability profile, with a lower incidence of treatment-emergent adverse events than levofloxacin. These findings strongly support the further study of solithromycin in Phase 3 clinical trials for the treatment of CABP.

**Funding:** Cemptra Pharmaceuticals.

## P720 Macrolides quinolones for community-acquired pneumonia: systematic review and meta-analysis of randomised controlled trials

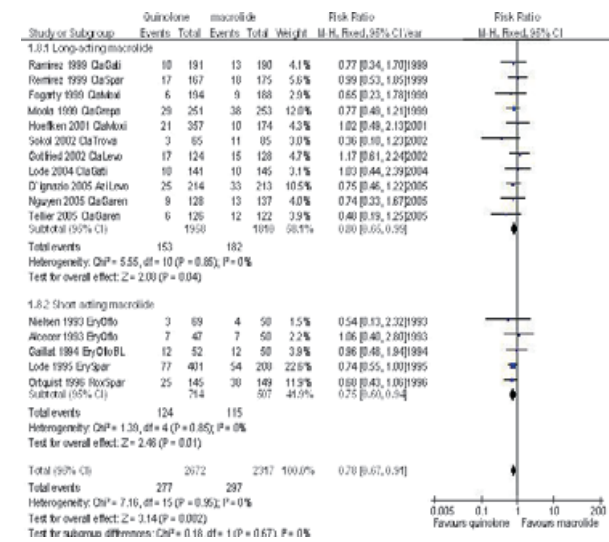
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**Objectives:** We sought to compare the efficacy and safety of macrolides vs. quinolones in the treatment of community-acquired pneumonia (CAP).

**Methods: Design:** Systematic review and meta-analysis. Data sources: PubMed, Central, LILACS databases, references of included studies, relevant conference proceedings, trial registries and new drug application documents. Eligibility criteria: We included randomized controlled trials comparing any macrolide vs. any quinolone, as monotherapy or both in combination with a beta-lactam, for the treatment of CAP among inpatients or outpatients. We did not limit inclusion by age, pneumonia severity, publication status, language or

date of publication. The primary outcomes assessed were 30-day all-cause mortality and a treatment failure. Two authors independently extracted the data. Fixed effect meta-analysis of risk ratios (RR) with 95% confidence intervals was performed.

**Results:** Sixteen trials (4989 patients) were included, mostly assessing outpatients with mild to moderate CAP. All-cause mortality was not significantly different for macrolides vs. quinolones, RR 1.03 (0.63–1.68, seven trials), with a low event rate (2%). Treatment failure was significantly lower with quinolones, RR 0.78 (0.67–0.91, 16 trials) (figure). The definition of success (and failure) used in the primary studies was not clearly representative of patients' benefit. Microbiological failure was lower with quinolones, RR 0.63, (0.49–0.81, 13 trials). All adverse events, adverse events requiring discontinuation and any premature antibiotic discontinuation were significantly more frequent with macrolides, mainly on account of gastrointestinal adverse events. Resistance development was not assessed in the trials.



**Conclusions:** Randomized controlled trials show an advantage to quinolones in the treatment of CAP with regard to clinical cure without need for antibiotic modification and adverse events. The clinical significance of this advantage is unclear.

## P721 Assessment of outcomes at an early time point may identify a differential effect of macrolide therapy on community-acquired pneumonia due to atypical pathogens

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**Objectives:** Controversy exists regarding the need to include empiric therapy for atypical pathogens for optimal treatment of patients with CAP. Several CAP studies failed to show a clinical benefit of adding coverage for atypicals. Two recent global, randomised, double-blind, multicentre trials (FOCUS 1 and FOCUS 2) assessed the efficacy and safety of ceftriaxone (CPT) fosamil vs ceftriaxone (CRO) in CAP. The trials were identical except for a 24-hour course of clarithromycin (CLR) on Day 1 in FOCUS 1. This difference in design offers a unique opportunity to assess the effect of a macrolide on the outcome of CAP caused by ≥1 atypical pathogens.

**Methods:** FOCUS 1 and FOCUS 2 trials included hospitalised (non-ICU) moderate to severe CAP patients (PORT risk class III or IV) requiring intravenous therapy. Patients were randomised to CPT fosamil 600 mg q12h or CRO 1 g q24h for 5–7 day. FOCUS one patients also received oral CLR 500 mg q12h for 1 day. Primary

Subjects with:	FOCUS Day 4 S&S Response and Investigator Determined Clinical Response at Specified Timepoint n/N (%)			
	FOCUS 1 (macrolide)		FOCUS 2 (No macrolide)	
	S&S Day 4	TOC (Investigator Assessment)	S&S Day 4	TOC (Investigator Assessment)
Typical pathogens (mMITTE)	105/155 (67.7)	126/155 (81.3)	114/178 (64.0)	138/178 (77.5)
Atypical pathogen only (includes <i>M. pneumoniae</i> , <i>C. pneumoniae</i> , <i>L. pneumophila</i> ) (MITTE)	49/64 (76.6)	57/64 (89.1)	38/66 (57.6)	58/66 (87.9)
<i>L. pneumophila</i> only (MITTE)	8/12 (66.7)	12/12 (100)	12/19 (63.2)	14/19 (73.7)
<i>M. pneumoniae</i> and/or <i>C. pneumoniae</i> only (MITTE)	38/49 (77.6)	42/49 (85.7)	24/43 (55.8)	41/43 (95.3)

CAP = community-acquired pneumonia; FOCUS = cePtarOline Community acquired pneumonia trial versus ceftriaxone in hospitalized patients; MITTE = subjects with PORT score III/IV; mMITTE = subset of MITTE who had  $\geq 1$  typical pathogen with or without an atypical pathogen; PORT = Pneumonia Patient Outcomes Research Team; S&S=clinical stability and symptom improvement; TOC = test of cure; US FDA = United States Food and Drug Administration.

endpoint was clinical response at TOC visit (8–15 day after end of therapy). Also, a US FDA-specified endpoint of clinical stability and symptom improvement on Day 4 was assessed post hoc. Patients with an atypical pathogen only (65 *Mycoplasma pneumoniae*, 21 *Chlamydia pneumoniae*, 31 *Legionella pneumophila*, 13 mixed atypicals) were summarized separately.

**Results:** Compared with the overall study population, patients with only an atypical pathogen were younger (mean age 50.3 years in CPT fosamil group and 55.9 years in CRO group vs 60.8 years and 61.6 years, respectively) and had less severe infections (proportion with PORT IV CAP: 28.6% in CPT fosamil group; 23.2% in CRO group vs 37.9% and 38.4%, respectively).

**Conclusions:** A differential clinical benefit was observed at Day 4 in patients with CAP due to an atypical pathogen only, specifically *M. pneumoniae* and/or *C. pneumoniae*, and who received 1 day of CLR (76.6% vs 57.6%). No difference was seen at the TOC visit, perhaps due to the self-limited course of many atypical pneumonias. The addition of macrolide made no difference for typical pathogens, either at day 4 or TOC. In patients with *L. pneumophila* only, a difference was not seen early, but a numerical difference favouring the CLR-group occurred at TOC. Although not a randomised comparison, our observations suggest that outcome assessment at an early time point may better identify differential effects of two treatments than a later evaluation and that empiric atypical coverage may impact early clinical response. Additional study is warranted.

#### **P722** Increased reports of *Mycoplasma pneumoniae* from laboratories in Scotland in 2010 and 2011

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**Objectives:** An increased incidence of *Mycoplasma pneumoniae* was noted in several countries in Europe in 2010. In Edinburgh during the winter of 2010/11, higher than expected *M. pneumoniae* report frequency was found from patients at the Royal Hospital for Sick Children, and this continued through 2011. This local experience prompted a national review of *M. pneumoniae* reporting data for Scotland.

**Methods:** Health Protection Scotland captures nationwide laboratory reports of *M. pneumoniae* through the Electronic Communication of Surveillance in Scotland (ECOSS) reporting system. Reports from 01/01/2008 until 20/09/2011 were analysed in this study. Multiple samples from the same patient and infection episode (defined as 56 days) were excluded, and data were anonymised.

**Results:** There were a total of 716 *M. pneumoniae* reports in Scotland during the period studied. There were 76 reports in 2008, 124 in 2009, 254 in 2010 and 262 to week 37 in 2011. Reports of *M. pneumoniae* were highest in the autumn/winter period 2010/11, peaking in week 50 of 2010 and week 4 of 2011. Reporting frequency declined through the spring and early summer of 2011, but rose to high levels again after week 27. In 2010 and 2011, the mean age of patients was 21.6 years (SD  $\pm$  19.5 years; range <1 month to 89 years), however, the mode age was <1 year. The majority (78.9%) of reports were generated by PCR testing of respiratory specimens (of these, 94.3% were from the upper respiratory tract), 20.3% by serology and 0.8% were not stated. However, 48.1% of reports from respiratory specimens were in children <10 years old, compared to 24.5% of serology specimens.

**Conclusion:** *M. pneumoniae* reports have increased in frequency in Scotland since autumn/winter 2010, compared to previous years, and this has been sustained in 2011. Most reports were from children, particularly those <2 years of age, and were mainly diagnosed through PCR testing of upper respiratory tract specimens. Testing methods differed between laboratories, however those using PCR reported more positives from younger patients than those using serology, reflecting the greater acceptability of PCR for the diagnosis of *M. pneumoniae* in young children. Denominator testing data is not currently available, thus a prospective surveillance programme for *M. pneumoniae* incidence would be of benefit in optimising future antibiotic policy for pneumonia. Use of molecular testing is resulting in a change in the epidemiology of this infection.

#### **P723** Was winter 2010/11 a *Mycoplasma pneumoniae* epidemic season in Germany?

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**Objectives:** *Mycoplasma pneumoniae* (Mp) epidemics occur every 4–6 years. In late summer 2010 reports of an incipient epidemic arrived from Denmark. The epidemic situation in north and south Germany was then analysed in a retrospective survey.

**Methods:** Frequency of Mp infections was analysed from laboratory data in four successive autumn-winter periods (AWPs) from 2007 to 2011 (Sept. to Feb.). Sera from 14 279 patients (2760–4234 patients per season, age 1–95 years), of whom 61% were children and adolescents (1–20 years) were tested for IgG, IgA and IgM antibodies (Ab) using Mp-ELISA medac. Acute infection (AI) was defined as IgM- and IgA- or IgG-positive, and current/persistent or past infection (CPI) as IgG- and IgA- or single IgG-positive. In 818 patients from south Germany respiratory specimens (throat swabs, secretions) were also tested for Mp-DNA.

**Results:** Over the four AWPs Ab prevalences for IgG, IgA and IgM in the entire study population were: 28% to 31%, 8% to 9% and 6% to 15% respectively. The 15% positive IgM cases were recorded in winter 2010/11. In AWP 2010/11 AI reached their highest level with a mean of 11%. In parallel, the percentage of CPI at 17% was comparable to that in previous years. The percentage of Mp-DNA-positive samples in winter 2010/11 at 10.0% was also markedly higher than in the two previous observation periods (0.6% and 2.1%). In terms of seasonal variations, most AI were noted between November and January (14%), with a minimum (7%) in September. In children and adolescents the mean percentage of AI was found to be 15% in all years. The 6–10-year age group was prominent with 22%. In December 2010 and January 2011 values of 29% and 27% respectively were even recorded in this age group. In north Germany AI were recorded earlier and in marginally greater numbers than in south Germany.

**Conclusions:** AWP 2010/11 was characterised by a high percentage of acute Mp infections. Children between 6 and 10 years old were

predominantly affected. The peak time for infection was from December to January. Serological and PCR data point to an Mp epidemic in winter 2010/11 in Germany.

#### **P724** Macrolide resistant *Mycoplasma pneumoniae* in England and Wales

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**Objectives:** *Mycoplasma pneumoniae* is a common cause of pneumonia in humans. Reports of macrolide resistant *M. pneumoniae* have been increasingly reported globally. Other than a limited study in winter 2010 no studies have been performed in the UK to assess the level of macrolide resistance. We sought to determine if known genetic markers of macrolide resistance were present in clinical strains isolated in England and Wales from 1965 to 2005 and in DNA extracted from clinical samples known to be PCR positive from 1991 to October 2011.

**Methods:** Erythromycin sensitive phenotype was confirmed by microbroth dilution on the first 37 freeze dried strains, the remaining 33 isolates and 117 clinical samples were tested by molecular methods only. DNA was extracted from 70 stored strains (46 freeze dried, 24 frozen) and 117 clinical isolates using the Qiasymphony and Magnapure compact respectively. Samples were analysed by PCR for *M. pneumoniae* (Pitcher et al., 2006). A 600 bp fragment of the 23srRNA sequence was obtained from positive samples by PCR and sequencing. For 17 stored DNA extracts amplification did not occur and subsequent qPCR indicated a reduction in MPN DNA indicative of sample degradation. The remaining 100 samples gave adequate sequence data that were analysed for known mutations conferring macrolide resistance (2063 A-G/C, 2064 A-G/C, 2067 A-G, 2167 C-A/G). The % resistant detected by sequencing was calculated in all samples tested from 2008 to October 2011.

**Results:** Macrolide resistance markers were not found in clinical strains from England and Wales isolated from 1960 to 2005. In clinical samples screened from 1991 to October 2011 a single mutation was detected in one sample only from 2008 (2063 A-G). The % resistant detected by sequencing was calculated from 2008 to October 2011 2.2% (95%CI 0.01–12.6) 1/45.

**Conclusion:** Macrolide resistance markers were not found in 70 clinical isolates in England and Wales prior to 2005, and erythromycin resistant phenotype was not detected prior to 1996. In 100 clinical samples from 1991 to 2011 a single case of macrolide resistance was detected in 2008. Although limited by the number of positive cases available for testing, macrolide resistance was not detected in England and Wales prior to 2008 and was detected in *M. pneumoniae* in England and Wales at a low level (2.2% (95%CI 0.01–12.6) from October 2008–2011).

#### **P725** Macrolide resistance in *Mycoplasma pneumoniae* during two successive years with epidemic in Denmark

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**Objectives:** Cases of *Mycoplasma pneumoniae* (M. pn.) infections occur throughout the year, with the highest incidence during autumn and winter. The highest prevalence is seen in children and younger adults. At Statens Serum Institut (SSI) the rate of PCR positive samples is calculated each week, and a rise from approximately 5% to at least 15% over a few weeks is considered indicative of an M. pn. epidemic. During the autumn/early winter of 2010, a relatively small epidemic was seen in Denmark (DK). This autumn (2011) it appears that we are heading for another epidemic. During an M. pn. epidemic the consumption of macrolides increases considerably. In theory this could lead to an increase in macrolide resistance in M. pn., especially in a situation where the epidemic seems to cover two seasons.

**Methods:** PCR is used as a routine diagnostic test for M.pn. infections at SSI. Several local clinical microbiology laboratories in DK also use this method as a routine test for M. pn. In this study, only cases

diagnosed by PCR at SSI were included. A selection of PCR positive samples from the two epidemics (242 in 2010 and >250 for 2011) were examined by a pyrosequencing method which detects mutations associated with macrolide resistance (domain V of the 23S rRNA gene).

**Results:** The duration of the 2010 epidemic was from August to January 2011. The second epidemic (2011) also had its onset in August and is still ongoing. At the beginning of the 2010 epidemic, we found 1.4% (2 of 140) macrolide resistant samples, and at the end of the epidemic 2.8% (3 of 108) had mutations detected (not significant). Macrolide resistance data for the 2011 epidemic is still pending.

**Discussion:** M. pn. epidemics have a high impact on the community, and a laboratory-based system for the surveillance of this disease is recommended. According to our knowledge, Denmark is the only country with a continuous PCR-based surveillance system for M. pn. based on routine samples. Macrolide resistance in M. pn. is an increasing problem worldwide. In Denmark, low frequencies of resistant samples have been seen, but continuous surveillance is important.

#### **P726** Community-acquired *Legionella pneumophila* pneumonia: single-centre experience with 214 hospitalised cases over 15 years

D. Viasus, C. Garcia-Vidal\*, S. Di Yacovo, J. Adamuz, R. Verdaguer, J. Dorca, F. Gudiol, J. Carratalà (Vandellòs i l'Hospitalet, ES)

**Objective:** *Legionella pneumophila* has been increasingly recognized as a cause of community-acquired pneumonia (CAP). We aimed to determine the epidemiology, diagnosis, clinical features, treatment and outcomes of sporadic cases of community-acquired *L. pneumophila* pneumonia (LP) requiring hospitalization over a 15-year period.

**Methods:** Observational analysis of prospective cohort of hospitalized adults with CAP at a tertiary teaching hospital in Spain (1995–2010).

**Results:** Of the 3933 hospitalized patients with CAP during the study period, 214 (5.4%) patients had LP. While the diagnosis of LP by urinary antigen test remained stable over the years ( $p = 0.42$ ), the use of seroconversion and culture decreased ( $p < 0.001$  and  $p = 0.001$  respectively). The median age of patients with LP was 58.2 years (SD 13.8) and 76.4% were males. Comorbid conditions were present in 119 (55.6%) patients, mainly chronic heart disease, diabetes mellitus and chronic pulmonary disease. The frequency of older patients and comorbidities among patients with LP increased over years ( $p = 0.06$  and  $p = 0.02$  respectively). In addition, 100 (46.9%) patients were classified into groups IV-V according to the PSI. Twenty-four (11.2%) patients received inappropriate empirical antibiotic therapy at hospital admission. The number of patients who received macrolides decreased over years, whereas the number of patients who were given levofloxacin increased significantly. Compared with macrolide use, levofloxacin therapy was associated with a trend to shorter time to reach clinical stability (median 3 vs 5 days;  $p = 0.09$ ) and a shorter length of stay (median 7 vs 10 days;  $p < 0.001$ ). Regarding outcomes, 38 (17.8%) patients required ICU admission and 8 (3.7%) patients died. There has been a trend to lower frequency of mechanical ventilation ( $p = 0.10$ ) and overall mortality ( $p = 0.04$ ) over the study years.

**Conclusion:** *L. pneumophila* is a relatively frequent causative pathogen among hospitalized patients with CAP. During the 15-year period study period, the annually number of LP cases has remained stable over years and they are associated with high morbidity. Similarly, significant changes have occurred in diagnosis, treatment and prognosis of LP.

#### **P727** Detection of atypical pneumoniae with the BD MAXTM-instrument compared with BioRad CFX96

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**Introduction and Objectives:** *Mycoplasma pneumoniae* (Mpn), *Chlamydia pneumoniae* (Cpn) and *Legionella pneumophila* (Lpn)

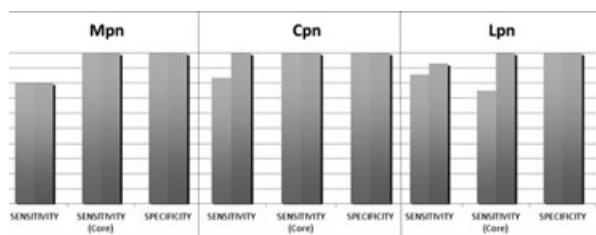
are the organisms responsible for most atypical pneumonia. Atypical pneumonia due to these bacteria causes mild forms of pneumonia and is characterized by a more protracted course of symptoms unlike other forms of pneumonia which can progress more quickly with more severe early symptoms. The BD MAXTM is a fully automated instrument for molecular diagnostics. The nucleic acid extraction and subsequent PCR is done by adding the appropriate reagent cartridges and the test tubes. Further intervention is not necessary (walk-away system). Performance comparison between classical extraction method with subsequent PCR and walk-away BD MAX allowed us to prove possible the transfer of classical assays to the BD MAXTM-instrument.

**Material and methods:** QCMD 2010 MCpn EQA Panel and QCMD 2010 Lpn EQA Panel were used on both systems to compare performance. This includes 12 MCpn samples (three Mpn cores, three Cpn cores, two Mpn positives, three Cpn positives and one negative) and 10 Lpn samples (four cores, three positives, and three negatives).

**Classical assay:** Four hundred microlitre of QCMD sample and 10 mL of Internal Control (IC) were extracted with QIAamp DNA Mini Handbook and eluted in 60 mL. Subsequent PCR was performed in 25 mL on BioRad CFX96.

**BD MAX assay:** 500 mL of QCMD sample and 10 mL of IC were extracted with DNA extraction kit DNA-3. PCR was performed automatically after sample preparation by BD MAXTM. Primers and probes were by Diagenode (Dia-MCpn-050 and Dia-Lpn-050). Master Mix was by BD.

**Results:** All positive Mpn samples but one (MCpn 08 – low positive) were correctly detected as such by BD Max (sensitivity 80%). All positive Cpn samples but one (MCpn 03 – low positive) were correctly detected as such by BD Max (sensitivity 83%). All positive Lpn samples but one (Lpn 09 – core) were correctly detected as such by BD Max (sensitivity 86%). All negative samples were detected as such; specificity was of 100% for all three parameters. Classical assay had a specificity of 100% for all three parameters and sensitivity of 80%, 100%, and 93% for Mpn, Cpn, and Lpn respectively (Fig. 1).



**Conclusions:** All samples were correctly detected, except low positive ones. Transfer from classical PCR systems to the BD MAXTM was easy. Sensitivity was overall the same on BD MAXTM and on CFX96. BD MAX is a true walk-away system that saves time in a routine molecular diagnostic laboratory.

**P728** **Elevated red cell distribution width predicts poor outcome in adult patients with community-acquired pneumonia: a cohort study**

*E. Braun\*, J. Kheir, Z.S. Azzam (Haifa, IL)*

**Objectives:** Community acquired pneumonia (CAP) is a major cause of morbidity and mortality. We recently demonstrated that among young patients (<60 years old) with CAP, elevated red blood cell distribution width (RDW) levels on admission was associated with significant higher rates of mortality and severe morbidity. We aimed to investigate the prognostic predictive value of RDW among CAP patients in general population of internal wards.

**Methods:** The cohort included patients of 18 years old or older who were diagnosed with CAP (defined as pneumonia identified 48 hours or

less from hospitalization) between January 1, 2005 and December 31, 2010. Patients were retrospectively analyzed for risk factors for primary endpoint of 90-day mortality. Secondary endpoint was defined as complicated hospitalization (defined as at least one of the following: In-hospital mortality, length of stay of at least 21 days or ICU admission). Binary logistic regression analysis was used for the calculation of the odds ratios (OR) with 95% confidence intervals (CI) and p values in univariate and multivariate analysis to identify association between patient characteristic, 90-day mortality and complicated hospitalization.

**Results:** The cohort included 3815 patients. In univariate analysis, patients with co-morbid conditions tended to have a complicated course of CAP. In multivariate regression analysis, variables associated with an increased risk of 90-day mortality included age>80 years, high Charlson comorbidity index (>7), blood stream infection (BSI), blood urea nitrogen (BUN) >30 mg/dL, abnormal white blood cells, systolic blood pressure <90 mmHg and elevated RDW >15% (OR 2.1 [1.8–2.5]). Variables associated with complicated hospitalization included high Charlson comorbidity index, abnormal WBC, BUN> 30 mg/dL, hemoglobin <10 g/dL, BSI on admission and elevated RDW (OR 1.5 [1.2–1.8]). Mortality rate and complicated hospitalization were significantly higher among patients with increased RDW regardless of the white blood cell count or hemoglobin levels.

**Conclusion:** Elevated RDW levels on admission are associated with significant higher rates of mortality and severe morbidity in adult patients with CAP. RDW as a prognostic marker was unrelated with hemoglobin levels.

**P729** **Obesity and metabolic syndrome as risk factors for community-acquired pneumonia**

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**Objectives:** Underweight, obesity or metabolic syndrome (MS) may be associated with an increased risk of community-acquired pneumonia (CAP), but available data on this relationship are sparse and inconsistent. The aim of our study was to evaluate the potential association between some anthropometric and metabolic data, such as body mass index (BMI), waist circumference (WC) and MS, and development of CAP.

**Methods:** We conducted a population-based case-control study. All patients aged ≥18 years diagnosed as CAP in the Emergency Department of Arnau de Vilanova Hospital in Lleida (Spain) between January 2009 and March 2010 were prospectively collected. Cases were matched by age and sex with control subjects randomly selected from a Primary Care Area in Lleida. Variables recorded were weight, height, WC, metabolic syndrome, smoking status, alcohol intake, influenza and pneumococcal vaccines, presence of underlying diseases such as diabetes mellitus, hypertension, dislipemia, chronic obstructive pulmonary disease (COPD), heart failure, liver and renal diseases, stroke and cancer, and current treatment with statins, proton pump inhibitors and angiotensin-converting enzyme inhibitors (ACE inhibitors). Subjects were stratified by BMI and divided into five categories according to the World Health Organisation classification. MS was defined by the National Cholesterol Education Program Adult Treatment Panel III 2001 (NCEP-ATP III). Univariate and multivariate analyses were performed with adjustment for confounding factors.

**Results:** A total of 164 cases and 164 controls (102 men and 62 women in each group, mean age 68 years) were enrolled in the study. In the univariate analysis, CAP was significantly associated with MS, smoking habit, absence of pneumococcal vaccination, COPD and ACE inhibitors use. In the multivariate analysis, COPD (Odds ratio [OR] 6.40; 95% confidence interval [CI] 2.95–13.90) and MS (OR 2.00; 95% CI 1.11–3.61) were significantly associated with an increased risk of CAP; conversely, pneumococcal vaccine (OR 2.00; 95% CI 0.29–0.80) and ACE inhibitors treatment (OR 0.51; 95% CI 0.27–0.94) were identified as protective factors. Neither subgroups of patients according to the BMI nor WC showed association with development of CAP.

**Conclusions:** Our study suggests that there is not a relation between BMI subgroups or WC and development of CAP. However, MS, in addition to other well-recognized predictive factors, could be a significant risk factor for CAP.

**P730 Integrated care pathway community-acquired pneumonia for hospitalised patients: how many patients could be included**

R.J. Pisco\*, C. Arnold, M. Ritter, S. Bassetti (Olten, CH)

**Objectives:** Considering the continuing increase in expenses in the health care system, more and more pressure to standardize procedures in care of patients with variable health problems is set up by local or national authorities. It is supposed that standardisation will reduce costs and improve quality of care. As these procedures were successfully implemented in surgical clinics, care pathways are also stipulated in internal medicine.

**Methods:** With regard to the conditions of the care pathway we calculated the percentage of patients with community acquired pneumonia that could be treated according to the schema. A principle precondition of the pathway is the absence of multiple comorbidities, defined as active diagnosis. Comorbidities were counted separately if they involved different organ systems. Hypertension alone or other comorbidities without impact in the management or duration of the hospitalization were not counted as comorbidities. The data were obtained retrospectively based on medical records of patients hospitalized with CAP from May 2007 till May 2009. We calculated how many patients would have been excluded due to comorbidities, admission to intensive care or immunosuppression.

**Results:** Three hundred and twenty-four patients with documented community acquired pneumonia and duration of hospitalization of at least 48 hours were analyzed. Eighty-seven patients had two, 70 three and 53 patients more than three comorbidities. Of the remaining 116 patients, 18 were admitted to the intensive care unit. Five of the remaining 96 patients admitted to a general ward were immunosuppressed. One due to a advanced HIV-infection, one due to chemotherapy for NSCLC and three due to immunosuppression for rheumatological diseases. Three more patients with a PSI class V were although estimated as not eligible for an integrated care pathway. 88/324 patients (27.2% 95% CI 22.3–32%) would have been estimated as eligible to be treated according to the integrated care pathway in our retrospective analysis.

**Conclusion:** The fraction of patients with CAP eligible to be treated according to a predefined integrated care pathway is limited. As most patients could not be treated according to this pathway, we are afraid that the introduction would rather cause confusion than help. Neither cost reduction nor improvement in care can be expected.

**P731 Evaluation of an antibiotic prescribing protocol for treatment of community-acquired pneumonia in a tertiary hospital**

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**Objectives:** Descriptive study of community-acquired pneumonia (CAP) admitted in Hospital Son Espases since January 1 to March 31, 2011, aiming to verify antibiotic prescription according to Committee on Infectious Diseases Guidelines.

**Methods:** Prospective observational study includes all patients admitted with CAP during the study period, pneumonia is defined by the presence of new lung infiltrates on chest radiography with one or more of the following symptoms: fever or hypothermia, cough with or without expectoration, dyspnea, and pleuritic pain. Patients with final diagnosis other than pneumonia are excluded.

Data are collected in a standardized form where the variables related to patient are: comorbidities, previous vaccinations, associated symptoms, signs on physical examination, antibiotic and antiviral treatment,

analytical and microbiological data, complications and related data to monitoring clinical protocol.

**Results:** This study included 102 patients with a CAP diagnosis, 56% men, age average 61 year old. The 37.3% were smokers, and comorbidity is present at 75%, from which 28.4% COPD. Five patients died, 3 with ARDS. Considering the prescription of several antibiotics at the same time, the most prescribed empirical antibiotic treatment were amoxicillin/clavulanate acid (55%), azithromycin (43%) followed by levofloxacin (31.6%).

Empirical treatment is prescribed according to protocol in 75.5% of cases. The 66.7% received antibiotic treatment within 4 hours after hospital admission. In 21.6% switch to oral administration the same day the patient reached clinical stability. Antibiotic treatment was adjusted according to the results of culture and sensitivity in 42.2% of cases. The date of completion of antibiotic treatment consisted in 60.8% of discharge summaries.

**Conclusion:** Specific standard therapeutic care protocols supported by scientific evidence and criteria of efficiency are a useful tool to improve the efficiency and effectiveness of antibiotics in hospitals. The adequacy of CAP protocol was 75.5%, with early antibiotic prescription and antibiogram adjustment. The point to be improved is the appropriate switch to oral administration.

**P732 Intravenous azithromycin – single dose 1.5 g vs. 500 mg once daily for 3 days in patients with community-acquired pneumonia: a prospective and randomised study**

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**Introduction:** Community-acquired pneumonia (CAP) is one of the most common infectious diseases and an important cause of mortality and morbidity worldwide. The objective of the clinical study was to evaluate efficacy and tolerance of intravenously (i.v.) administered azithromycin (AZM) single dose (1.5 g) vs. 500 mg once daily for 3 days in patients with CAP. Single dose application of i.v. AZM could be an alternative for Outpatient Parenteral Antimicrobial Therapy (OPAT) of CAP.

**Methods:** Adults (18–90 years; written informed consent; achieving inclusion criteria) suffering from CAP were enrolled in this study. Severity assessment by CRB-65 score was performed. Effectiveness of single i.v. dose of 1.5 g AZM (diluted in 1000 mL Lactated Ringer's Solution, duration of infusion 4 hours) compared to a standard dose schedule of 500 mg i.v. once daily for 3 days was evaluated. The primary endpoint was clinical cure, defined as: disappearance of fever within 72 hours; disappearance of other clinical and laboratory signs of inflammation; no necessary administration of an additional antimicrobial therapy. The study was an open, comparative, prospective and randomized trial.

**Results:** A total of 72 patients with CAP were randomized. Table 1 summarizes demographic characteristics of the study population and study results. The study drug was well tolerated in all patients. The most causative pathogens were *S. pneumonia* (22.2%), followed by *M. pneumonia* (6.9%), *L. pneumophila* (6.9%), *C. burnetii* (1.4%) and

	All	A	B
No. of patients, n (%)	72 (100)	39 (54.2)	33 (45.8)
Sex m/f, n	47/25	30/9	17/16
Age, years, mean (SD)	44.6 (+/-18.7)	45.3 (+/-19.4)	43.8 (+/-18)
CRB-65: 0, n (%)	24 (33.3)	11 (28.2)	13 (39.4)
CRB-65: 1, n (%)	37 (51.4)	21 (53.8)	16 (48.5)
CRB-65: 2, n (%)	11 (15.3)	7 (17.9)	3 (9.1)
Prior oral antibiotic, n (%)	13 (18.1)	7 (17.9)	6 (18.2)
Clinical cure, n (%)	63 (87.5)	34 (87.2)	29 (87.9)



CMV (1.4%). In 59.7% of the cases no causative pathogen could be diagnosed. No differences in cure rates were seen between the two groups. For 13% of the patients an additional antimicrobial therapy was necessary to get cured.

**Conclusion:** The results of this study indicate that single administration of 1.5 g AZM i.v. seems not to be inferior to standard dosage of 500 mg i.v. once daily for treatment of CAP. Treating patients with a single dose of AZM in an OPAT setting ensures high and adequate drug levels independent from patients compliance. Oral macrolides often show bad bioavailability, mainly oral AZM (about 30%). Single dose administration of AZM could provide a cheap and safety possibility for OPAT in patients suffering from an allergy to betalactam antibiotics. Data are limited by the low number of patients included. Further prospective studies including more patients need to be done.

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**P733** **Empiric use of fluoroquinolone in patients with pulmonary tuberculosis presenting as severe community-acquired pneumonia may improve survival**

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**Introduction:** Empiric treatment with a fluoroquinolone (FQ) is recommended for community-acquired pneumonia, but may delay the treatment for tuberculosis and induce resistance. This study aims to evaluate the impact of empiric fluoroquinolone use in patients with tuberculosis presenting as severe community-acquired pneumonia in an endemic area of tuberculosis.

**Method:** All patients who presented as severe community-acquired pneumonia requiring intensive care from 2005 to 2010 and were finally laboratory confirmed to have pulmonary tuberculosis were included. Patients were separated into two groups according to the empiric antibiotics (FQ vs. non-FQ groups).

**Results:** A total of 79 tuberculosis patients were identified. Among them, 44 (55.7%) received empiric FQs. The clinical characteristics were similar between FQ and non-FQ groups except there were more patients with chronic pulmonary diseases among non-FQ group and more patients presented with fever among FQ group. Ninety-five percent (n = 42) and 97% (n = 34) had underlying systemic diseases (p > 0.9). The most initial presenting symptoms included respiratory symptoms (93% and 94%) and fever (63.6% and 34.3%) among FQ and non-FQ groups separately. Sputum samples were smear-positive for acid-fast bacilli in 10% (n = 8) overall. Among FQs used and non-FQs used group separately, the mean of APACHE scores and SOFA scores were 21.3 ± 6.6 vs. 22.1 ± 7.7 (p = 0.6), and 7.9 ± 3.6 vs. 7.3 ± 3.8 (p = 0.5), the mean duration of ICU stay and hospital stay were 30.57 ± 21.07 days vs. 17.57 ± 17.63 days (p = 0.05), and 75.70 ± 67.72 days vs. 39.66 ± 26.61 days (p = 0.004). Cox regression analysis and Kaplan–Meier analysis showed FQs use was the only associated factors for benefit of survival.

**Conclusion:** Due to its excellent bactericidal activity against *Mycobacterium tuberculosis*, empiric use of fluoroquinolone for patients with severe community-acquired pneumonia may improve survival.

**P734** **Aetiologies of prolonged cough in Thai adults: the roles of *Bordetella pertussis* and atypical pathogens**

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Although pertussis traditionally has been considered a disease of childhood, it was well-documented in adults nearly a century ago and is currently recognized as an important respiratory tract infection in adolescents, adults and elderly. The epidemiology of pertussis in the Asian region seems poised for a similar change. *B. pertussis* infections are well known to cause prolonged cough, often this diagnosis is

overlooked because of atypical presentation of the disease, low diagnostic suspicion and limited use of confirmatory tests. In countries with routine vaccination against pertussis with high coverage, pertussis is not usually taken into consideration for the etiology of prolonged cough in adults. Studies in a variety of populations have documented that pertussis is quite common, ranging from 12% to 26% of adolescents and adults with prolonged cough. The anticipation and early recognition of this change in the epidemiology is important because the affected adolescents and adults act as reservoirs of the disease to the vulnerable population of infants, for whom the disease can be life threatening. We conducted a prospective study to determine the incidence of pertussis and atypical pathogens in Thai adults with prolonged cough.

**Methods:** Seventy-six patients with cough lasting for more than 2 weeks (range, 14–180 days) were recruited, from October 2010 through February 2011. Patients had a mean age of 55 years (range, 15–85 years) with a sex ratio F/M of 1.7. At enrollment, nasopharyngeal swabs were taken for detection of nucleic acid of *Bordetella pertussis*, *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* by PCR and paired serum samples were collected and tested for IgG antibody to pertussis toxin by use of ELISA.

**Results:** Fourteen patients (18.4%) with mean age of 59 years (range, 28–85 years) and mean duration of cough were 34 days (range, 14–120 days) had evidence of acute infection of pertussis; among them, PCR was positive in one patient and 13 cases were diagnosed serologically. The possible *M. pneumoniae* and *C. pneumoniae* infections among patients with prolonged cough diagnosed by PCR were 1.3% and 25% respectively. Both PCR for *C. pneumoniae* and serology for pertussis were positive in two patients.

**Conclusion:** *B. pertussis* is being increasingly recognized as a cause of prolonged, distressing cough without whooping symptoms in adults in countries where childhood pertussis vaccination is universal.

**Fungal infections: from sensitive diagnosis to rapid species identification**

**P735** **Comparison of MycAssay™ *Aspergillus* real-time PCR kit and “in-house” real-time PCR assay on culture confirmed respiratory samples**

R. Kofol\*, T. Matos (Ljubljana, SI)

**Objective:** The MycAssay™ *Aspergillus* real-time PCR kit (Myconostica, Mancheste, UK) is a commercially available real-time PCR diagnostic assay that enables detection of *Aspergillus* spp. in respiratory samples. We compared a commercially available real-time PCR diagnostic assay and »in house« real-time PCR on culture confirmed respiratory samples.

**Methods:** We included 35 respiratory samples, sent for routine microbiology diagnostic procedures. Material was divided in two, one part was cultured using standard cultivation methods, the other was used for DNA extraction. DNA was extracted and purified using MagNA Pure Compact instrument (Roche Diagnostics, Mannheim, Germany) and MagNA Pure Compact Nucleic Acid isolation kit I (Roche Diagnostics, Mannheim, Germany), following the manufacturer's instructions. Isolated DNA was amplified with MycAssay™ *Aspergillus*, according to manufacturer's instructions and with »in-house« PCR targeting 18S rRNA genes as described by Sanguinetti et al., 2003. Both amplifications were performed using SmartCycler (Cepheid, Sunnyvale, CA) system.

**Results:** All culture positive respiratory samples were identified as *Aspergillus* spp. 43% (15/35) of samples were positive with MycAssay™ *Aspergillus* real-time PCR kit and 23% (8/35) with in house real-time PCR. All in-house positive samples, were also positive with MycAssay.

**Conclusion:** Sensitivity of both PCR assays in comparison to culture is quite poor. Explanation can be found in (i) inappropriate DNA extraction method for respiratory samples or (ii) presence of inhibitors in DNA isolates or (iii) non-optimal PCR reaction mixture and

conditions of both PCR assays. PCR assay for the detection of *Aspergillus* DNA could be an important additional diagnostic approach. It's faster, but need a suitable extraction method.

#### **P736** Molecular identification of *Candida* spp. from positive blood cultures

R. Kofol\*, V. Marcic, T. Matos (Ljubljana, SI)

**Objective:** For the detection of *Candida* blood stream infection blood culture remains the gold standard. Selection of the appropriate therapy is largely dependent on the accurate species identification. Standard microbiological methods of species identification are time-consuming. Here we present the TaqMan real-time PCR assay which enables detection and identification of six most frequently isolated *Candida* species from blood cultures: *C. albicans*, *C. glabrata*, *C. krusei*, *C. parapsilosis*, *C. tropicalis* and *C. kefyr*.

**Methods:** In the study were included 136 blood cultures (six negative, 130 positive) processed with BacT/Alert 3D automated blood culture system (bioMérieux, Marcy l'Etoile, France). Positive blood cultures were subcultured using Sabouraud agar and CHROMagar *Candida* (Mast Diagnostics, Merseyside, UK). One millilitre of suspension from positive blood culture was transferred into a new tube and centrifuged. DNA from supernatant was extracted automatically on MagNA Pure Compact Instrument (Roche Diagnostics, Mannheim, Germany). Species-specific TaqMan probes and primers were designed from the variable internal transcribed spacer region ITS2 as described previously by Guiver et al. (2001). Real-time PCR was performed in a 25 µL reaction mixture volume using the SmartCycler instrument (Cepheid, Sunnyvale, CA).

**Results:** Comparing classical identification and real-time PCR detection and identification concordant results were found in 123/136 (90%) samples (117 positive, six negative). Using real-time PCR we determined *Candida* spp. in another eight samples, which we couldn't determine with standard cultivation methods. In five samples we isolated fungi other than *Candida* which were PCR negative. Results were expected, and additionally confirmed PCR specificity.

**Conclusion:** TaqMan probe based real-time PCR is fast, because it allows species identification in few hours after positive blood cultures detection, is sensitive and specific.

#### **P737** Rapid identification of *Candida glabrata* cryptic species using real-time PCR combined with denaturing high-performance liquid chromatography

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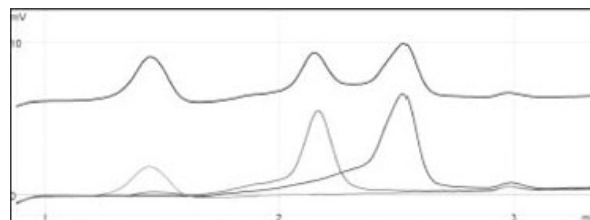
**Introduction:** During last years, the increment in incidence, and associated high morbi-mortality, has converted invasive fungal infections in one of the most important public health associated problems. Besides, non-albicans *Candida* species have emerged as etiological agents of invasive candidiasis. Since the description of two new cryptic species (*C. bracarensis* and *C. nivariensis*) phylogenetically related to *C. glabrata* with different phenotype and antifungal susceptibility profile, it seems to be necessary to develop a rapid and accurate identification technique in order to distinguish between these three microorganisms.

**Objective:** We studied the performance of real-time PCR combined with Denaturing High Liquid Chromatography (DHPLC) as an alternative, fast and novel method to perform such identification accurately.

**Methodology and results:** Fungal DNA from pure cultures of *C. glabrata* (04.229), *C. bracarensis* (NCYC-3133) and *C. nivariensis* (04.228) reference strains was extracted using a MagNAPure Compact system. A small amplicon of the ITS2 region was amplified using the ITS86-F and ITS4-R primers previously described by Grutzner et al. employing a real-time PCR scheme with SYBR Green I in a LightCycler 2.0. The PCR products obtained were purified using

Ultra Clean™ PCR Clean –Up DNA purification kit according to manufacturer's instructions.

The identification technique of the three species was performed by DHPLC using WAVE-MD System. In order to achieve a clear separation of the three species, 5 µL of a mixture of the reference strains PCR products were injected in the chromatographic system under different partially denaturing conditions predicted by the system software. The identification of the species was carried out analyzing each sample separately at optimal conditions, as shown in the figure. Once optimized, this technique was applied to check the identification of 96 strains that were previously phenotypically identified as *C. glabrata* and all the system confirmed this identification in all the samples analyzed.



**Conclusions:** Real-time PCR combined with DHPLC was an accurate, fast and reliable method to confirm *C. glabrata*, *C. bracarensis* and *C. nivariensis* identification simultaneously in culture samples.

#### **P738** Invasive aspergillosis: evaluation of several diagnosis tests

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**Objectives:** Invasive aspergillosis (API) is a disease of high mortality and difficult to diagnose. The aim of our study is to compare the results obtained by detection of ag. Galactomannan (AG) fungal culture and molecular techniques to detect DNA (PCR), in order to establish a protocol for diagnosing and monitoring the evolution of API infection in risk patients or clinical suspicion patients.

**Materials and Methods:** In the period of 8 months, were prospectively studied 472 samples of 271 adult patients from: Infectious, Pneumology, Oncology Services and Critical Care Unit, The processed samples were: 388 respiratory (104 sputum, 263 BAS, BAL 21.) and 84 serum samples. Respiratory specimens were processed for fungal culture and detection of AG.

In cases of disagreement between them, or both negative results, was performed to detect fungal DNA in respiratory specimens; PCR and AG in serum (66).

AG test was determined by the Platelia *Aspergillus* test (Bio-Rad Ref 62 794) according to manufacturer's recommendations.

A serum samples and/or BAL negative tests can not exclude the diagnosis of API.

Molecular diagnosis is based on the detection of *Aspergillus* genomic DNA by Real-time PCR. Kits were used for the qualitative detection of genomic DNA of *Aspergillus* in respiratory specimens of lower respiratory tract and serum, using a computer SmartCyclerR (Ref Myconostica MYCO-080-045).

**Results:** Considering culture as the gold standard, AG test in respiratory samples, showed a positive predictive value (PPV) of 23%, negative predictive value (NPV) of 100%, sensitivity (S) 100% and a specificity (E) of 52%.

Comparing the results of PCR with culture, we obtained a PPV of 69%, a NPV of 89%, S of 64% and E of 82%.

In serum, the AG and PCR tests showed a 60% of discrepancy.

**Conclusions:** AG detection is a highly sensitive and useful as an adjunct to culture for diagnosis and/or possible warning API. The specificity could be improved by increasing the rate of positivity in respiratory specimens AG.

In case of disparity of results or negative determination in patients with symptoms suggestive of API, the combination of several tests in both respiratory and serum sample simultaneously would increase the specificity of the diagnostic. In order to assess the diagnostic utility of the PCR, we believe that it's needed further studies to get it. Then, it could be improving the samples tests also using the quantitative technique.

**P739 Comparison of microscopic diagnosis and a real-time PCR test for the diagnosis of *Pneumocystis jirovecii* pneumonia**

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**Objectives:** *Pneumocystis jirovecii* pneumonia (PCP) is a common opportunistic infection, with high mortality when an adequate treatment is not performed, so empirical therapy is necessary but not extent of toxicity. Microscopic diagnosis has limitations, real-time PCR may assist in diagnosis but this technology is not still validated. We compared these two techniques in respiratory samples from patients with clinical suspicion of PCP.

**Methods:** We analyzed a total of 41 respiratory samples, 23 bronchoalveolar lavage (BAL) and 18 induced sputa (IE), received in our microbiology laboratory from April to October 2011. These samples belonged to 39 patients with strong clinical suspicion of PCP, six children (<14 years) and 33 adults. All the children's samples were BAL. Samples were investigated with microscopically examination using a direct immunofluorescence assay (IF) to detect *P. jirovecii* cysts (MONOFLUO *Pneumocystis jirovecii* IFA Test Kit, Biorad®). DNA was extracted from samples and was tested with the MycAssay *Pneumocystis* kit (Myconostica®) on a SmartCycler real-time PCR platform (Cepheid®), according to the manufacturer's instructions; this is a qualitative real-time PCR test utilizing molecular beacons, whose target sequence is the *Pneumocystis* mitochondrial ribosomal large subunit. Human DNA is not detected and the kit contains an internal amplification control sequence to confirm amplification.

**Results:** Comparing both techniques, we obtained 24 IF-/PCR-samples, 9 IF+/PCR+ samples and 8 IF-/PCR+ samples, none IF+/PCR-, what meant a concordance of 80%.

Analyzing by type of sample, concordance was 100% for BAL, whereas all the discrepant cases happened with IE. Two patients with two samples showed IF-/PCR+ IE and IF+/PCR+ BAL, so they were correctly diagnosed of PCP.

We realized that PCR recovered many cases of IF- IE, what can be explained by a low-level of microorganisms present in sputum. The problem is to differentiate between infection and colonization; a positive PCR result must be supported by clinical signs and symptoms and by results in other complementary tests, such as radiography. A negative test is helpful for excluding the diagnosis of PCP.

**Conclusion:** Multidisciplinary approach is required for early PCP diagnosis. Real-time PCR seems to be very helpful in diagnosis in a compatible clinical context.

**P740 Rapid identification of yeast species by MALDI-TOF MS compared to the Prove-it™ PCR assay**

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**Objectives:** The aim of this study was to determine the usefulness of MALDI-TOF MS to identify yeasts of clinical importance compared to a newly developed molecular method (Aittakorpi et al, ECCMID 2011 P2098).

**Methods:** One hundred and twenty eight yeasts from clinical specimens which had been previously identified by a combination of

conventional and molecular methods, were cultured for 18–24 hour on Sabouraud Dextrose Agar at 37°C. These samples were processed using the Bruker Biotyper alcohol extraction method. Using the Bruker Real Time Classification (RTC) software (Biotyper database 3995), samples were analysed automatically and an ID produced.

**Results:** One hundred and twenty eight isolates were analysed of which 88% (113/128) were identified. Nine isolates were not reliably identified. The PCR method gave the expected ID to species for 82% (105/128) of the comparative isolates.

**Conclusion:** MALDI-TOF is a rapid identification technique with a turnaround time of 24–36 hour, and could identify a greater diversity of yeasts compared to the Prove-it™ method. MALDI-TOF correctly identified a greater percentage of the yeasts, although this was not statistically significant. Assuming the user has access to a MALDI-TOF MS, the running costs are far lower and the process much simpler.

**P741 Molecular identification of *Malassezia* species isolated from dermatological patients in Saint Petersburg, Russia**

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**Objectives:** The aim of this study was an identification of *Malassezia* species isolated from patients with various skin diseases in Saint Petersburg, Russia.

**Methods:** *Malassezia* strains were isolated from skin lesions or pustule content of patients on Leeming-Notman agar (de Hoog et al., 2000) at 32°N during 4–14 days or more. rDNA extraction from *Malassezia* cultures was performed by PrepMan Ultra kit (Applied Biosystems, USA). PCR was performed with primers NL1 and NL4 (Kurtzman et al., 1998). Amplicons were purified and sequenced on both strands using BigDye Terminator V 3.1 Kit according to manufacturer protocol. Products were analyzed on Genetic Analyzer 3500 (Applied Biosystems, USA). Comparative sequences analysis and GenBank searches were made by MEGA 5 software (Tamura et al., 2011) and nucleotide-nucleotide BLAST (Basic Local Alignment Search Tool, <http://blast.ncbi.nlm.nih.gov/>).

**Results:** Eighty seven patients with following diseases were examined: 25 – pityriasis versicolor (29%), 23 – seborrheic dermatitis (27%), 20 – atopic dermatitis (23%), 11 – *Malassezia folliculitis* (13%) and 7 (8%) – other (pemphigus vulgaris, otitis, neurodermatitis, psoriasis, dyskeratosis). For molecular identification D1/D2 sequences were obtained. Based on intraspecific sequences comparison the limit of identity for species identification by BLAST analysis was chosen as 98%.

*M. sympodialis* were 37 (43%) isolates, *M. globosa* – 32 (37%), *M. restricta* – 9 (10%) and *M. obtusa* – 4 (5%). Four isolates (5%) showed identity <98% and were considered as *Malassezia* sp. *M. sympodialis* was the most frequent species in patients with pityriasis versicolor (52.0% cases) and *Malassezia folliculitis* (72.7%). *M. sympodialis* and *M. globosa* were associated with seborrheic dermatitis and atopic dermatitis nearly at the same rate (34.8% and 43.5% of seborrheic dermatitis cases, 35.0% and 40.0% of atopic dermatitis cases, respectively).

**Conclusion:** Unambiguous identification of 95% of *Malassezia* isolates was performed using D1/D2 region sequences of 26S rDNA. The most frequent species isolated from patients with *Malassezia*-associated skin diseases were *M. sympodialis* and *M. globosa*.

**P742 Genetic diversity of *C. parapsilosis* complex**

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**Objectives:** *Candida parapsilosis* is an opportunistic pathogen which is a cause fungemia, vaginitis, endocarditis, endophthalmitis, septic arthritis and peritonitis. The aim of our study was to examine the

genetic diversity of isolates of *Candida parapsilosis* complex, obtained from clinical samples and to evaluated matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry for the rapid identification of *Candida parapsilosis*, *C. orthopsilosis* and *C. metapsilosis*.

**Methods:** We analysed 122 *C. parapsilosis* complex strains previously identified using standard cultivation methods (CHROMagar *Candida* (Mast Diagnostics, UK) and biochemical tests [ID 32 C and API *Candida* (bioMérieux, France)]. Reference strains of *C. parapsilosis* ATCC 22019, *C. metapsilosis* ATCC 96144 and *C. orthopsilosis* ATCC 96139 were also included. The genetic diversity of *C. parapsilosis* was assessed using BanI digestion at SADH fragment as previously published by Tavanti et al, 2005. MALDI-TOF (Bruker Daltonik GmbH, Germany) was done according to manufacture's instruction.

**Results:** Using BanI digestion of SADH fragment we were able to differentiate 122 *C. parapsilosis* complex strains into 109 *C. parapsilosis* (89.35%), 10 *C. metapsilosis* (8.2%) and 3 *C. orthopsilosis* (2.45%). MALDI-TOF results were in complete concordance.

**Conclusion:** *C. parapsilosis* complex strain can be divided in *C. parapsilosis*, *C. metapsilosis* and *C. ortopsilosis* using any at the described methods.

#### P743 *C. haemulonii* complex: a human multi-resistant pathogenic yeast with a high genetic biodiversity

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**Background:** *C. haemulonii* is one of the rarest yeast species that can be isolated from human clinical sources. Fungal infections due to this yeast have been described in the literature, varying from superficial to deep infections. Cases of catheter related fungemia, blood stream infection, osteitis and outbreaks in intensive care units have been reported recently.

The susceptibility profile of this *C. haemulonii* shows that is resistant to Amphotericin B (AMB) and Fluconazole (FLC) with high Minimal Inhibitory Concentrations (MIC) for both compounds, which can hinder the management of patients with deep infections caused by this yeast. This antifungal profile has often been associated with clinical failure.

**Materials and methods:** Thirty strains of the *C. haemulonii* complex belonging to the Mycology Department of the National Centre for Microbiology (CNM, Majadahonda, Spain) and the CBS Fungal Biodiversity Centre (CBS-KNAW, Utrecht, The Netherlands) collections were analyzed. The amplification and sequencing of the ITS domain (ribosomal DNA), D1/D2 region and DNA Polimerase II gen (RPB2) were done.

**Results:** From the 30 isolates of the *C. haemulonii* complex used in the present study, 19 were assigned to the most commonly encountered group (*C. haemulonii* group I), seven isolates were assigned to *C. haemulonii* group II, and the other four Isolates were assigned to a new cluster. Based on the ITS sequences analysis, three main clusters were distinguished. The first one included 23 strains, four out of these 23 formed a subcluster of "atypical" strains which were positive distantly from the other 19 *C. haemulonii* group I isolates. The third cluster included strains that belong to the *C. haemulonii* group II. On the other hand, the analysis of the RPB2 gene showed the same results as the ones showed in the ITS analysis. Whereas the analysis of the D1-D2 26S rRNA gene fragment sequences only showed two different clusters. The group formed by the four "atypical isolates" was included in the same cluster as those of *C. haemulonii*.

**Conclusions:** (i) the molecular data of the RPB2, D1/D2 and ITS regions demonstrate that the *C. haemulonii* complex has a high genetic diversity. These data suggest that *C. haemulonii* complex is composed by three different clusters. (ii) Due to the resistant antifungal profile, more studies would be needed in order to establish a relationship between the susceptibility profile and each one of these three clusters.

#### P744 Genotyping of *Candida albicans* by CDC3 microsatellite length polymorphism and high-resolution melting analysis: comparison between Tunisian and Parisian clinical isolates

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**Objectives:** Invasive candidiasis has emerged as a major problem in neonatal intensive care units (NICUs) over the last decades. The aim of our study was to assess whether there is a specific ecology to a Tunisian NICU and to evaluate the performance of the CDC3 microsatellite MLP and HRM analysis recently described as a typing system of *Candida albicans*.

**Methods:** We genotyped 82 isolates obtained from 40 neonates collected in the NICU of Farhat Hached hospital in Sousse city (Tunisia), by using CDC3 microsatellite MLP and the HRM analysis. We compared the results with those previously reported on 95 isolates collected in some remote Parisian hospitals to identify any specific ecology.

**Results:** Twelve genotypes were identified in the Tunisian isolates and 16 genotypes in the Parisian isolates. Eleven genotypes were common to both collections of isolates. Only one genotype from the Tunisian collection was not detected among the Parisian isolates. However five genotypes detected in Parisian collection were not shown among Tunisian isolates. In addition of the 16 genotypes observed, three were significantly more frequent in the Tunisian NICU. These results argue for a specific *C. albicans* population to the investigated NICU and represents an indirect evidence of nosocomial transmission of *C. albicans* in the unit. However, we cannot rule out the fact that the difference in the patients' population may partly explain the difference in the genotypes' distribution between the compared collections in addition to the difference in the ethnic origin and the age of the patients as the Tunisian isolates were obtained from neonates whereas the Parisian isolates were obtained from adults.

**Conclusion:** The differences in genotypes distribution among Tunisian and Parisian isolates argue for a specific *C. albicans* population to the Tunisian NICU. Combined microsatellite length polymorphism (MLP) and high-resolution melting (HRM) analysis are a highly reproducible and transportable genotyping method very suitable for comparison of the *C. albicans* ecology in different hospital facilities.

#### P745 Clonal diversity of *Candida albicans* isolates causing candidaemia over a 4-year period: patients located in different departments can be infected by identical genotypes

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**Objectives:** Most episodes of fungaemia are caused by *Candida albicans*. Genotyping of *C. albicans* strains isolated from blood may clarify the genotypic diversity of this species, although the technique is rarely performed. We studied the clonal diversity of *C. albicans* isolates using a highly reproducible and discriminatory microsatellite marker panel.

**Methods:** We studied 186 *C. albicans* strains isolated from the blood cultures of 174 patients with candidaemia (January 2007 to December 2010). Each isolate represented one episode of candidaemia. Multiple episodes were defined as isolation of *C. albicans* in further blood cultures taken  $\geq 7$  days after the last isolation in blood culture. The isolates were identified after amplification and sequencing of the ITS1-5.8S-ITS2 region and further genotyped using a panel of six microsatellite markers (Botterel JCM 2001, Sampaio JCM 2003, Sampaio JCM 2005). Patients had one episode (n = 160), two episodes (n = 9), or 3 (n = 1) episodes. Patients with mixed genotypes in the same culture were excluded (n = 4). Identical genotypes showed the same alleles for all six markers. A similarity dendrogram was

constructed using the remaining 181 strains from the 170 patients included.

**Results:** An inpatient analysis revealed that the genotypes causing both episodes were identical in most patients with two episodes (7/9). In contrast, two different genotypes were found in the patient with three episodes, one causing the first and second episodes and the other causing the third episode (isolated 6 months later). An interpatient analysis revealed that 124 of the 143 different genotypes found were involved only in one episode (n = 121 patients); the remaining genotypes grouped in 19 clusters (n = 49 patients) including two to five patients each. In 13 of the 19 clusters, the patients were infected by the same genotype but had been admitted to different departments. In contrast, each of the remaining six clusters grouped isolates from patients infected by the same genotype in the same department; interestingly, four of the clusters had a different genotype and involved patients admitted to the same unit (neonatology).

**Conclusion:** We showed that patients admitted to hospital could develop candidaemia caused by an identical genotype of *C. albicans*. In up to 70% of cases, patients were not located in the same department at diagnosis. In contrast, in patients with multiple episodes of *C. albicans* candidaemia, the genotype causing the first episode was found in the subsequent episodes.

**P746** Catheter-related fungaemia caused by *Candida albicans*: are genotypes from the catheter tip and blood the same?

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**Objectives:** *Candida albicans* is a major cause of catheter-related fungaemia (CRF). The definition of CRF requires the isolation and identification of the same fungal species from both the catheter tip and blood cultures. However, strains are rarely genotyped to prove the presence of identical genotypes in both types of sample. The aim of this study was to investigate the presence of identical *C. albicans* genotypes in blood and tip samples from patients with CRF.

**Methods:** We retrospectively studied 22 patients admitted to hospital (January 2008 to December 2010) with *C. albicans* isolated simultaneously from blood and catheter tip cultures (48 isolates: 22 from blood cultures and 26 from catheter tip cultures [an additional tip culture with isolation of *C. albicans* was studied in four patients]). In 17 cases, the tip was received in the microbiology laboratory a mean of 2 days (range, 0–5 days) after receipt of the blood sample. Isolates were genotyped using a panel of six microsatellite markers (Sampaio JCM 2003, Sampaio JCM 2005, Botterel JCM 2001). Matches between catheter and blood genotypes were defined when they both showed identical alleles for all six markers.

**Results:** We found matches between genotypes in both samples in 20 of the 22 (91%) patients. Most patients (19/20) had only one genotype, but one patient had two different genotypes found simultaneously in both the tip culture and the blood culture. Only 2 of 20 patients had different genotypes both samples. In one, the catheter tip genotype differed in two markers from the blood genotype (the catheter was received in the laboratory 5 days after the blood culture); in the other patient, the catheter tip genotype differed from the blood genotype in five markers. In the case of the four patients in whom a second catheter tip was analyzed, we again found matches between the catheter tip and blood genotypes.

**Conclusions:** The use of a microsatellite panel with high discriminatory power enabled us to show – albeit in a low proportion of cases of CRF – that the *C. albicans* genotypes colonizing catheter tips are different from those causing candidaemia. Future prospective studies including several colonies from both tip cultures and blood cultures are warranted. Jesús Guinea (CP09/00055) and Pilar Escribano (CD09/00230) are supported by a contract from FIS.

**P747** Fungal environmental control: usefulness of a pan-fungi NASBA in a protected haematology unit

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**Objectives:** The fungal surveillance of environmental contamination is necessary in protected haematology wards housing patients at high-risk of invasive filamentous fungal infections. This monitoring is usually performed with mycological culture-based method. Several PCR have been described to evaluate the fungal load but mainly in highly contaminated environment.

We describe a quantitative NASBA targeting fungal rRNA (molds and yeasts) and an original application of this method on environment samples to monitor the fungal load in protected haematology unit.

**Methods:** This quantitative pan-fungi NASBA (Zhao et al, 2009) was used in combination with an internal control (Weusten et al, 2002) and was fully developed on NucliSENS EasyQ (bioMérieux). The sample preparation and extraction were performed with a miniaturized prototype specifically designed for processing environmental samples derived from the NucliSENS easyMAG (bioMérieux).

Two different locations in a protected haematology ward were sampled: the corridor and the patient's rooms with HEPA filters and LAF. Surface samples were performed by contiguous sampling using contact plates (bioMérieux) and swabs (Copan).

**Results:** During this 12 weeks prospective study, 132 surfaces were studied (table). The percentage of agreement between culture and NASBA were 78% and 92% for samples performed in corridors and in rooms, respectively. Among discrepancies between these two methods, the positivity of NASBA associated with a negative culture (9.8%) was more frequent than negative NASBA with a positive culture (2.3%). In the five positive samples performed in corridor, the load was 2.8 CFU ± 2.5/25 cm<sup>2</sup> and 3.9 ± 2.7 Geq/25 cm<sup>2</sup> for culture and NASBA, respectively.

Haematology corridor (n=36)

n° of samples	UFC/25cm <sup>2</sup>	Geq/25cm <sup>2</sup>
23	0	0
5	7	4
	3	8
	2	2
	1	4,6
	1	1
1	1	0
7	0	1
	0	1,4
	0	2
	0	2,6
	0	3
	0	5,6
	0	6,8

Haematology patient's rooms (n=96)

n° of samples	UFC/25cm <sup>2</sup>	Geq/25cm <sup>2</sup>
88	0	0
2	1	0
	1	0
6	0	1
	0	1,6
	0	1,8
	0	2
	0	3,8
	0	4,6

CFU = Colony Forming Unit, Geq: genome equivalent.

**Conclusion:** Pan-fungi NASBA used in this study allows to evaluate rapidly the global fungal load (3 hours instead of 1 week) and could be applied to the surface fungal environmental surveillance in protected unit, since the percentage of agreement between culture and NASBA is high. In protected area the fungal load is very low. Two surfaces although contiguous could be not strictly contaminated by the same number of spores explaining the discrepancies observed between culture and NASBA in part. Furthermore all molecular methods detect also non cultivable fungi but NASBA has the advantage to detect only viable organisms. Further, the evaluation of NASBA in air samples is necessary as well as the evolution of NucliSens technology towards an integrated system.

**P748 FGD PET features of pulmonary histoplasmosis**

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**Objective:** Pulmonary histoplasmosis (H) is an endemic mycosis that often radiographically resembles lung cancer (LCa) and complicates the evaluation of pulmonary nodules (PN). This study aims to describe the fluorodeoxyglucose positron emission tomography/computed tomography (FDG-PET) features of H and pathologic correlation.

**Methods:** The pathology records of our hospital were retrospectively reviewed over a 10-year study period in order to identify patients who had histopathologically diagnosed H and who underwent FDG-PET imaging. We then reviewed their demographic, clinical, laboratory and FDG-PET features.

**Results:** Fourteen patients (pts) (eight men; mean age 50; range 24–69 years) were included. Twelve pts (85%) had underlying lung disease. Most frequent clinical manifestations were cough in 5 (35.7%), and dyspnea in 2 (14.3%) pts. Histopathologically, fibro-caseating nodule (inactive lesion) was the most frequent finding (9 pts; 64.3%), followed by active granulomatous inflammation (5; 35.7%) and acute pulmonary inflammation (1; 7.1%). Three pts were found to have simultaneous H and LCa. The diameter of PN and lymph nodes (LN) ranged from 0.6 to 4.4 cm (mean 1.7) and 0–3 cm (mean 1.47), respectively. Positive FDG uptake was identified in 11 pts (78.6%), and negative uptake in three pts (21.4%). The mean standardized uptake values (SUV) of 14 PN and 13 LN were  $7.37 \pm 10.8$  (0–39), and  $2.93 \pm 1.8$  (0–5.2), respectively. When H-LCa cases were excluded, the diameter of PN and lymph nodes (LN) ranged from 0.6 to 2.0 cm (mean 1.24) and 0–3 cm (mean 1.5), respectively. The mean SUV of 11 PN and 10 LN were  $3.4 \pm 4.15$  (0–15), and  $2.87 \pm 2.03$  (0–5.2), respectively. Pathologically active H lesions had marginally significantly higher FDG radioactivity than inactive lesion ( $p = 0.056$ ). The mean SUV of the active PN lesions was  $5.56 \pm 5.31$  vs.  $1.2 \pm 1.71$  in inactive PN lesions. No statistical significance was found on the Spearman correlation between the density of the yeast in the lesions and SUV measures.

**Conclusion:** This retrospective study provides FDG-PET – pathologic data on H, in patients residing in H-endemic area, who underwent FDG-PET for evaluation of chest imaging abnormalities concerning for neoplasm. To our knowledge this is the largest case series of FDG-PET findings in pts with H.

**P749 Species distribution and anti-fungal susceptibility of *Candida* spp. at the Heart of England Foundation Trust, United Kingdom**

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**Introduction:** Invasive candidiasis has an attributable mortality of 40%. Fluconazole is extensively used for the prevention and treatment of *Candida* infections, despite its frequently reduced or absent activity against some non-albicans species. The IDSA guidelines advise using echinocandins in patients with severe illness, as well as those with prior azole exposure. Susceptibility testing guides patient management and epidemiological surveillance. This is increasingly necessary in the light of proposed new antifungal breakpoints.

**Methods:** *Candida* species were collected prospectively, identified using the AUXACOLOR™<sub>2</sub> Kit (Bio-Rad, USA) and susceptibility tested using the YeastOne® Sensititre (TREK Diagnostics, USA) Kit. Susceptibilities were interpreted using national reference laboratory breakpoints.

**Results:** Hundred and one isolates were tested in the study, of which 61% were from non sterile sites and 39% were from blood cultures. Species distribution for blood cultures showed 44% were *C. albicans* and 45% from the non-sterile sites. No fluconazole resistance in *C. albicans* from blood cultures was identified, although the rate of intermediate resistance was 12% with the new breakpoints. The non-albicans strains from blood cultures had high levels of fluconazole resistance increasing from 55% to 82% when interpreted with the new

breakpoints. Clinical data of these patients is summarised in Figure 1. Isolates of *C. albicans* from non-sterile sites, 45% ( $n = 28$ ) were from high vaginal swabs, the rest were from sputa and superficial swabs. One percent of all specimens were noted to be fluconazole resistant, with intermediate susceptibilities increasing from 25% to 32% when interpreted with the new breakpoints. In the non-albicans strains from non-sterile sites fluconazole resistance levels increasing from 15% to 41% for using the new breakpoints.

Age (yrs)	51.5±24.2
Sex (male n,%)	26, 67%
Isolate from Critical Care (n,%)	7, 18%
Fluconazole used appropriately as a first agent (n,%)	32, 82%
30 day mortality	57%
Duration of Treatment (days)	11.1±8.1

**Discussion:** There is a variation in the local epidemiology of *Candida* species from sterile and non-sterile sites. Less than 50% of organisms recovered from blood cultures were found to be *C. albicans*, and, this coupled with >50% resistance rates to fluconazole in the non-albicans suggests that patients with a probable or proven candidaemia should be managed with an echinocandin empirically and then de-escalated to fluconazole if proven susceptible. Monitoring of resistance patterns in non-sterile samples is necessary to assess the effect of systemic antifungals available without a prescription.

**P750 Early prediction of dose–response and PK/PD relationships of antifungals by quantitative measurement of tissue burden using real-time quantitative PCR in an animal model of invasive aspergillosis**

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**Objectives:** Experimental models of invasive aspergillosis (IA) have been used to explore pharmacokinetic and pharmacodynamic (PK/PD) properties of antifungal agents. Survival is still considered the most reliable effect measure to determine exposure-response. We here study the feasibility of quantitative PCR (qPCR) to measure fungal load in target tissues for early assessment of antifungals efficacy in an experimental model of IA.

**Methods:** We studied in vivo pharmacokinetics and antifungal efficacy of voriconazole (VCZ) vs. anidulafungin (AFG) in an immunocompetent model of *Aspergillus fumigatus* (AF) infection (VCZ-susceptible and VCZ-resistant isolates). Groups of 17 mice were randomized for doses regimens of 2.5, 5, 10 and 20 mg/kg/body weight. Therapy was started 24 hour after fungal inoculation for seven consecutive days, once daily intraperitoneally. The therapeutic efficacy was investigated using animal survival at 7 and 14 days postinfection, compared to the decrease in tissues fungal burden at 48 and 72 hour postchallenge, utilizing real-time qPCR targeting the 28S region of AF. Kidneys were collected from three treated and three control mice at each timepoint and also from all surviving mice at the end of the experiment.

**Results:** The mean number of genome copies detected in untreated animals was  $3 \times 10^4$  in kidneys ( $n = 3$ , range =  $1 \times 10^3$ – $2 \times 10^5$ ) at days 2 and 3 post infection. There was a mean 2–3 log<sub>10</sub> ( $n = 3$ , range =  $1 \times 10^2$ – $2 \times 10^4$ ) reduction of AF genome copies in infected animals treated with highest dosage of VCZ (100% survival at days 7 and 14). A stronger correlation between 7 days survival and qPCR was observed at day 3 post infection ( $r^2 = 0.90$ ,  $p = 0.01$ ) compared to day 2 ( $r^2 = 0.84$ ,  $p = 0.02$ ). Survival due to AFG therapy maximized at 72% and qPCR showed a significantly lower reduction (1–2 log<sub>10</sub>,  $p < 0.05$ ). The relationship between reduction in tissue burden at day 3 postinfection and 7 days survival were similar for VCZ-susceptible and VCZ-resistant isolates with highest dosage of VCZ ( $r^2 = 0.90$  vs.  $R^2 = 0.95$ ,  $p < 0.05$ ).

**Conclusion:** Our results indicate that real-time qPCR assay is a reliable and promising technique to detect *Aspergillus fumigatus* DNA. This assay could be used to measure the fungal burden in organs and thus monitor the efficacy of antifungals in animal model of IA at day 3 post challenge. Targeting molecular markers by real-time quantitative PCR can reduce the number of animal needed for dose–response and PK/PD studies of antifungals in experimental model of IA.

**P751** Detection of *Pneumocystis jirovecii* in adult patients with acute and chronic respiratory tract infections

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**Objectives:** *Pneumocystis jirovecii* is usually considered an opportunistic pathogen. However, recent studies show that *P. jirovecii* can also be detected in the nonimmunocompromised populations. This fungus can stimulate pulmonary inflammation, and has been implicated in the pathogenesis of chronic obstructive pulmonary disease. In this study, we aim to study the prevalence of *P. jirovecii* in patients with acute and chronic respiratory tract infections.

**Methods:** This study included adult patients aged 18 years or above. Group 1 consisted of patients with nasopharyngeal specimens sent to our laboratory for respiratory virus detection by direct immunofluorescence or viral culture between November 2010 and April 2011. Group 2 consisted of patients with laboratory-confirmed *Mycobacterium tuberculosis* infection by polymerase chain reaction (PCR) between January 2010 and June 2011. The decision for specimen collection was made by the clinician, and therefore group 1 and group 2 were representative of patients with acute and chronic respiratory tract infections, respectively. Nested PCR targeting the mitochondrial large subunit ribosomal RNA of *P. jirovecii* was performed in archived nasopharyngeal specimens for group 1, and in bronchial or sputum specimens for group 2. This study was approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster.

**Results:** A total of 181 patients were selected. Group 1 consisted of 131 patients, in which 37 had laboratory-confirmed 2009 pandemic H1N1 virus infection, and 94 were negative for any respiratory viral pathogens by direct immunofluorescence or viral culture. Group 2 consisted of 50 patients. *P. jirovecii* was detected in the nasopharyngeal specimens from five patients (3.8%) in group 1; and in the lower respiratory tract specimens from 8 (16%) patients in group 2 (Table 1). Nine of 13 (69.2%) of *P. jirovecii* positive patients did not have high risk immunosuppressive conditions (HIV, transplant, malignancy, connective tissue disease, immunosuppressants) nor chronic obstructive pulmonary disease.

Patient no.	Group	Sex / Age (years)	Underlying disease	Outcome at 30 days
1	1	F/27	Good past health	Survival
2	1	F/81	Hypertension, gastrointestinal stromal tumour, proteinuria	Survival
3	1	M/74	Right side pleural effusion during childhood of unknown cause	Survival
4	1	F/84	Carcinoma of the breast with curative mastectomy, cerebrovascular accident with left hemiparesis, osteoporosis, gastric ulcer	Survival
5	1	M/59	Hypertension, left basal ganglion infarct, neurogenic bladder	Survival
6	2	M/79	Partial gastrectomy for peptic ulcer	Survival
7	2	M/81	Atrial fibrillation, cerebrovascular accident, spondylolisthesis	Survival
8	2	M/48	Lung cancer	Death
9	2	M/79	Chronic obstructive pulmonary disease, diabetes mellitus, benign prostatic hyperplasia, lung cancer	Death
10	2	F/78	Alzheimer's disease	Survival
11	2	F/84	Hypertension, bilateral cataract, paranoid schizophrenia, genital prolapse	Death
12	2	F/19	Anorexia nervosa	Survival
13	2	M/77	Myocardial infarction, lung cancer	Survival

**Conclusions:** *P. jirovecii* can be detected in patients with both acute and chronic respiratory tract infection, even in

nonimmunocompromised patients or those without chronic pulmonary diseases. The clinical significance of *P. jirovecii* in these patients should be further investigated.

## Invasive bacterial infections: diagnosis and clinical presentation

**P752** Analysis of 554 cases of bacterial meningitis within nationwide survey in Slovakia, 1990–2010

J. Sokolova\*, V. Krčmery on behalf of Slovak Meningitis Study Group

**Objective:** Purpose of this study was to assess whether differences in aetiology and risk factors of meningitis acquired in hospital (HAM) or in community (CAM) have impact on outcome of infected patients.

**Methods:** Among 554 cases of bacterial meningitis within last 20 years from 10 major Slovak hospitals (Bratislava, Trnava, Kosice, Ruzomberok, Nitra, Banská Bystrica, Nove Zamky, Presov, Zilina, Lucenec), 261 (47.2%) were HAM and 293 (52.8%) were CAM. Aetiology, risk factors and outcome were compared in both groups. Differences were assessed by Chi-square test and Fisher's exact test computerized with the open source statistical package "R" and  $p$  value  $< 0.05$  was considered statistical significant.

**Results:** Patients with HAM had more frequently coagulase negative staphylococci (18.5% vs. 0%), Enterobacteriaceae (17.6% vs. 5.5%;  $p = 0.001$ ), *Acinetobacter baumannii* (10.7 vs. 3.1;  $p = 0.002$ ) and *Staphylococcus aureus* (9.2% vs. 4.4%;  $p = 0.03$ ) isolated from cerebrospinal fluid (CSF) and *Streptococcus pneumoniae* (6.5% vs. 35%;  $p < 0.001$ ), *Neisseria meningitidis* (3.8% vs. 33%;  $p < 0.001$ ) and *Haemophilus influenzae* (1.2% vs. 4.8%;  $p = 0.001$ ) were more commonly isolated from cerebrospinal fluid in CAM. Concerning risk factors, neurosurgery (82% vs. 1.7%;  $p < 0.001$ ), prior sepsis (24.1% vs. 14%;  $p = 0.002$ ), neonates (12.6% vs. 3.4%;  $p = 0.001$ ) and cancer (14.6% vs. 3.7%;  $p = 0.001$ ) were more common in HAM and alcohol abuse (1.9% vs. 14%;  $p < 0.001$ ) as well as otitis/sinusitis (3.5% vs. 20.1%;  $p < 0.001$ ) in CAM. Initial treatment success (88.2% vs. 87.4%,  $p = 0.79$ ) and cure after treatment modification (13.14% vs. 14.3%,  $p = 0.75$ ) was similarly frequently observed in both groups of meningitis. Overall mortality (11.8% vs. 12.6%,  $p = 0.88$ ) in both groups was similar too. However when analysis risk factors for mortality in the groups of HAM and CAM was done separately, different risk factors could be identified for mortality. In CAM alcohol abuse ( $p = 0.01$ ), diabetes mellitus ( $p = 0.02$ ), staphylococci ( $p = 0.02$ ) and *Pseudomonas aeruginosa* in aetiology ( $p = 0.01$ ) were at risk factor for death. In HAM any risk factor or specific aetiology was significant associated with death.

**Conclusion:** Despite of numerous differences in aetiology and risk factors between CAM and HAM within 20 years of Slovak nationwide survey, no differences in outcome, neither in mortality nor in neurological post-meningitis sequelae were observed.

**P753** Increase in 2011 in Denmark of invasive meningococcal disease due to a higher number of serogroup C

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**Objectives:** The aim was to present data on invasive meningococcal disease (IMD) with focus on observed changes in 2011.

**Methods:** Data were obtained from the clinical and laboratory notification systems, which both are mandatory. All isolates submitted by the Departments of Clinical Microbiology to the reference laboratory were characterized by serogroup, porA and feta type. Data presented for 2011 are based on 62 isolates and 9 PCR verified cases from 10/12 of the year (January through November) and the estimated incidences for 2011 were calculated from the number of these isolates and the population size for 2011.

**Results:** The incidence per 100 000 of laboratory confirmed IMD cases decreased from 1.61 (yearly average  $n = 86$ ) during the time period 2001–2005 to 1.18 (average  $n = 65$ ) in 2006–2010. However, in 2011 the estimated incidence increased to 1.53 per 100 000 (estimated  $n = 85$ ). The incidence decline from 2001–2005 to 2006–2010 was due to a decrease in the incidence of serogroup B IMD from 1.14 to 0.69 per 100 000 whereas the incidence of serogroup C IMD only changed from 0.36 to 0.41 per 100 000. In contrast, the estimated increase in the incidence of IMD in 2011 was due to an elevated number of sporadic cases of serogroup C. In 2011 the incidence of serogroup C IMD was 0.94 per 100 000 (estimated  $n = 52$ ) whereas it was 0.47 per 100 000 (estimated  $n = 26$ ) for serogroup B IMD. Among serogroup C isolates the prevalent finetype (porA: FetA) was 5,2: F3-3 representing 70% (21 of 30 typed serogroup C isolates) in 2011. This type was also prevalent in the previous 3 years.

**Conclusions:** In 2011 the estimated incidence of IMD in Denmark increased and for the first time became more prevalent than serogroup B IMD. This increase was due to an increase in serogroup C, porA: FetA type 5,2: F3-3.

#### **P754** *Mycoplasma hominis* meningitis – a case report and review of the current literature

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We present a case of a term neonate with *Mycoplasma hominis* meningitis following Group B Streptococcal sepsis. 16S rDNA PCR and sequencing played a critical role in confirming the diagnosis and directed appropriate antibiotic therapy. We determined minimal inhibitory concentrations for single and combinational antibiotics and reviewed the limited existing literature on *M. hominis* central nervous system (CNS) infection.

A 23-day-old term neonate was transferred to our tertiary healthcare facility with meningitis of unknown origin and subdural collections. Immediately after birth the infant had developed signs of respiratory distress necessitating admission to the neonatal unit. Blood cultures grew Group B streptococci. Initially intravenous (i.v.) cefotaxime was started. Following a sterile CSF culture and negative CSF group B streptococcal antigen assay, treatment was switched to i.v. amoxicillin. C-reactive protein declined over the next 2 weeks but rose again with intermittent pyrexias. A repeat CSF sample on day 23 of life was suggestive of bacterial meningitis (WCC: 1760/mm<sup>3</sup>; protein: 4.34 g/L; glucose <0.1 mM). A CT brain showed multiple bilateral extra-axial collections and evidence of non-communicating hydrocephalus. Due to clinical symptoms suggestive of raised intracranial pressure he was transferred to our hospital for extra ventricular drain placement.

At the time of transfer the CSF showed colonial growth on blood agar plates but the organism did not take up Gram or Ziehl–Neelsen stain. The sample was referred for 16S rDNA PCR and was positive for *M. hominis*. Following identification of the causative organism the antibiotic treatment was modified to intravenous ciprofloxacin, which was changed to intravenous moxifloxacin after 4 days. Within 2 days the infant became afebrile, and the inflammatory markers declined.

The published literature suggests that *M. hominis* CNS infection is an uncommon entity that mainly affects neonates. This case highlights that isolation of an organism from the CSF of a neonate that cannot be stained by routine methods should prompt colleagues to consider *M. hominis* and request molecular diagnostic tests that allow correct identification and appropriate choice of antibiotic therapy.

#### **P755** Meningitis caused by *Campylobacter jejuni*

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**Objective:** *Campylobacter jejuni* infection is one of the most common bacterial causes of acute gastroenteritis worldwide. Extraintestinal

infections are rare and most reports of meningitis due to *C. jejuni* species describe only a small number of cases. A case of meningitis caused by *C. jejuni* in a newborn is described in this report.

**Case report:** A newborn infant (15-days-old) presented to the hospital with a 5-hour history of fever (38.8°C) and no other symptoms. Initial investigation showed that total WBC count 10750/mm<sup>3</sup>, hemoglobin 13.5 g/dL and the other biochemical examinations were within normal limits. The cerebrospinal fluid (CSF) analysis revealed leukocyte count 1150/mm<sup>3</sup> (polymorphonuclear 85%, lymphocytes 15%), protein 95 mg/dL and glucose 31 mg/dL. CSF microscopy was negative for microorganisms, but conventional CSF culture yielded a curved Gram(–) organism. Also, blood and faecal specimens were cultured and they were negative. The organism, that was isolated from CSF culture, was identified as *C. jejuni* and was resistant to cephalothin and sensitive to ampicillin, erythromycin, cephalexin and ceftriaxone. The patient was treated with a combination of cephalexin 150 mg/kg and ampicillin 200 mg/kg daily intravenously. After the 12 day, the treatment with cephalexin was continued for other 9 days. In order to determine the source of infection faecal specimens from six family members were cultured and *C. jejuni* was isolated from the faecal specimen of the father and that supports the domestic transmission. Also, the brother of the neonate suffered from acute gastroenteritis some days ago. The patient's condition gradually improved and he remained afebrile and was discharged.

**Conclusion:** To our knowledge this is the first case of *C. jejuni* meningitis that was reported in Greece. This case shows that *C. jejuni* can cause meningitis in neonates and that it should be considered in the differential diagnosis of meningitis, since the clinical symptoms are not specific in the meningitis caused by *C. jejuni*, in neonates.

#### **P756** Case series of brain abscesses and empyemas: 11-year experience of a Portuguese infectious diseases department

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**Introduction:** Abscesses and empyemas in the Central Nervous System (CNS) are rare but potentially fatal. Early diagnosis is associated with better prognosis, favored by new imaging techniques, antibiotic regimens and neurosurgical approaches.

**Objectives and methods:** A retrospective study was performed aiming the characterization of clinical presentation, treatment and outcome of patients hospitalized at the Department of Infectious Diseases between January 2000 and December 2010, who were diagnosed with ‘brain empyemas and/or abscesses’.

**Results:** The authors identified sixty-eight patients, 76.4% male, with a mean age of 47 years [13, 80]. It was not possible to determine the prior duration of symptoms in 23.5% patients, while within 25% of total were symptomatic 4 days before admission in Emergency Room (ER), and 32.4% more than 2 weeks prior to it.

Upon admitted in the ER, 44.1% patients had made previous antibiotic therapy. 63.2% presented with headache, 57.3% with altered mental status, 51.5% with fever and 47% with neurological deficits. All patients underwent image studies: 33.8% had frontal lobe lesions, 19.1% temporal lesions and multifocal lesions in 5.9%. Fifty-four patients (79.4%) underwent at least for one neurosurgical drainage.

As predisposing conditions: a contiguous focus was identified in 54.4% (paranasal sinus infection 18 cases, otogenic infection 14 cases and dental infection five cases), while 19.1% of the patients underwent prior neurosurgery.

From the specimens collected ( $n = 62$ ), Gram negative bacilli rods were isolated in 6.5%, Streptococcus rods in 9.7%, Staphylococcus rods in 14.5% and anaerobes in similar percentage. No species were identified in 17.7% while culture was polymicrobial in 30.6% of the total.

The antibiogram was initiated empirically to all patients and kept for a period of 6 weeks in 25% and 20.6% in 8 weeks.

On average, patients remained hospitalized during a 53 days period. It was possible to detect sequelae in 14.7% of patients, while a mortality rate of 10.3% was registered.



**Conclusion:** Despite technical advances, poor specificity of signs and symptoms require a high degree of suspicion so that the diagnosis won't be delayed. Establishing an empirical antibiotic considering the predisposing factors and early surgical intervention where both crucial to the low morbidity and mortality registered.

**P757 Intracranial abscess: epidemiology and local antimicrobial guideline development**

*J.S. Cargill\*, K. Sethi (Leeds, UK)*

**Objectives:** Brain abscesses and subdural empyemas are associated with mortality rates of up to 24%. The aetiology has changed over time, with decreasing otogenic abscesses and an increase in those associated with immunosuppression. Therapeutic outcomes vary greatly in brain abscesses, according to different pathogens. We reviewed the laboratory processing of specimens from intracranial abscess, and report here the bacterial aetiology and the design of an empirical antimicrobial regime based on this local data.

**Methods:** Thirty-two patients (42 procedures; 18 excisions, 26 burr-hole drainage) procedures were identified by the neurosurgical department. All but one procedure had samples sent for microbiology. The laboratory records for the samples received were reviewed to obtain microscopy and culture results. The patients' clinical notes were not obtained and so correlation with potential sources of infection is not possible.

**Results:** A total of 42 pus samples, nine tissues and 10 swabs were identified. Forty-one pus samples (97.6%), nine tissues (100%) and five swabs (50.0%) received Gram stains, (90.2% of all samples). Gram stains were performed on at least one specimen sent from forty procedures (95.2%); the two procedures from which no Gram-stains were available each had one sample only sent to the laboratory. In the case of the swab specimens, some were not clearly labelled as operative samples and were processed as superficial swabs.

The most frequently identified bacterial pathogens are *Streptococcus* species and *Staphylococcus aureus*, with no bacterial cause identified in one third of cases. There were small numbers of other Gram-positive organisms, including *Nocardia*, and of Enterobacteriaceae. Mixed infections were not unusual.

**Conclusions:** Gram staining of intracranial tissue and pus samples should be routine; staining of pus swabs is not indicated when other specimens are available. Only one episode was identified where additional organisms were isolated from a swab compared to pus or tissue samples. Molecular identification may be useful in unusual cases and where conventional techniques fail to identify an organism; 16S ribosomal RNA gene sequencing was used in several local cases, either to identify an isolate, or to identify bacteria where no isolates were recovered but organisms were seen on Gram stain.

This local data was used to develop an empirical antimicrobial regime.

**P758 Co-infection with *Borrelia burgdorferi* and tick-borne encephalitis virus in humans, ticks and tick cells – analysis of clinical cases, literature and experimental possibilities**

*A. Moniuszko\*, P. Czupryna, L. Bell-Sakyi, J. Fazakerley, J. Zajkowska, S. Pancewicz (Bialystok, PL; Edinburgh, UK)*

**Objectives:** *Borrelia burgdorferi* sl (Bb) and tick-borne encephalitis virus (TBEV) are both transmitted in central and eastern Europe by the same tick, *Ixodes ricinus*. An individual tick can be infected simultaneously with both pathogens, and therefore a patient could be infected simultaneously with Bb and TBEV. This should be taken into account in the diagnosis of patients who suffer from meningoencephalitis and who live in or have visited tick-endemic regions, because the treatments of these two infectious diseases differ, and co-infection may intensify the clinical course of both diseases. We aimed to determine the incidence of simultaneous infection in patients, influence on clinical picture, and to explore the use of tick cells

coinfected in vitro with Bb and an arbovirus to test whether the pathogens act synergistically or antagonistically in the vector.

**Methods:** We examined our clinical records concerning influence of co-infection with Bb and TBEV on patients hospitalized in our department. Additionally we analyzed the literature concerning prevalence of multiple pathogen species in *I. ricinus* ticks in Poland. Moreover, we used a model system to study co-infection with Bb and the arbovirus Semliki Forest virus (SFV) in *Ixodes* spp. cell lines.

**Results:** Between 1993 and 2008 in eastern Poland we observed that, out of 687 patients with TBE, 116 (16%) were coinfecting with Bb. Among them 13 (2%) were diagnosed with neuroborreliosis. The clinical picture and results of cerebrospinal fluid examination differed between TBE patients with and without coinfection. Coinfection of *I. ricinus* ticks with different pathogens is quite common. In Poland, the prevalence of *I. ricinus* ticks co-infected with at least two pathogens varies from 0.1% to 8.3%. In preliminary experiments we found that the presence of Bb spirochaetes in tick cell lines tended to enhance short-term replication in vitro of SFV RNA, while the presence of the virus had no effect on spirochaete DNA replication.

**Conclusion:** We propose that not only clinical observation, but also experimental research may be useful to discover more about coinfection with Bb and TBE virus in humans and ticks. Very little is known about the influence of multiple infections on either the tick life cycle or the coinfecting pathogens reactions. Tick cell lines offer the possibility to study in vitro the effect of coinfection with multiple pathogens on ticks at the cellular and molecular level.

**P759 Osteoarticular infections in children: an Italian cohort**

*C. Bertaina\*, S. Aguzzi, M. Pinon, M. Cuzzo, F. Mignone, S. Virano, S. Garazzino, C. Gabiano, P.-A. Tovo (Turin, IT)*

**Objectives:** Prompt diagnosis and proper treatment of osteoarticular infections (OAIs) are essential to prevent short- and long-term complications in children, including growth impairment. We performed a retrospective analysis of children with OAIs to define their epidemiology, aetiology, clinical manifestations, treatment and outcome.

**Methods:** Clinical records of children discharged with a diagnosis of OAI from Regina Margherita Children's Hospital (Turin, Italy) between January 2006 and January 2010 were retrospectively analyzed. T-student test was used for statistical significance assessment.

**Results:** Fifty-six children (mean age 6.4 years; 36 male, 20 female) were evaluated. 71% was diagnosed with osteomyelitis (OM), 22% with septic arthritis (SA) and 7% with spondylodiscitis (SD). Mean age at presentation was significantly lower in children with SA vs those with OM (3.3 years vs 7.7;  $p < 0.05$ ). The most frequent trigger event of OAI was a recent traumatic event in OMs (33%) and a concomitant focus of infection in SAs (42%). The most common symptoms at presentation were pain (96.4%) reduced motility (87.5%) and fever (64.2%). OMs and SAs involved lower limbs in 75% of cases. All SDs involved lumbar column. Nuclear Magnetic Resonance was the most reliable diagnostic technique for OMs (100% sensibility), joint ultrasound for SAs (90% sensibility). In 42% of OMs and 67% of SAs the causative pathogen was not detected. Methicillin-sensible *Staphylococcus aureus* was the most frequently isolated pathogen (22.5%). *Mycobacterium tuberculosis* was detected in one case of SD. Most of the patients was treated with an association of antibiotics, initially administered intravenously and then switched to an oral formulation. Mean duration of antimicrobial treatment was 1.8 months (range 0.5–6.7 months); in particular 2.2 months in OMs, 1.2 months in SAs and 2.7 months in SDs. Mean follow-up after treatment completion was 16 months. Recovery was achieved in 83.4% of patients. Infection became chronic in two children. No growth defect was recorded.

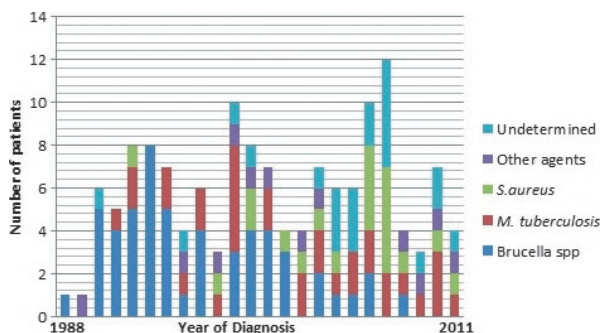
**Conclusions:** In our study we found a high rate of therapeutic success in children with OAIs. This finding suggests that a proper and

prolonged course of antimicrobials (at least 14 days) is essential to prevent recurrences and complications. OAI aetiology may be difficult to identify, but *S. aureus* is often the causative pathogen: therefore, empiric treatment with *S. aureus* coverage should always be considered.

**P760 Spondylodiscitis: case review, 1988–2011**

A. Lebre\*, J. Velez, E. Rabadão, J. Oliveira, J. Saraiva da Cunha, A. Meliço Silvestre (Coimbra, Aveiro, PT)

The authors conducted an analysis of Spondylodiscitis (SD) cases at their institution from January 1988 to September 2011. The aim was to evaluate epidemiological, etiological, clinical, imaging and therapeutic aspects of this pathology. The inclusion criteria were SD evidenced by imaging study. Clinical records of 140 patients were reviewed. Sixty three (63%) were male; average age was 57 years [17–83]; mean cases/year ratio was 5.8; mean hospital stay was 46 days [1–190]. The most frequent symptoms at admission were pain (96%), fever (46%) and neurological symptoms (26%). Average duration of symptoms was 4.5 months [3 days–48 months]. MRI was the imaging technique more often performed. It was done in 116 of the patients, showing abnormalities compatible with SD in 115. The lumbar segment was predominantly involved (55%). In 61% of cases a certainty diagnosis was achieved (*Brucella* spp 47 cases; *Staphylococcus aureus* 19 cases; other bacteria 11 cases; *Mycobacterium tuberculosis* seven cases; *Candida albicans* one case). Certainty diagnosis criteria were: *Brucella* spp. (Wright  $\geq 160$  or blood culture yielding *Brucella* spp.); bacteria (blood culture or abscess needle puncture/vertebral biopsy specimen yielding the agent); *M. tuberculosis* (abscess needle puncture/vertebral biopsy specimen yielding the agent); *C. albicans* (abscess needle puncture specimen yielding the agent). Etiology was presumed in 26%, and considered undetermined in 13% of the patients. The most prevalent etiological agent -certainty or empirical diagnosis- was *Brucella* spp. (39%)



mostly with a serological diagnosis, followed by *M. tuberculosis* (24%) with diagnosis made mainly by abscess needle puncture, *S. aureus* and other bacteria (23%) with diagnosis made mainly by positive blood cultures and *C. albicans* (1%) diagnosed by abscess needle puncture. The etiological agent changed over the years (Grap.1) as well as treatments. The outcome was favourable in 82% of the patients, 5% died and 10% were lost to follow-up. In 3% there was no clinical improvement. SD is a typically complex and time-consuming pathology to diagnose. Although it is associated with low mortality, a prompt diagnosis and adequate therapy are important determinants that might improve the long term prognosis. In Portugal, like in other Mediterranean countries, *Brucella* spp. and *M. tuberculosis* were frequently implicated. In the last years a sharp decline in *Brucella* etiology was observed probably due to better control of the animal endemy.

**P761 Diagnostic clues for Brucella spondylodiscitis**

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**Aim:** Brucellosis, which was once described as the great imitator, is an endemic zoonosis in Turkey and causes complications affecting multiple systems. *Brucella spondylodiscitis* may cause diagnostic obstacles for the physician. Symptoms are subtle and nonspecific at the beginning. This study aims to determine predictive factors for brucellar spondylodiscitis.

**Methodology:** This case-control study included 227 consecutive brucellosis patients who applied to Adiyaman State Hospital and Adiyaman 82nd Year State Hospital Infectious Diseases Clinics between January 2010 and July 2011. Demographic figures and clinical findings of patients were recorded in follow up sheets. Spondylodiscitis was diagnosed radiologically by Contrast Enhanced Magnetic resonance imaging. Data were entered to SPSS 16.0 package program and statistical analyses were made using T test, Mann-Whitney U test and chi-square test. p values < 0.05 were accepted to be statistically significant. Logistic regression analyses was done in order to find diagnostic risk factors.

**Results:** Among 227 brucellosis patients included in this study, 88 (38.7%) were males, 139 were females, and mean age was 43.1 ± 15.2. Ninety one patients were housewives, 81 were breeding stock and 40 were public servants. Route of transmission could be determined in 98.7% of patients. The most frequent three symptoms were arthralgia, malaise, and anorexia. Hepatomegaly was detected in 56, splenomegaly in 32 and lymphadenopathy in three patients. Spondylodiscitis, sacroileitis and neurobrucellosis were diagnosed in 54 (23.7%), 21 (9.2%) and 1 (0.45%) patients, respectively. Laboratory tests revealed anaemia, thrombocytopenia and leucopenia in 27.3%, 14.1% and 10.6% of the patients, respectively. C reactive protein (CRP) was elevated in 167 and erythrocyte sedimentation rate (ESR) was increased in 136 patients. Blood cultures yielded *Brucella* spp. more frequently in patients with spondylodiscitis (p = 0.021). ALT, AST, ESR, and CRP were found to be higher (p = 0.005, p = 0.001, p = 0.047, p = 0.033, respectively) and leukocyte number was significantly lower (p = 0.029) in patients with spondylodiscitis. Mean STA titre was also higher in patients with spondylodiscitis (p = 0.005). Results of logistic regression analyses was shown on table 1.

	B	p	Odds Ratio
Sweating	1.3	0.008	3.69
AST level (> 40 iu/ml)	1.4	0.003	4.41
Leucopenia (<2000/mm <sup>3</sup> )	1.4	0.009	4.12

In conclusion, treating physicians should consider spondylodiscitis when treating leucopenic brucellosis patients with high ALT, AST, ESR and CRP values.

**P762 Clinical presentation, complications, and treatment outcome of spondylodiscitis caused by brucellosis**

A. Ulu-Kilic\*, M. Sayar, E. Tutuncu, F. Sezen, I. Sencan (Kayseri, Ankara, TR)

**Objective:** Spondylodiscitis is a frequent and important complication of brucellosis. Attention is drawn to this disease given the need for prolonged duration of treatment in order to avoid possible sequelae.

**Methods:** Twenty patients with brucellar spondylodiscitis were retrospectively evaluated in terms of their clinical, laboratory, and radiologic features and their response to antibiotic regimens.

**Results:** Of the 96 patients with brucellosis, 20 (20.8%) were diagnosed with spondylodiscitis. Patients who had brucellar spondylodiscitis aged 15–81 years (average 54 + 14.6 years). The demographic and clinical characteristics of these patients were compared with those of all patients with brucellosis who did not have spondylodiscitis (Table 1). Patients with spondylodiscitis were more likely to be older ( $p = 0.001$ ), have erythrocyte sedimentation rates (ESRs) higher ( $p = 0.01$ ) and were more likely to have anemia ( $p = 0.017$ ) than those patients with brucellosis who did not have spondylodiscitis. Back pain (95%) and debility (65%) were most common symptoms in patients with spondylodiscitis ( $p < 0.05$ ). *Brucella melitensis* biovar III was isolated from blood cultures of six (30%) patients. A computed tomography-guided needle aspiration of the paravertebral collection of two patients yielded purulent material that also grew *B. melitensis* biovar III. Magnetic resonance imaging (MRI) showed that the lumbar segment (19/20) was the most frequently involved region. Sacral (8/20), thoracic (3/20) and cervical (1/20) involvement of vertebral column were seen less common. Spondylodiscitis was accompanied by paravertebral or epidural abscess in seven, psuedo abscess in five and radiculitis in six of cases. Antibiotic regimens including two or three antibiotics with combination of doxycycline, rifampin and streptomycin were used. In this series, the mean duration of antimicrobial therapy was 22.6 weeks (range, 12–56 weeks) varied according to clinical response and the presence of epidural or paravertebral masses and radiculitis. Surgical intervention was required for four patients. At the end of treatment all patients had a complete response.

**Conclusions:** Brucellar spondylodiscitis should be considered particularly in elderly patients with back pain and debility in endemic areas. The patients can be treated effectively with appropriate antibiotic combinations at least 12 weeks. Prolonged treatment is suggested for patients with spondylodiscitis accompanied by abscess or radiculitis.

#### **P763** The prevalence of brucellosis in adults in northeastern region of Turkey

G. Arvas, Y. Akkoyunlu, M. Berktaş, B. Kaya, T. Aslan\* (Iğdir, Istanbul, Van, TR)

**Objectives:** Brucellae are primarily animal pathogens, infecting humans after contact with infected animals or their body products. Brucellosis exists in animals worldwide. Human brucellosis presents when the bacteria enter the body via abrasions in the skin, via the alimentary tract or most commonly, via the respiratory tract. The disease is prevalent in the Mediterranean basin, the Indian subcontinent, the Arabian peninsula, and in parts of Central Asia, Africa, Central and South America. However it continues to be one of the major health problems in developing countries, as well as in Turkey. The treatment of the disease leads to loss of time, labour, and results economical burden. The purpose of this study is to determine the prevalence of brucellosis which is previewed to be very common in the northeastern region of Turkey and make an emphasis on the problem.

**Material and method:** In this study, seroprevalence of brucellosis has been examined in sera of a total of 2913 adult patients (18 years of age and older). Of those, 889 were men and 2024 were women who applied to Iğdir State Hospital between February and December in year 2010. The existence of brucellosis antibody has been investigated in sera samples taken from patients using the method of Standard Tube Agglutination Test (STA, Wright's Agglutination Test). The attained results have been evaluated statistically using chi-square trend analysis method.

**Results:** At the end of the survey, significantly high level (1/40 dilution) of specific antibodies have been detected in 380 (18.8%) women and 145 (16.3%) men with a total of 525 (18%) patient sera ( $p = 0.111$ ). Positive test results have been found in 2 (0.4%) with 1/40 dilution, in 3 (0.6%) with 1/80 dilution, in 216 (41.1%) with 1/160 dilution, in 303 (57.7%) with 1/320 dilution and in 1 (0.2%) with 1/640 dilution ( $p = 0.258$ ). There was no significant difference between the positivity distribution in men and women.

**Discussion and conclusion:** According to the test results, brucellosis prevalence in the northeastern region of Turkey is one of the major public health problems. Existing region of country has borders with three different countries. Therefore, we should fight against the disease with measures like controlling animal transportations, improving public awareness upon processing and consuming milk and milk products, paying attention to co-operation between sectors.

#### **P764** Serological follow-up after laboratory exposures to *Brucella* in the UK and Ireland

S. Murray\*, R. Cooke, N. Beeching, P. Lal (Liverpool, UK)

**Objectives:** Brucellosis is a zoonosis transmitted to humans through contact with infected animals or ingestion of infected unpasteurised cheese. However brucellosis has also been reported in staff after laboratory exposure incidents. Following such an incident, current UK guidance suggests serological testing on all staff encountering a "high risk" exposure, at 0, 6 and 24 weeks. These individuals should also receive post exposure antibiotic prophylaxis. Though current guidance lacks an evidence base, the incubation period of brucellosis is known to range from a few days to 6 months. Serological testing in the UK is currently provided at the Brucella Reference Unit (BRU) in Liverpool. The aim of this study was to review the results of serological samples sent to the BRU for testing following laboratory exposures and assess the appropriateness of current guidance.

**Methods:** A retrospective review of all the serological samples sent for testing following laboratory exposure incidents over a 38 month period. Results were stored under ILog numbers (each representing a single exposure incident). Samples from 01/11/2007 until the most recent closed ILog (21/01/2011) were reviewed from the microbiology computer database. Standard interpretation criteria were used to assess serological results.

**Results:** Seven ILog numbers were identified representing exposures in Dublin, Derby, Leicester, Birmingham and 3 London laboratories. The total number of serum samples reviewed was 296. The number of laboratory staff included under each ILog ranged from 0 to 106, and the number of specimens received under each ILog varied from 0 to 212. All the specimens were found to be negative, showing no evidence of brucellosis. The majority of sera were collected at 0 and 6 weeks post exposure.

**Conclusions:** Over the period studied, no exposed laboratory staff showed serological evidence of brucellosis. In addition, figures suggest that there was poor compliance with the recommended number of follow up tests. Based on these findings, current guidance needs to be revised and should recommend that baseline blood is taken for storage, and only follow up serum taken if symptoms consistent with brucellosis occur. This would bring the laboratory exposure guidance in line with existing UK guidance on exposure following a deliberate release of brucella. This approach should also reduce staff anxieties and laboratory costs.

#### **P765** Antimicrobial susceptibility of *Brucella* spp. isolates from blood samples

S. Aljohani\* (Riyadh, SA)

**Aim:** Brucellosis is a worldwide zoonotic disease that remains an important public health problem in rural Saudi Arabia. The aim of the present study was to assess the antimicrobial susceptibility of isolates from blood samples.

**Materials and methods:** The study included 401 *Brucella* isolates grew in culture at microbiology laboratory between 2003 to 2008 at King AbdulAziz Medical City, a 1000 beds tertiary care center. The minimal inhibitory concentration (MIC) values of tetracycline, rifampin, streptomycin, ciprofloxacin, and tigecycline were determined using the E test method.

Testing was under BSC at our level III laboratory.

**Results:** Isolates showing different susceptibility patterns against different antibiotics, tetracycline and rifampin remain the most potent agents against *Brucella* isolates, but their susceptibility patterns start to show some resistance in the last few years. (Table 1) MIC90 values of tetracycline, streptomycin, rifampin, ciprofloxacin, and tigecycline were 0.25, 0.50, 1.0, 0.25, and 1.0 mg/L, respectively.

National Guard Health (Spain)  
King Abdul Aziz Medical City  
Section Of Microbiology

Brucella Antibigram Year 2003 to 2008 - All Departments (Department & outpatient)

Total Number Of Isolates Relative To The Percentage Of Sensitive Strains

YEAR	TOTAL # OF ISOLATES	% SUSCEPTIBLE ISOLATES					
		Ciprofloxacin	Rifampin	Streptomycin	Tetracycline	Trimeth/Sulfa	* Tigecycline
2003	44	100	100	--	100	59	--
2004	79	98	100	100	100	64	--
2005	66	100	100	100	100	59	--
2006	79	97	100	100	100	79	--
2007	74	98	98	93	100	95	--
2008	59	88	81	84	86	74	57

**Conclusions:** In recent years there has been tremendous interest in the identification of *Brucella* strains and their antimicrobial susceptibility. According to antimicrobial susceptibility test results, we have few of the isolates were resistant to the currently recommended antibiotics. This data need to studied further in collaboration with other centers as our number of isolates worth further studies.

#### P766 A strange case of co-infection from *Leishmania infantum* and *Brucella melitensis*

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**Objectives:** Visceral leishmaniasis is the most severe form of leishmaniasis, constituting the second largest parasitic killer in the world. On the other hand, although great efforts have been made in order to extinct brucellosis from the cattle population, it still stands as a problem in public health. We are going here to present a case of illness from visceral leishmaniasis and brucellosis.

**Methods:** A 37 year old male of Romanian descent, immigrant, working in livestock occupations, presented in our emergency department, febrile (38.9oC) and reporting pain in his left side abdomen along with fatigue and anorexia for the last week. During the clinical examination, the patient was hemodynamically stable and liver and spleen were palpable (15 cm under abdominal ribs). The laboratory findings were indicative of pancytopenia (white blood cells 2240/mm<sup>3</sup>, hematocrit 20.6%, platelets 71 000/mm<sup>3</sup>) but showed no significant change in blood biochemistry except for a slight increase in alkaline phosphatase, gamma-glutamyltransferase and reverse albumin/globulin ratio. An ultrasound check of the abdomen confirmed the clinical findings. Blood and urine cultures were taken along with a full serologic and virus check.

**Results:** Serologic and virus control was negative for HIV, hepatitis A, B, C, cytomegalovirus, toxoplasmosis. The clinical and laboratory findings raised the suspicion of leishmaniasis. The patient started receiving treatment with liposomal amphotericin B (Ambisome®) along with supportive treatment. After 24 hours he became afebrile and his clinical condition started improving. On the same time rK39 *Leishmania infantum* dipstick test proved positive and so was the indirect fluorescent antibody test (1:1600). *Brucella melitensis* was cultivated in blood culture and treatment with ciprofloxacin, rifampicin and doxycycline was commenced. After one week the patient the patient was dismissed from the hospital in a much improved condition, and continued receiving treatment for brucellosis for a total duration of

six weeks and amphotericin B for leishmaniasis on day 14 and day 21 after his admission.

**Conclusion:** The recent movement of population and living in poor sanitary conditions should always raise the possibility of a zoonosis infection. Therefore diseases considered extinct from Europe should always been taken into account in differential diagnosis.

#### P767 Invasive pneumococcal disease in adults older than 59 years in the autonomous region of Madrid Spain, 2008–2010

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**Objectives:** The pneumococcal capsular polysaccharide vaccine (PCPV) is recommended in the Region of Madrid in specific risk groups and in adults over 59 years. Furthermore, pneumococcal conjugate vaccine (PCV) is included into the routine childhood schedule, changing heptavalent (PCV7) to 13-valent (PCV13) in 2010. The objectives of this study are to describe the incidence and the epidemiological characteristics of invasive pneumococcal disease (IPD) in adults older than 59 in the Region of Madrid in the period 2008–2010.

**Methods:** IPD is a notifiable disease in the Region of Madrid. The case definition of the disease includes the identification of pneumococcus in a normally sterile site. Pneumococcal strains were serotyped by latex agglutination assay and Quellung reaction. Clinical and epidemiological data are collected through a structured questionnaire for every case. Incidence and mortality annual rates per 100 000 inhabitants were calculated. We compared the rates in 2010 to 2008, and also the vaccinated and non-vaccinated patients by risk ratio (RR).

**Results:** IPD incidence rate was 19.99/100 000 in 2008 and 15.45/100 000 in 2010 (RR = 0.77 p = 0.007) and mortality was 3.48/100 000 in 2008 and 2.04/100 000 in 2010 (RR = 0.59 p = 0.029). Of the 662 cases registered in this period 33.8% were older than 79, and 55.9% were males. Pneumonia (64.3%), bacteraemia (14.6%) and septicaemia (9.3%) were the most frequent diagnoses. The case-fatality rate for this period was 15.2%. Underlying risk factor for pneumococcal was observed in 56.2% of the patients, being the most frequent chronic respiratory disease (16.1%). Among all patients 38.3% were vaccinated with PCPV, this proportion being 41.4 among those with risk factor. Serotype was identified in 91.6% of the cases. The six most frequent serotypes were 3, 19A, 7F, 1, 14 and 8 that account approximately 50% of cases, with those included in PCV7 causing 12.6%, those included in PCV13 55.4% and those included in PCPV 76.6% of cases. Vaccinated patients had lower risk of PCPV serotypes disease (RR = 0.63 p = 0.020).

**Conclusions:** The incidence and mortality decreased in this period. The low proportion of patients with risk factor that were vaccinated makes necessary to improve de vaccine coverage in this group. The lower risk of disease by PCPV serotypes in vaccinated patients is compatible with the vaccine efficacy described.

#### P768 Invasive pneumococcal disease in the autonomous region of Madrid, Spain: from 2008 to 2010

M.E. Rosales Statkus\*, M.A. Gutiérrez Rodríguez, M. Martínez Blanco, M.D. Lasheras Carbajo, F. Martín Martínez, A. Arce Arnáez, B. Ramos Blázquez, M. Ordoñas Gavín (Madrid, ES)

**Objectives:** Invasive pneumococcal disease (IPD) was declared a notifiable disease in the Autonomous Region of Madrid in 2007, after the inclusion of the pneumococcal conjugate vaccine (PCV) into the child immunization schedule, first as the heptavalent vaccine (PCV7) in 2006, replaced by the 13-valent vaccine (PCV13) in 2010. Pneumococcal polysaccharide vaccine (PCPV) has been recommended in Spain since 2001 for persons 2 years and older with certain underlying medical conditions. In the Region of Madrid this

vaccine is also recommended in adults older than 59 since 2005. The objectives of this study are to describe the incidence and the characteristics of IPD cases in the period 2008–2010.

**Methods:** We selected the cases from 2008 to 2010 registered in the notifiable disease system. Case definition includes the identification of the pneumococcus in a normally sterile site. Data cases are collected through a structured questionnaire. Pneumococcal strains were serotyped by latex agglutination assay and Quellung reaction. We estimated annual incidence rates per 100 000 inhabitants according to age, sex and serotypes included in the pneumococcal vaccines. We compared incidence in 2010 to 2008 by risk ratio (RR).

Age group (years)	2008 N=764		2009 N=491		2010 N=827		RR 2010/2008
	%	Rate	%	Rate	%	Rate	
< 5	21.1	47.42	19.4	36.02	21.0	26.81	0.57*
5-14	6.4	6.44	7.4	8.51	4.3	3.24	0.30*
15-39	13.1	4.11	15.5	4.39	10.5	2.04	0.50*
40-59	27.9	12.46	25.8	10.16	22.1	5.74	0.46*
60-74	16.4	16.31	15.1	14.13	17.8	10.34	0.63*
> 74	15.2	26.39	16.9	26.60	24.4	24.12	0.91
Total	100.0	12.2	100.0	10.8	100.0	7.2	0.59*
<b>Sex</b>							
Male	57.9	14.54	59.3	13.25	58.7	8.77	0.60*
Female	42.1	9.97	40.7	8.54	41.3	5.79	0.59*
<b>Serotypes identified</b>	94.4		92.8		93.4		
Included in PCPV	72.1	8.79	77.7	8.41	74.1	5.36	0.61*
Included in PCV13	55.6	6.79	53.9	6.42	57.0	4.12	0.61*
Included in PCV7	10.3	1.26	9.1	0.99	6.6	0.40	0.30*
<b>Case-fatality rate</b>	10.3		8.7		8.4		
<b>Underlying medical conditions</b>	32.2		39.4		49.3		
<b>Vaccinated patients</b>	29.7		33.7		43.5		
PCPV	11.1		15.3		23.6		
PCV	18.5		18.5		19.9		
<b>Clinical presentation</b>							
Pneumonia/pneumonia	59.9		64.8		64.7		
Bacteremia	16.6		12.4		13.1		
Sepsis	6.4		6.9		7.7		
Meningitis	9.2		8.0		7.9		
Other	3.8		4.5		5.6		
Unknown	4.1		3.3		1.1		

\*p<0.05

**Results:** (results are shown in table submitted).

**Conclusion:** Incidence rates are higher in extreme age groups and in males. Incidence in all age group and case-fatality rates decreased along the study period. Incidence for serotypes included in vaccines also decreased. This is compatible with the efficacy of pneumococcal vaccines. Proportion of patients older than 59, cases with underlying medical conditions and vaccinated with PCVP increased, which is compatible with the limited effect of PCVP.

#### **P769** Kinetics of old and novel biomarkers in severe bacterial infections

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**Objectives:** Accurate diagnosis and management of severe bacterial infections are still important issues. Therefore, we analyzed the kinetics of novel and routinely used biomarkers in severe bacterial infections and compared the usefulness of these parameters in monitoring of the empirical antibiotic therapy efficacy.

**Methods:** This prospective observational study, which included 54 patients hospitalized with community-acquired bacterial infection was conducted in an infectious disease department of a teaching hospital. As controls, 27 patients with viral infections and 19 healthy persons were enrolled. Paired samples of whole peripheral blood and serum were obtained upon the admission to the study – day 1 (D1), at D4 and D8. Next parameters were evaluated: neutrophil and lymphocyte counts, neutrophil to lymphocyte count ratio (NLCR), C-reactive protein (CRP), procalcitonin, cortisol, interleukin (IL)-1beta, IL-6, IL-8, IL-10, IL-12 and TNF-alpha. Statistical analysis was performed using SPSS software™ software.

**Results:** The highest sensitivity and specificity for the diagnosis of bacterial infection demonstrated increased values of PCT, NLCR, cortisol and IL-6. Appropriate empirical antibiotic therapy was

associated with a more rapid normalization of IL-6, IL-8, lymphocyte count and NLCR compared to neutrophil count, C-reactive protein, procalcitonin and cortisol at D8. At D8, significant differences between patients with bacterial infections and controls were still observed for CRP (p < 0.001), PCT (p = 0.001), neutrophil count (p = 0.007) and cortisol (p = 0.007).

**Conclusion:** Our results demonstrate that successful antibiotic therapy is associated with the earlier normalization of lymphocyte count, NLCR, IL-6 and IL-8 compared to other parameters during severe bacterial infections. Further studies are needed to assess if the use of these biomarkers with the faster kinetics can improve to rationalize antibiotic therapy.

Acknowledgment: The study was supported by the grant IGA NT/11390-5.

#### **P770** Bacterial agents in the aetiology of today's diabetic foot infections: shift to more Gram-negative pathogens

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**Background:** Identification of organisms and effective, early and appropriate treatment is important to avoid complications of diabetic foot infections. This study was undertaken to determine the organisms associated with diabetic foot infection (DFI) and their antibiotic sensitivity pattern.

**Methods:** A total of 107 patients having diabetes mellitus with Wagner's grade 1–5 foot ulcers attending our tertiary hospital between the period of May, 2005 and July, 2010 included in the retrospective designed study. Specimens were processed for aerobic culture. The bacteriological isolation and antimicrobial sensitivity tests of the isolates (Quantitative culture) were done by standard microbiological methods according to the CLSI criteria. Gram negative bacilli were tested for inducible betalactamase positivity (IBL) and extended spectrum beta lactamase (ESBL) production. *S. aureus* and coagulase negative staphylococci were screened for methicillin resistance.

**Results:** Of the 107 patients, 74 (69%) were male and 33 (31%) female, mean age was (62 ± 13). The mean HbA1c level was 9 ± 2.5. Culture taken from 298 samples; 165 (55%) wound, 108 (36%) tissue and 25 (9%) bone yielded 311 bacteria. There was no growth in the 31 (10%) samples. A total of 311 bacteria were isolated from 267 samples and an average of 1.16 isolates per culture was reported. Of the positive culture, (223/267) 83.5% had monomicrobial, (44/267) 16.4% had multiple organisms. Gram negative bacteria (n = 192; 62%) were isolated more often than gram-positive ones (n = 119; 38%) in our retrospective study. Most frequently isolated microorganisms were *Pseudomonas* spp. (30.5%), *Staphylococcus aureus* (17%), *Enterococcus* spp. (11.3%), *E. coli* (7.1%) and *Enterobacter* spp (7.1%). IBL and ESBL positivity detected in 25% (48/192) of the gram negative bacteria. Methicillin resistance was detected in 45% (24/53) of *S. aureus* isolates.

**Conclusions:** This study has shown that infection with multidrug resistant gram negative bacilli is the most common cause of DFI in our tertiary care hospital and this is a new phenomenon which is widely occurring in the nowadays world. Amikacin, piperacillin-tazobactam, cephaperazon-sulbactam and imipenem were active against gram-negative bacilli, while vancomycin was found to be active (100%) against gram-positive bacteria including enterococci. Fusidic acid was found to be active against MRSA. The choice of empirical antimicrobial therapy can be done according to this data.

#### **P771** Leptospirosis associated with Hashimoto thyroiditis: a case report

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**Objective:** Leptospirosis is a worldwide zoonosis. Mild anicteric form is observed in 90% of the patients with leptospirosis, however Weil's disease occurs in 5–10% of the patients. The disease has been rarely

shown to be related with autoimmune disorders. In this report a patient with leptospirosis who developed acute renal failure, thrombocytopenia and Hashimoto thyroiditis was presented.

**Case:** A 38-year old previously healthy male patient with fever, fatigue, nausea, vomiting, abdominal pain and diarrhoea with 10 days of history, referred to our clinic due to rapid deterioration and increase in serum creatinine. The patient's history revealed that he had interested in hunting. He had been to a lake for hunting 20 days before symptoms have started. Intravenous metronidazole treatment was administered for 2 days in another hospital. On physical examination the patient was conscious, cooperate, and temperature was 38.7°C, blood pressure was 90/50 mmHg, and pulse was 90/minute. Liver was palpated 2 cm below the ribs. Laboratory findings were as; platelets:118.000/mm<sup>3</sup>, CRP:465 mg/L, procalcitonin:26.5 ng/mL, creatinine:5.3 mg/dL, AST:99 U/L, ALT:59 U/L and microscopic hematuria. After blood cultures were taken empiric intravenous meropenem 2 × 1 g was started with unknown aetiology of sepsis. All culture samples were negative. Brucella standard tube agglutination and Gruber–Widal tests were negative. Abdominal ultrasonography revealed mild hepatomegaly and grade 1–2 increase in both renal parenchymas. Hepatitis serology were negative. Serum sample was positive for *Leptospira Bratislava Jez Bratislava* antibody at 1/100 titer by MAT. Since Anti-Tg (236.2 U/mL) and Anti-TPO (317.8 U/mL) antibodies were high, a thyroid ultrasonography was performed and Hashimoto thyroiditis was diagnosed. Serum creatinine levels increased in the 2nd day of admission and hemodialysis was performed. Fever decreased on the third day of treatment, serum creatinine began to decline on the 4th day of admission and normalized on 7th day as thrombocyte count and liver function tests. Serum sample was positive for *Leptospira Bratislava Jez Bratislava* antibody at 1/800 titer in the second week of hospitalization. The patient was discharged healthy on 9th day of admission.

**Conclusion:** Leptospirosis diagnosis was confirmed in the second week of admission as patient history was consistent with the disease. Although seem rarely, autoimmune disorders may be point at issue in leptospirosis.

#### P772 Haemolytic uraemic syndrome associated with *Streptococcus dysgalactiae* ssp. *equisimilis*

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**Objectives:** The haemolytic uraemic syndrome (HUS) includes haemolytic anaemia, thrombocytopenia, and acute renal failure. HUS can be distinguished in typical HUS and atypical HUS (aHUS). Enterohaemorrhagic *Escherichia coli* and *Shigella dysenteriae* are the cause of typical HUS. Atypical HUS defines non-Shiga-toxin HUS and designated a primary disease due to a disorder in complement alternative pathway regulation. Although extremely rare, infections due to *Streptococcus pyogenes* (GAS) have been associated with HUS. *Streptococcus dysgalactiae* subsp. *equisimilis* (SDSE) causes invasive streptococcal infections. Similarly to GAS, SDSE possesses virulence factors. We report the first case of bloody diarrhoea and HUS due to SDSE.

**Methods:** A 3-year-old girl was transferred to University Hospital Puerta del Mar with a history of bloody diarrhoea. On admission, BUN and serum creatinine were 167 and 3.1 mg/dL, respectively, haemoglobin was 11.3 g/dL and platelet count was 100 000/μL. She was transferred to the intensive care unit. Blood smear showed polychromasia with the presence of schistocytes. By the fourth hospital day, C3 levels were low (79 mg/dL). Her renal function continued to deteriorated and peritoneal dialysis was started. Blood and stools samples were sent on admission and 1 day after for culture and detection of Shiga toxin by PCR (GenoType EHEC, Hain LifeScience).

**Results:** tools cultures showed pure growth of SDSE. Identification was made in accordance to phenotypical, serological and biochemical characteristics. No *Salmonella*, *Shigella*, *Campylobacter*, *Vibrio*, *Aeromonas* or *Yersinia* were isolated. The presence of Shiga toxins genes, intimin gene *eae*, and virulence factor *ipah* gene, to detect enterohaemorrhagic, enteroinvasive and enteropathogen *E. coli* and *Shigella* were negative.

**Conclusion:** The prevalence of invasive and noninvasive SDSE infections has increased gradually year by year. The association of β-hemolytic streptococcus with HUS is not well known. The release of inflammatory mediators in the presence of SDSE infection may play a role in the pathogenesis of HUS. Hypocomplementemia typically is found in aHUS and low C3 levels predict a poor outcome. Although aHUS has been demonstrated to be a disorder of the regulation of the complement alternative pathway, an interesting point is the high frequency of an infectious triggering event, which suggests that complement anomalies are risk factors rather than the only cause of the disease.

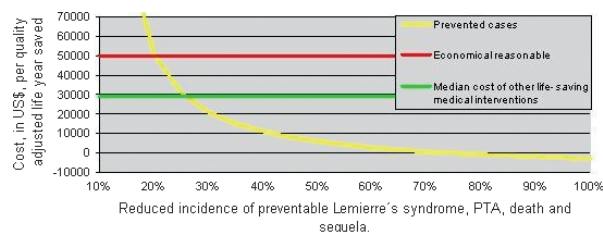
#### P773 A cost-effectiveness analysis of identifying *Fusobacterium necrophorum* in throat swabs followed by antibiotic treatment to reduce the incidence of Lemierre's syndrome and peritonsillar abscesses

S. Bank\*, K. Christensen, L. Hagelskjær Kristensen, J. Prag (Viborg, Aarhus, DK)

**Objectives:** The main objective was to estimate the cost per quality adjusted life year (QALY) saved by identifying *Fusobacterium necrophorum* in throat swabs followed by proper antibiotic treatment, to reduce the incidence of Lemierre's syndrome and peritonsillar abscesses (PTA) originating from a pharyngitis. The second objective was to estimate the population size required to indicate that antibiotic treatment has an effect.

**Methods:** Data from publications and our laboratory were collected. Monte Carlo simulation and 1-way sensitivity analysis were used to analyse cost-effectiveness.

**Results:** The cost-effectiveness analysis shows that examining throat swabs from 15 to 24 years old for *F. necrophorum* followed by antibiotic treatment probably will be less costly than most other life saving interventions, with a median cost of 8 795US \$ per QALY



saved. To indicate a reduced incidence of Lemierre's syndrome and peritonsillar abscess in Denmark, the intervention probably has to be followed for up to 5 years.

**Conclusion:** Identifying *F. necrophorum* in throat swabs from 15 to 24 years old followed by proper antibiotic treatment only requires a reduction of 20–25% in the incidence of Lemierre's syndrome and PTA to be cost-effective.

This study warrants further examination of the effect of antibiotic treatment on the outcome of *F. necrophorum* acute and recurrent pharyngitis as well as the effect on Lemierre's syndrome and PTA.

## Epidemiology, risk factors and clinical presentation of fungal infections

### P774 Clinical score of candidaemia in non-ICU, non-neutropenic patients

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**Background:** *Candida* spp. is the leading cause of invasive fungal infections in hospitalized patients and the fourth most common isolates recovered from patients with bloodstream infection. Few data exist on risk factors for candidemia in non-ICU patients, so we performed a population based case-control study of patients with candidemia to evaluate main predictors for candidemia in non-ICU patients.

**Methods:** This retrospective, multicenter study included all non-neutropenic, non-surgical and non-ICU adult patients with candidemia between January 2006 and January 2011. Cases and controls were identified using laboratory records. Patient with positive, non-candidal blood culture obtained at the same day were selected as controls. Patients were matched according to hospital ward, age and clinical characteristics. A multiple regression analysis was performed to identify risk factors.

**Results:** We identified 60 patients with candidemia. Median age was 67 years (25–90). Most patients were male (35; 58%). *Candida albicans* was the most frequent specie (29 cases; 48%) followed by *C. parapsilosis* (13 cases; 22%). Time to positivity was significantly shorter in patients with bacteraemia than in those with candidemia (10.2 ± 14 days vs 17.6 ± 14.1 days; p = 0.043). Mortality rate was significantly higher for patients with candidemia than that for patients with bacteraemia [22/60, (37%) vs. 12/60 (18%); p = 0.04. OR 2.57 (95%CI 1.11–5.96)].

Univariate analysis identified prior use of antibiotics (p < 0.001; OR 9.34 95%CI 3.6–23.8), total parenteral nutrition (p = 0.014; OR 3.37 95%CI, 1.29–8.77), central venous catheter (p = 0.033; OR 2.4 95%CI 1.09–5.29) and subcutaneous implantable devices, (p = 0.025; OR 4.58 95%CI 1.22–17.2). In multivariate analysis factors independently associated with candidemia included were prior use of antibiotics [p < 0.001; Exp (B): 9.26; (95%CI 3.52–24.39)], central venous catheter [p = 0.032; Exp (B): 2.36; (95%CI 1.22–16.83)] and presence of subcutaneous implantable devices [p = 0.045; Exp (B): 4.52; (95%CI 1.12–19.6)]. Predicted probability of having various combinations of the aforementioned factors ranged from 12% to 45%.

**Conclusion:** Crude mortality was 37% highlighting the need for prompt identification and initiation of therapy in these patients. We identify a set of easily determinable independent predictors of the occurrence of candidemia in non-ICU patients. Our results provide a rationale for initiating early antifungal treatment in high-risk non-ICU patients.

### P775 Invasive aspergillosis in onco-haematology: environmental study in central Tunisia

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**Objectives:** Invasive aspergillosis (IA) is a major infectious complication in patients with prolonged neutropenia. Difficulties in diagnosis and treatment underline the importance of preventive approach. The aim of our study was to assess the spectrum of filamentous fungi isolated in respiratory samples of patients suffering from haematological malignancies and the environmental mycological contamination in their rooms of hospitalization in Onco-Haematology Department of Farhat Hached University hospital at Sousse City, Tunisia.

**Methods:** A 2-year prospective study (December 2009 to September 2011) was carried out. We collected weekly environmental samples (air,

surfaces) in 11 rooms (with two to four beds each) and, three times a week, sputum from 70 patients suffering from haematological malignancies and admitted in the same rooms over the study period. Suspected IA cases are classified as proven, probable or possible according to the EORTC criteria.

**Results:** We collected 1398 surfaces samples, of which, 1229 (87.9%) had a positive cultures with filamentous fungi. Of 439 air samples collected, 339 (90.9%) were positive. The most frequently isolated genus in all environmental samples was *Aspergillus* sp (21%), followed by *Alternaria* sp (16.4%), *Penicillium* sp (14.1%), *Cladosporium* sp (9.3%) and *Rhizopus* sp (7.8%). *Aspergillus* sp was isolated in 60.1% of air samples and in 52.6% of surfaces samples. *Aspergillus niger* was the most frequent species (52.4%), followed by *A. flavus* (27%), *A. nidulans* (5.4%) and *A. fumigatus* (2.9%).

Of the 70 investigated patients, 19 were diagnosed with probable IA and 19 with possible IA. The overall estimated incidence of probable IA was 13%.

Of 660 sputum collected from these 70 patients, *Aspergillus* sp species were isolated in 49 samples (7.4%): *A. niger* in 22 sputum (44.8%), *A. flavus* in 21 sputum (42.8%), *A. nidulans* in three sputum, and *A. fumigatus* in two sputum.

**Conclusion:** Our findings showed that the mycological contamination of the Onco-Haematological Department of our hospital is high. This highlights the pertinence of the HEPA filters' use in reducing the aspergillosis risk. In both, hospital environment and sputum samples, *A. niger* and *A. flavus* are the most common species. *A. fumigatus* seems rare in our region.

### P776 Epidemiology and clinical manifestations of candidaemia in non-neutropenic patients

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**Objective:** To describe the predisponent factors, clinical features and outcome of non neutropenic patients with *Candida* bloodstream infections diagnosed in a community teaching hospital.

**Methods:** Retrospective analysis of the records of patients diagnosed of candidemia according to the database of Microbiology department from January 2003 to October 2011.

**Results:** Fifty-three episodes of candidemia corresponding to 52 patients were included on the period of study. Seventy percent were men, the mean age was 67 years (12–85) and 77.4% had underlying diseases. The most frequent conditions were solid neoplasms (51%) chronic renal failure (24.5%) and diabetes mellitus (19%). Previous to fungemia, 43 patients (81%) had undergone surgery, mainly gastrointestinal and urological (92%) and 96.2% of the cases had received broad spectrum antibiotics. Forty-five subjects (85%) had central line catheters, 75.5% had indwelling urinary catheters and 70% received total parenteral nutrition. Sixty-eight percent of the patients presented clinical manifestations of sepsis and septic shock. Of the total cases of candidemia, 39.6% were catheter related infections, 20.8% were from intra-abdominal infections and 32.1% were due to a primary source.

*C. albicans* was the most common isolate (43%), followed by *C. tropicalis* (18.9%), *C. parapsilosis* (17%), *C. glabrata* (15.1%) and other species (5.7%). Twelve of non albicans isolates (22%) showed decreased susceptibility to fluconazole (MIC ≥ 4 µg/mL). Thirty-five patients received fluconazole therapy (66%) and only 20.8% of the cases begun antifungal treatment on day 0 from the culture date of the first blood sample positive for yeast. Nineteen patients died within the first week after candidemia (36%) and six cases (11%) were included as late mortality (8–30 days). Mortality was considered to be related to candidemia in 37.7% of the cases. Non-albicans *Candida* species isolation was statistically significant related to early mortality [p < 0.000, (RR : 13.73 (IC 95% : 2.71–69.39)].

**Conclusions:** It is crucial to suspect candidemia in abdominal surgical patients with persistent fever and/or criteria of sepsis and prior antibiotic therapy.

2 The leading cause of fungemia in our institution were non- albicans *Candida* species.

3 There is a high mortality rate associated to non-albicans *Candida* species and that may be related to a decreased sensitivity to empirical fluconazol therapy.

**P777** **Epidemiology of *Candida* bloodstream infections and antifungal susceptibility profiles: 10-year experience with 381 candidaemia episodes in a tertiary care university centre**

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**Objectives:** 1 To review the epidemiology of candidemia and its frequency among bloodstream infections over the last 10 years in a 1000-bed university hospital.

2 To evaluate the antifungal susceptibility among candidemia isolates.

**Methods:** We retrospectively evaluated our database from January 2001 to December 2010. Patients with at least one positive blood culture yielding *Candida* species were included in the study. In case of multiple candidemia episodes, only the first episode was included. Available antifungal susceptibility test (AST) results were interpreted according to currently approved CLSI breakpoints.

**Results:** During the study period, there were 18.426 positive blood cultures of which 858 grew *Candida* spp. For the current analysis 381 candidemia episodes were included. *Candida* ranked the fifth (ranged 4–7 during individual years) frequent cause of bloodstream infection. The distribution of species in 381 candidemia episodes and ranking of *Candida* isolates among all positive blood cultures are shown on the Table. *C. albicans* was always the dominant one [p-value for trend test between years 2001–2010 for *C. albicans* and non-albicans *Candida* species (NAC) was non-significant (p > 0.05)]. There were 213 *Candida* strains with available AST results (123 *C. albicans*, 37 *C. parapsilosis*, 27 *C. tropicalis*, 12 *C. glabrata*, 5 *C. krusei*, 3 *C. kefyr*, 3 *C. guilliermondii* and one of each *C. dubliniensis*, *C. lusitanae*, *C. rugosa*). Not all drugs were tested for each isolate. The number of resistant-(susceptible-dose-dependent [SDD])/total strains to fluconazole were as follows: *C. albicans* 1-(2)/122, *C. glabrata* 1-(11)/12, *C. parapsilosis* 0-(2)/22, and *C. tropicalis* 0-(1)/18. The respective values for voriconazole were: *C. albicans* 1-(4)/93, *C. glabrata* 1-(6)/8, *C. krusei* 1-(1)/3, *C. parapsilosis* 0-(2)/18 and *C. tropicalis* 4-(6)/21. Except for one intermediate *C. krusei*, all *Candida* isolates were susceptible to voriconazole. No resistance was detected against caspofungin, the number of intermediate/total strains were: *C. glabrata* (2)/4, *C. parapsilosis* (1)/29, and *C. krusei* (1)/3.

Species	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	Total
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
<i>C. albicans</i>	13 (50.0)	21 (61.8)	14 (66.7)	23 (65.7)	34 (58.6)	35 (62.5)	31 (65.9)	16 (42.1)	16 (61.6)	19 (54.3)	222 (58.3)
<i>C. parapsilosis</i>	3 (11.5)	8 (23.5)	2 (9.5)	3 (8.6)	6 (10.3)	6 (10.7)	9 (19.1)	5 (13.2)	4 (12.9)	8 (22.9)	38 (10.2)
<i>C. tropicalis</i>	3 (11.5)	2 (5.9)	5 (23.8)	4 (11.4)	4 (6.9)	11 (19.6)	4 (8.5)	6 (15.8)	4 (12.9)	1 (2.9)	31 (8.1)
<i>C. glabrata</i>	3 (11.5)	1 (2.9)	-	3 (8.6)	2 (3.4)	3 (5.4)	1 (2.1)	1 (2.6)	4 (12.9)	4 (11.4)	26 (6.8)
<i>C. krusei</i>	-	-	-	-	1 (1.7)	-	1 (2.1)	3 (7.6)	-	1 (2.9)	5 (1.3)
<i>C. kefyr</i>	1 (3.8)	-	-	-	-	-	-	-	2 (6.5)	1 (2.9)	4 (1.1)
Other NAC*	3 (11.5)	2 (5.9)	-	2 (5.7)	4 (6.9)	1 (1.8)	-	-	1 (3.2)	1 (2.9)	14 (3.7)
<b>Total</b>	<b>26</b>	<b>34</b>	<b>21</b>	<b>35</b>	<b>58</b>	<b>56</b>	<b>47</b>	<b>38</b>	<b>31</b>	<b>35</b>	<b>381</b>
Ranking among all positive blood cultures	6	4	7	7	4	4	5	6	4	6	5

\*Other non-albicans *Candida* species (NAC) included in this study were: 4 *C. guilliermondii*, 4 *C. lusitanae*, 2 *C. nonspouanonegensis*, 2 *C. rugosa*, 1 *C. dubliniensis*, 1 *C. colubrosa*.

**Conclusions:** *Candida* spp. play a prominent role among the common bloodstream isolates in our center. Among all *Candida* species, *C. albicans* has been the most common cause of candidemia and this trend

has not changed during the study period. Resistance to tested antifungal drugs is not common among our candidemia isolates except for itraconazole. Decreased susceptibility to fluconazole in *C. glabrata* is remarkable.

**P778** **Candidaemia in hospital, Como, Italy, from 1997 to July 2011 and sensitivity to antifungal drugs**

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**Objectives:** Candidemia 1997–July 2011 and evaluation of antifungal drugs referring to EUCAST and CLSI breakpoints are reported.

**Methods:** Blood culture was performed by automated system, and those samples positive for yeast were subcultured on Sabouraud agar and Chromagar *Candida* (BD); *Candida* identification: Auxacolor (Remel) and Yeast card VITEK2 (bioMerieux); antifungal susceptibility: Sensititre Yeastone.

**Results:** Since 1997 to July 2011, 155 candidemia were detected: *C. albicans* 82 (53%), *C. glabrata* 26 (17%), *C. parapsilosis* 29 (19%), *C. tropicalis* 10 (6%), other species eight cases (5%). Eighty-two cases occurred since 1997 to 2007, with 7.4 cases/year; since 2008 there was an increase with 73 candidemia until July 2011 and 20.3 cases/year. Candidemia cases April 2010–July 2011: 39 cases, 16 males (41%) and 23 females (59%). *C. albicans* is the species most frequently found with 20 isolates (51%); *C. glabrata* 10 isolates (26%); minor surveys of candidemia from other *Candida* species (*C. famata*, *C. krusei*, *C. lusitanae*, *C. parapsilosis* and *C. tropicalis*). Sensititre provides MIC for different antifungal drugs. *C. albicans*: 17 isolates susceptible to fluconazole and voriconazole based on CLSI, 16 based on EUCAST; referring to posaconazole, 17 susceptible with CLSI breakpoint, 13 based on EUCAST; there are no differences for anidulafungin and amphotericin-B with all the isolates susceptible. All *C. glabrata* isolates result susceptible to anidulafungin and amphotericin-B. Two out of three isolates of *C. parapsilosis* and two isolates of *C. tropicalis* are resistant to posaconazole with EUCAST and susceptible with CLSI. There were no differences for *Candida* spp. with anidulafungin and amphotericin B.

**Conclusions:** Over the past 14 years, there has been a significant increase in *Candida* spp. bloodstream infections; non-albicans *Candida* species reach almost half of the *Candida* isolates; *C. albicans* EUCAST breakpoints show an increase of isolates resistant to fluconazole, posaconazole and voriconazole; the use of antifungal susceptibility test is now mandatory.

**P779** **Candidaemia in Polish hospitals – a multicentre survey**

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**Objectives:** The aim of the study was to examine the epidemiology of candidemia in selected Polish hospitals in years 2006–2007.

**Methods:** Nineteen microbiological laboratories distributed in sixteen Polish cities reviewed retrospectively the documentations from years 2006–2007 and collected the information about each episode of isolation of *Candida* from blood. The patient's data were obtained in cooperation with the staff of particular hospitals. Blood cultures were performed using Bact/ALERT (BioMerieux) or Bactec (Becton Dickinson) systems. Statistical analysis of the data was performed using the STATISTICA software system, version 8.0. (StatSoft, Inc., 2008).

**Results:** A total of 308 episodes in 300 patients (145 women and 155 man) were identified in 94 hospital's units of 20 hospitals. Eight patient showed two candidemia episodes, divided by 2–12 months long period of recovery with blood negative cultures. A total number of examined blood samples was 2783, of them fungi were cultured from 774 (27.8%). Most blood samples were described as being "venous"



(2402), 334 as ‘‘catheter-taken’’ and in 59 samples the method of sampling was unknown. The higher number of the infections were noted in Intensive Care Units – ICU (30.19%), and surgical (28.8%), followed by hematological (16.5%), ‘‘others’’ (61; 19.8%) and neonatological (14; 4.5%) units. *C. albicans* represented main fungal blood pathogen, responsible for almost half of reported episodes (49.84%), however its prevalence was significantly lower in hematology (20.78%) ( $p < 0.0015$ ), and higher in ICU and neonatology (60.61%, and 73.33%, respectively). The frequency of *C. krusei* and *C. tropicalis*, was significantly higher (23.53 and 16.98%) in hematology, than in the rest units ( $p < 0.02$ ), whereas distribution of *C. glabrata* (13.79%) and *C. parapsilosis* (12.85%) do not differ statistically between compared departments. Similarly, analysis of distribution of fungal species in relation to patient underline disease indicated highest frequency of *C. albicans* isolation in patients with traumas (83.33%) and the lowest in patients with blood neoplasms (21.95%), who showed the highest percentage of *C. krusei* and *C. tropicalis*.

**Conclusion:** Obtained data indicated that the species distribution of *Candida* blood isolates in polish hospitals reflects the worldwide trends of increase in the number of infection due to *Candida* non-albicans, especially these with resistance to azoles.

#### **P780** Risk factors for *Candida* infections in a neurology intensive care unit: a case-control study

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**Objectives:** *Candida* species has an emerging importance in intensive care units (ICU) in recent years. The aim of this study was to describe the epidemiologic and microbiologic characteristics as well as the risk factors for *Candida* infections in the neurology ICU (N-ICU).

**Methods:** All adult patients who were hospitalized over 48 hours in the N-ICU between 1st January 2010 and 30th October 2011 were included into the study. Patients group was composed of all patients with proven *Candida* infection. Control group comprised of other ICU patients who didn't have any *Candida* infection at all. For the statistical evaluation, the first *Candida* attack of infection was selected for each patient. Identification and antifungal susceptibility of *Candida* species was performed by VITEK II system (Biomerieux, France). CDC criteria's were used for the definition of hospital infection (HI).

**Results:** During the study period a total of 693 patients were hospitalized into the N-ICU. The patient day was 6170 days. Two hundred and ninety one HI were detected in 137 patients. HI rate was 42.0 per 100 patients and HI density was 47.2 per 1000 patient days. The control group comprised of 243 patients. Fifty-seven *Candida* strains were isolated in 44 patients. Among isolates, 11 *C. albicans*, 8 *C. tropicalis*, 31 *C. parapsilosis*, 5 *C. glabrata*, and 2 *C. krusei*. Twenty two of all *Candida* infections were catheter related bloodstream infections while remaining were urinary tract infections. When patient and control groups were compared, older age; having any of the following: nasogastric tube, central venous catheter, arterial catheter, enteral feeding and parenteral feeding were found as risk factors for

	Patient Group n=44 (%)	Control Group n=243 (%)	p	OR (%95 CI)
Age (mean)	73.0±12.1	64.2±18.1	<b>0.002</b>	
Gender (M/F)	17/27	126/117	0.107	0.7 (0.50-1.12)
Underlying diseases				
Malignity	4 (9.1)	7 (2.9)	0.070	3.9 (0.96-10.33)
Diabetes mellitus	10 (22.7)	34 (14.0)	0.170	1.6 (0.87-3.04)
Chronic heart diseases	10 (22.7)	30 (12.3)	0.067	1.8 (0.97-3.49)
Chronic Lung Diseases	2 (4.5)	15 (6.2)	0.674	0.7 (0.17-3.10)
Invasive procedure				
Urinary catheter	40 (90.9)	198 (81.5)	0.126	1.2 (0.99-1.25)
Nasogastric tube	29 (65.9)	52 (21.4)	<b>0.000</b>	3.1 (2.23-4.25)
Central venous catheter	30 (68.2)	30 (12.3)	<b>0.000</b>	5.5 (3.73-8.17)
Arterial catheter	12 (27.3)	17 (7.0)	<b>0.000</b>	3.9 (2.00-7.58)
Ventilatory	12 (27.3)	42 (17.3)	0.177	1.6 (0.90-2.75)
Tracheotomy	2 (4.5)	5 (2.1)	0.292	2.2 (0.44-11.03)
Enteral feeding	7 (15.9)	7 (2.9)	<b>0.001</b>	5.5 (2.03-14.91)
Parenteral feeding	36 (81.8)	94 (38.7)	<b>0.000</b>	2.1 (1.71-2.61)
Peripheral venous catheter	34 (77.3)	163 (67.1)	0.244	1.5 (0.96-1.38)
Hospital stay (days-mean)	44.8	7.4	<b>0.000</b>	37.4 (25.8-49.1)
Mortality	37 (84.1)	72 (29.6)	<b>0.000</b>	2.8 (2.2-3.5)

*Candida* infection ( $p < 0.05$ ) (Table 1). When these factors were evaluated on multivariate analysis, only having central venous catheter and parenteral feeding were detected as independent risk factors for *Candida* infections. Overall mortality in patient and control groups were 84.1% and 29.6% respectively. Mean length of the hospitalization was also longer in the patient group. These differences were found to be statistically significant ( $p < 0.000$ ).

**Conclusion:** *Candida* infections caused by non-albicans species occurred more frequently in our N-ICU. Medical device usage increases the risk for *Candida* infections. *Candida* infections has to be kept in mind particularly for those patients with central venous catheter and parenteral feeding. *Candida* infections were also associated with higher mortality.

#### **P781** Epidemiological trends of nosocomial candidaemia over a 7-year period at a Nantes University Hospital, France

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**Objectives:** To determine the main epidemiological characteristics of candidemia in our hospital over a 7-year period.

**Methods:** All episodes of candidemia occurring between February 2004 to December 2010 were analysed retrospectively. Demographic and clinical data (age, sex, medical unit, risk factors) as well as antifungal therapy were recorded. Species identification was performed using phenotypical methods. Isolates belonging to the *C. parapsilosis* and *C. glabrata* species complex and rare *Candida* species were confirmed by ITSrDNA sequencing. In vitro susceptibility to fluconazole, voriconazole, amphotericin B and caspofungin were determined by E-test®.

**Results:** One hundred and eighty-seven episodes of candidemia ( $n = 193$  *Candida* spp. isolates) occurring in 184 patients were analysed (sex ratio = 1.5, mean age = 53.4 years). Global incidence of candidemia was 0.37 per 1000 admissions. Incidence rate increased from 0.27‰ to 0.45‰ between 2005 to 2009. Higher incidences were noted in Hematology (6.65‰) and intensive care units (ICU, 2‰). Central venous catheter and antibiotherapy were the most frequent risk factors (67% and 75% respectively). *Candida albicans* was the predominant species (51.8%) followed by *C. parapsilosis* (14.5%), *C. glabrata* (9.8%), *C. tropicalis* (9.8%), *C. kefyr* (3.6%) and *C. krusei* (3.1%). No trend toward an increased prevalence of non-albicans species was noted over the time. Species distribution among episodes clearly differed between units, *C. tropicalis* being the most prevalent in Hematology (27%) whereas *C. albicans* was the dominant species in the ICU (73%). Prevalence of non-albicans was higher in Hematology compared to any other medical units (33 of 37 episodes, 89.2%). Fluconazole followed by caspofungin were the main antifungals given at the time of diagnosis (43% and 28% of the episodes). Notably, over the study, a clear trend over an increased use of caspofungin was observed. Overall in vitro susceptibility to the four antifungal drugs was noted. Twelve-weeks mortality rate was 29%, being lower for *C. parapsilosis* (18%) than for *C. tropicalis* (47%). Despite the limited number of patients, a trend to a better survival was noted in patients with catheter removal.

**Conclusion:** This study highlights that *C. albicans* is the main species responsible for candidemia at our hospital as well as the significant prevalence of *C. kefyr* ranking at the fifth common *Candida* species. Important differences in species distribution was noted between medical units.

#### **P782** A retrospective study of candidaemia in a Greek hospital, 2007–2011

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**Objectives:** The aim of this study was to investigate the prevalence of *Candida* spp. in blood stream infections (BSI) during the last 5 years in

a tertiary Greek hospital, to determine the species distribution among *Candida* isolates and their susceptibility to antifungals and finally to evaluate the risk factors associated with candidaemia.

**Methods:** A total of 18,802 blood cultures were incubated in BacT/Alert system (bioMerieux). Isolation of *Candida* and species identification carried out according to conventional methods. Susceptibility testing to Amphotericin B (AB), Fluconazole (FZ), Itraconazole (IZ), Voriconazole (VZ), Posaconazole (PZ), Caspofungin (CS) and 5-Flucytosine (FC) performed with Sensititre Yeast One Test Panel (Trek Diagnostics). MIC's were evaluated according to CLSI criteria. Clinical and demographic data obtained from patients' files.

**Results:** *Candida* sp were recovered from 220 blood cultures (1.17%). *C. albicans* remained the most prevalent species, accounting for 50.4% of all isolates, followed by *C. parapsilosis* (25.9%), *C. glabrata* (10.8%) and *C. tropicalis* (10.8%), while only 2.1% of the strains were identified among other species. Patients had a mean age of 73 years (range from 41 to 89) and BSI was established after an average hospitalization of 25 days. Most of them were hospitalized in Medical Wards (44.3%), 29% in ICU and 26.7% in Surgical Wards, 91.3% had previously received antibiotics and 43.7% had central IV catheters; however only in 19.8% of BSI, central IV catheters were determined as the source of candidaemia, followed by urinary tract infections in 8.4%, while in most cases no source could be identified. Antifungal MIC's ranged for AB: 0.06–1, FZ:  $\leq 0.125$ –32, IZ:  $\leq 0.008$ –4, VZ:  $\leq 0.008$ –1, PZ: 0.03–2, CS: 0.03–1 and FC:  $\leq 0.03$ –2  $\mu\text{g/mL}$ .

**Conclusions:** Candidaemia's prevalence was low (1.17%) with *C. albicans* being considered as the causative agent in half of the infections. Previous administration of antibiotics and presence of IV catheters were the most frequent risk factors. All *Candida* isolates were susceptible to AB, FC, VZ and CS, 26.7% of *C. glabrata* exhibited resistance to IZ, while reduced susceptibility to FZ encountered at 73.3% of *C. glabrata* and 5.5% of *C. parapsilosis* and to IZ at 53.3% of both *C. glabrata* and *C. tropicalis*, 36.1% of *C. parapsilosis* and 5.7% of *C. albicans*.

**P783** **Is the incidence of candidaemia caused by *Candida glabrata* increasing in Brazil? Five-year surveillance of *Candida* bloodstream infection in a university reference hospital in southeastern Brazil**

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**Objective:** To determine the epidemiology of *Candida* bloodstream infection in a 5-year surveillance.

**Methods:** From 2006 to 2010, a retrospective study was conducted in a university referral tertiary care hospital to study the frequency and distribution of *Candida* species in different medical specialties. The use of mechanical ventilation, central venous catheter and urinary catheter were recorded per 1000 patient-days and the use of antifungal was calculated using defined daily dose (DDD).

**Results:** A total of 313 episodes were identified and the overall incidence was 0.57 (0.43–0.71) episodes per 1000 patients-day. *C. albicans* caused 44% of the overall episodes, followed by *C. tropicalis* (21.7%), *C. parapsilosis* (14.4%), *C. glabrata* (11.2%) and *C. krusei* (3.5%). The incidence of *C. glabrata* significantly increased from 2006 to 2010 (range: 4.3–23.5%) ( $p = 0.024$ ) and *C. parapsilosis* decreased ( $p = 0.046$ ). *C. glabrata* was associated with malignancies ( $p = 0.004$ ) and *C. krusei* with hematologic malignancies ( $p < 0.0001$ ). The use of antifungal was higher in the hematology/bone marrow transplant units and represented 40% of all fluconazole prescription in the hospital. There was no correlation with the use of fluconazole and the increasing ratio of *C. glabrata* ( $r = 0.60$ ). The use of invasive devices was significantly higher in the ICUs than the medical and surgical emergencies units ( $p < 0.001$ ). In contrast, the emergencies had higher incidence of candidemia (2–2.1 episodes/1000 patients-day) than the ICUs (1.6 episodes/1000 patients-day).

**Conclusion:** *C. glabrata* showed a significant raise differently from the current national literature where *C. parapsilosis* remained the most

important non-albicans species in Brazilian hospitals. Our findings suggested that the increasing incidence of *C. glabrata* was not associated with use of fluconazole and other risk factors might play an important role.

**P784** **Risk factors and outcome of mixed *Candida*/bacterial bloodstream infections: a case-control study**

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**Objectives:** Invasive *Candida* infections are increasingly problematic in critically ill patients as a result of the high crude mortality. Mixed *Candida*/bacterial bloodstream infections (BSIs) have been reported to have an incidence among cases of candidaemia ranging between 7% and 27%. However, the clinical impact of mixed *Candida*/bacterial BSIs is unknown.

**Methods:** We reviewed medical records of all consecutive patients developing candidemia at a tertiary care center from July 2006 to June 2011. Mixed *Candida*/bacterial BSIs were defined as isolation of bacterial species growing concomitantly within the same blood culture bottles or within the 48 hour of each other. We performed a case-control study comparing mixed *Candida*/bacterial BSIs with monomicrobial candidaemia.

**Results:** During the 5-year study period, 37 (29.3%) of 126 patients with *Candida* BSIs met the definition for mixed *Candida*/bacterial BSIs. Non-albicans *Candida* species (74 of 126 isolates, 58.7%) were more frequently identified. The most common bacteria were coagulase-negative staphylococci (10 of 44 isolated, 22.7%). In multivariate analysis, previous bacteraemia originated from gastrointestinal tract within 30 days prior to positive blood cultures for *Candida* (OR 6.249, 95% CI 1.892–20.639,  $p = 0.003$ ) and corticosteroid administration (OR 3.584, 95% CI 1.364–9.415,  $p = 0.010$ ) were significantly associated with mixed *Candida*/bacterial BSIs. Persistent candidaemia  $\geq 3$  days in duration occurred more frequent in patients with mixed *Candida*/bacterial BSIs (36.7% vs. 15.8%, OR 3.088, 95% CI 1.104–8.638,  $p = 0.035$ ). The 12-week mortality was not different between mixed *Candida*/bacterial BSIs and monomicrobial candidaemia (70.3% vs. 56.2%). Independent predictors for 12-week mortality were persistent positive blood culture for *Candida* (HR 7.721, 95% CI 4.154–14.350,  $p < 0.001$ ), SOFA score  $\geq 5$  (HR 5.509, 95% CI 3.033–10.004,  $p < 0.001$ ), no administration of antifungal agents (HR 4.172, 95% CI 2.291–7.596,  $p < 0.001$ ), total bilirubin  $\geq 2$  mg/dL (HR 2.422, 95% CI 1.442–4.068,  $p = 0.001$ ), corticosteroid administration (HR 2.166, 95% CI 1.235–3.800,  $p = 0.007$ ), and Charlson's comorbidity index  $\geq 4$  (HR 2.088, 95% CI 1.251–3.487,  $p = 0.005$ ).

**Conclusion:** Mixed *Candida*/bacterial BSIs were associated with recent bacteraemia originated from gastrointestinal tract and corticosteroid administration. However, mixed *Candida*/bacterial BSIs did not show a higher mortality rates compared with monomicrobial candidaemia.

**P785** **Changing roles of non-albican *Candida* species in candidaemia in Western China hospital from 2007 to 2010**

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**Objectives:** To investigate the pathogenic and clinical characteristics of candidaemia in a teaching hospital in Western China from 2007 to 2010.

**Methods:** A retrospective analysis of the microbiological results for candidaemia from 1 January 2007 to 31 December 2010 was conducted. Clinical data of candidaemia in intensive care unit (ICU) were reviewed and statistics analyses were performed to compare the characteristics of the cases due to albicans and non-albicans *Candida* species.

**Results:** A total of 198 episodes of candidaemia were defined during the study period. Overall, the species distribution of *Candida* spp.

has changed. The non-*albicans Candida* species were predominant, and increased rapidly from 52.6% (10/19) in 2007 to 81.0% (17/21) in 2010 among all isolates recovered in ICU ward. In non-ICU wards, non-*albicans Candida* species accounted for 72.7% (24/33) of all isolates in 2010. Of non-*albicans Candida* spp. isolated, *C. parapsilosis* was the leading species, followed by *C. tropicalis* and *C. glabrata*, but *C. krusei* remained rare. All the *C. albicans* isolated in 2007, 2009 and 2010, were susceptible to amphotericin B and 5-Fluorouracil, while a few isolated in 2008 were non-susceptible to them. During the last 4 years, azole susceptibility has changed but didn't make a statistical significance for the *C. albicans* isolates ( $p > 0.05$ ). In terms of the non-*albicans Candida* species, non-susceptibility of azole in 2007 and itraconazole in 2008 were observed ( $p < 0.05$ ). No statistically significant differences with regard to age, sex, admission service (medical or surgical), underlying condition, usage of antibiotics, immunosuppressive therapy, total parenteral nutrition, major operation during ICU stay, catheterization, and mechanical ventilation for the 48 ICU patients studied were found ( $p > 0.05$ ).

**Conclusion:** Change in the species distribution of *Candida* spp. causing candidaemia in West China Hospital from 2007 to 2010 has been observed. The non-*albicans Candida* species played more important roles, especially for the patients admitted to ICU, while the factors associated with candidaemia caused by non-*albicans Candida* species vs. *C. albicans* remained to be found.

#### P786 Secular trends in fungaemia in a large teaching hospital over a 26-year period

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**Objectives:** Data on bloodstream infections caused by yeasts (YBSI) are usually restricted to short periods, specific clinical backgrounds, and specific pathogens or they are simply outdated. The high mortality of YBSI and the impact of efficacious empirical therapy make microbiological surveillance of invasive fungal infections essential. We evaluated the workload, incidence and etiology of YBSI in a general hospital over a 26-year period (1985–2010).

**Method:** We prospectively analyzed the laboratory workload and trends in incidence per 1000 admissions of different YBSI in a general 1750-bed hospital. All yeasts isolated in blood from the same patient within 1 week were considered a single episode.

**Results:** We recorded 1205 episodes of significant YBSI in 1050 patients (1129 *Candida* spp. and 78 other yeasts; 3.44% of all BSI). The species involved were 534 *Candida albicans*, 364 *Candida parapsilosis*, 101 *Candida glabrata*, 83 *Candida tropicalis*, 18 *Candida krusei*, 16 *Candida guilliermondii*, 8 *Candida dubliniensis*, 6 *Candida lusitanae*, 2 *Candida kefyr*, and 15 *Candida* spp. The other yeasts were 40 episodes of *Cryptococcus neoformans*, 14 *Saccharomyces cerevisiae*, 10 *Trichosporon* spp., 6 *Blastoschizomyces capitatus*, 6 *Rhodotorula* spp. and 2 *Hansenula anomala*. The departments in which the yeasts were isolated were adult medical (37.9%), adult intensive care unit (22.7%), adult surgical (15.5%), and pediatrics (23.9%).

Incidence increased from 0.20 episodes/1000 admissions in 1985 to 1.51 episodes/1000 admissions in 2010, ie, an annual increase of 0.07 episodes/1000 admissions (95%CI, 0.05–0.09;  $p < 0.0001$ ). The global incidence/1000 admissions and the annual increase in different yeast species are summarized in Table 1.

Microorganism	N. of episodes	Annual average (per 1,000 admissions)	Annual increase (CI95%)	p
Yeasts	1,205	46.35 (0.88)	0.069 (0.05-0.09)	<0.0001
<i>Candida</i>	1,129	43.42 (0.82)	0.071 (0.05-0.09)	<0.0001
<i>C. albicans</i>	534	20.54 (0.39)	0.038 (0.02-0.05)	<0.0001
<i>C. parapsilosis</i>	364	14.00 (0.27)	0.019 (0.01-0.03)	0.002
<i>C. glabrata</i>	101	3.88 (0.07)	0.009 (0.00-0.01)	0.001
<i>C. tropicalis</i>	83	3.19 (0.06)	0.004 (0.00-0.01)	0.043
<i>C. krusei</i>	18	0.69 (0.01)	-	0.402
Other yeasts	78	3.00 (0.06)	-	0.647

**Conclusions:** The long-term, unbiased perspective of a large teaching hospital shows a significant increase in the incidence of fungaemia, mainly that caused by *C. albicans*, *C. parapsilosis*, *C. glabrata* and *C. tropicalis*.

#### P787 Candidaemia in critically ill patients, 2005–2010

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**Objectives:** To provide a picture of local epidemiology and to assess whether appropriate antifungal therapy is administered in a timely fashion. To evaluate risk factors, outcome and the associated costs.

**Methods:** Retrospective analysis of all candidaemias between January 2005 and December 2010 at a 460 bed district general hospital.

**Results:** There were a total of 60 candidaemias between 2005–2010 resulting in a prevalence of 0.24 per 1000 patient admissions. The most predominant species were *C. albicans* which accounted for 43 cases (73%). From a total of 60 candidaemias, 55 cases were investigated further. It was found that 56% of cases were diagnosed in intensive care (ITU) resulting in ITU prevalence of 9.4 per 1000 admissions compared to a non ITU prevalence of 0.10 per 1000 admissions. This equates to approximately a 100-fold difference between ITU and non ITU cases, consistent with previous reports. The most common risk factor was a central venous pressure line (67%). Forty out of 45 (88.9%) patients received appropriate antifungal treatment within 24 hours of positive blood culture.

From a total of 54 candidaemias, crude mortality rate was 36.6% (15/41) and 46.2% (6/13) for *C. albicans* and non-*albicans Candida* respectively. The difference between the crude mortality is 9.6% and is not significant ( $p = 0.75$ , Fisher's exact test). Hospitalization plus antifungal costs were £48 698/pt and £67 809/pt for the *C. albicans* and non-*albicans Candida* groups respectively. The average length of stay per patient was 58 bed days however, the *C. albicans* group accounted for 18.6 bed days/pt in ITU and 34.5 bed days/pt in non ITU wards, compared to the non-*albicans Candida* group who accounted for 23.8 bed days/pt in ITU and 50.7 bed days/pt in non ITU wards.

**Conclusions:** 88.9% of patients received timely antifungal treatment. There is a high mortality rate in patients with candidaemias. The care of *C. albicans* infections was on average £19 111/pt cheaper. Trust outcome would be to devise and implement a candidaemia score card to improve outcomes and costs.

#### P788 Clinical characteristics of ocular candidiasis: when should fundoscopy be performed to rule out ocular candidiasis?

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**Objectives:** Ocular candidiasis is a major complication of *Candida* bloodstream infection (BSI), but little information is known about the clinical characteristics. This study was conducted to investigate the incidence, risk factors and the outcome of ocular candidiasis and the optimal timing of fundoscopy.

**Methods:** We analyzed 216 cases with positive blood cultures of *Candida* species from January 2005 to June 2011 in two teaching hospitals to determine the incidence and the clinical characteristics of ocular candidiasis. Infectious diseases physicians in these hospitals performed proactive intervention in all of the *Candida* BSI cases and recommended fundoscopic examinations by ophthalmologists.

**Results:** One-hundred sixty three of 216 *Candida* BSI cases had ophthalmology consultations during the course of the antifungal therapy. Fifty-three (32.5%) cases had findings consistent with the diagnosis of ocular candidiasis, consisting of 44 cases with possible/probable chorioretinitis and nine cases with vitritis. The groups with and without ocular involvement did not differ in age, sex, steroid use or diabetes mellitus, but more cases had digestive organ abnormalities or malignancies. Of these 53 cases, 42 (79.2%) were diagnosed within 7 days after a positive blood culture. The average time from a positive fungal culture to the diagnosis was 5.5 days. Twelve ocular candidiasis

cases died during antifungal therapy and 40 of 41 cases completed antifungal therapy without any worsening of visual acuity. Four cases were negative for ocular manifestation on the first examination, but were subsequently diagnosed as ocular candidiasis on a second examination more than 10 days after a positive fungal culture. Among the patients with ocular candidiasis, more cases were due to *Candida albicans* ( $p = 0.004$ , OR; 2.99 95%CI 1.42 – 6.33) and had higher beta D-glucan values ( $p = 0.002$ , OR; 4.17 95%CI 1.05–5.52). Thirty-day mortality rate was relatively higher in the ocular candidiasis cases, although the difference was not statistically significant. (26.4% vs 18.8%  $p = 0.303$ ).

**Conclusions:** When determining the optimal antifungal drug of choice and duration of therapy, fundoscopic examination should preferably be performed soon after a positive blood culture especially in those who had BSIs by *C. albicans* and had higher beta D-glucan values, and follow-up fundoscopic examination should also be considered in such high risk patients.

**P789** **Mannose-binding lectin gene polymorphisms. Impacts on the colonisation by *Candida* spp. and on the anti-*Candida* immune response**

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**Objectives:** Invasive candidiasis (IC) is a persistent public health problem linked to difficulties of diagnosis. Several recent observations suggest an “immunogenetic disparity” against IC. Among anti-*Candida* host defenses, the mannose-binding lectin (MBL) has a major role in clearance of yeasts by opsonisation and activation of complement pathway. The aim of this study is to assess serum MBL levels in IC vs. controls and to analyse relationship between serum MBL, mannanemia and anti-mannan antibodies during the time course of IC.

**Methods:** Between January 2005 and December 2007, 112 sera was retrospectively collected in different wards of CHRU of Lille coming from 49 IC patients (22 females and 27 males) proved by positive blood culture. Two control groups were also included in this study: 70 presumed healthy subjects (HS) and 82 hospitalized patients (HP) without evidence of infection.

Polymorphism of MBL-2 gene was analyzed through PCR-SSP, circulating levels of MBL were determined by ELISA.

**Results:** Association between MBL-2 gene mutations and MBL serum levels in sera of controls patients was confirmed during this study. On the other hand, serum MBL concentrations were significantly higher in IC than HP (2700 vs. 1200 ng/mL;  $p < 0.0001$ ) and HS (2700 vs. 1850 ng/mL;  $p = 0.0035$ ). In HP, significantly lower level of MBL seems to be associated with colonization ( $p = 0.0291$ ). During the time course of IC, significant variations of MBL levels was observed before ( $p = 0.013$ ) and during candidemic episodes ( $p = 0.006$ ). This dynamic evolution of MBL is correlated with anti-mannan antibody and both decrease in case of detectable mannanemia.

**Conclusion:** This study is the first evidence of variation in serum MBL levels during the time course of IC. MBL levels were paralleled to anti-mannan antibodies and vary according to circulating *Candida* mannan. More exploration of these serological/mycological and genetic analyses could help in understanding the role of MBL among IC risk factors.

**P790** **DHPS gene mutations in *Pneumocystis jirovecii* and clinical outcome in non-HIV patients with *Pneumocystis* pneumonia in China**

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**Backgrounds:** *Pneumocystis* pneumonia in non-HIV immunocompromised patients usually present with severe disease with high mortality. It is unknown whether treatment failure was

associated with *pneumocystis* resistance. The purpose of our study is to investigate dihydropteroate synthase (DHPS) gene mutations in our non-HIV patients with *Pneumocystis* pneumonia (PCP) and their clinical context.

**Methods:** DHPS gene from HIV-negative patients with PCP presented between January 2008 and April 2011 were amplified by polymerase chain reaction (PCR) and sequenced. Clinical data from medical records were reviewed.

**Results:** (i) DNA was extracted successfully from 20 of 22 patients' respiratory samples. The most common point mutation at codons 55 and 57 were not detected. (ii) Two other nonsynonymous point mutations, Asp90Asn and Glu98Lys were identified in *P. jirovecii* from two patients. (iii) The percentage of circulating lymphocytes was significantly lower in non-survivors than in survivors (4.2%[IQR2.4–5.85] vs. 10.1%[IQR5.65–23.4]  $p = 0.019$ ). Neutrophil proportion in BALF was significantly higher in non-survivors than in survivors (49.78 + 27.67% vs. 21.33 + 15.03%  $p = 0.047$ ). Apart from the co-trimoxazole as the first line therapy, nine (50%) patients received Caspofungin during treatment (one monotherapy and eight in combination), but only two patients (22.22%) survived.

**Conclusions:** No common DHPS gene mutations of *Pneumocystis jirovecii* were found in our HIV-negative PCP patients. However, other mutations did exist, in which the significance needs to be identified. The reduction of lymphocyte in peripheral blood and elevation of neutrophil counts in BALF seem to be associated with poor outcome. The beneficial effect of adjunctive caspofungin to non-HIV PCP patients requires further investigation.

**P791** **Imported Tinea capitis in adopted children in Spain: a 6-year retrospective study**

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**Background:** With human migration, some species uncommon in developed countries are being diagnosed currently. To analyze the epidemiology and clinical features of these infections, a 6-year retrospective study of imported dermatophytoses in adopted children from underdevelopment regions diagnosed in the Pediatric department of our centre was performed.

**Methods:** A retrospective analysis of samples from adopted immigrant children with scalp lesions with clinical suspicion of mycosis from January 2005 to October 2011 was performed. Specimens (hair or skin) were pressed on plates of agar medium with added chloramphenicol with/without cycloheximide, which were sealed and incubated at 30°C for 21 days. Readings were conducted once a week. In case of growth, the identification was made by macroscopic and microscopic morphology.

**Results:** A total of 71 samples were studied. The mean age of the study population was 4.5 years old (range: 6 months to 11 years). Forty-five (63.3%) samples yielded dermatophytes. The etiologic agent isolated more frequently was *Trichophyton violaceum* ( $n = 29$ , 64.4%) followed by *T. rubrum* ( $n = 6$ , 13.3%), *T. mentagrophytes* ( $n = 4$ , 8.8%), *T. soudanense* ( $n = 4$ , 8.8%), *Microsporum audouinii* ( $n = 2$ , 4.4%) and *T. schoenleinii* ( $n = 1$ , 2.2%). Two different dermatophytes in a single lesion were two patients: *T. rubrum* with *M. audouinii*, and *T. violaceum* with *T. mentagrophytes*. Regarding the origin of the adopted children, most of them came from Ethiopia ( $n = 30$ ) and *T. violaceum* was the most frequent dermatophyte isolated ( $n = 26$ , 76.6%).

**Conclusion:** Tinea capitis is a public health issue because of their transmissibility from human to human that involved mainly children of school age. In developed countries, incidence of dermatophytoses has showed a change in their clinical and epidemiological pattern with an increase of anthropophilic dermatophytes. Although the reasons are not clear, immigration of other geographical areas has a remarkable role. The accuracy detection and diagnosis of these exotic species is essential for its therapeutic implications, prevention measures and an update on their epidemiology.

**P792 Fungal malignant external otitis**

B. Hammami\*, A. Ben amor, D. Lahieni, I. Maaloul, F. Makni, A. Ayadi, M. Ben jema (Sfax, TN)

**Objective:** To investigate the clinical characteristics and outcome of fungal malignant external otitis (MOE).

**Methods:** We report in this retrospective study, seven cases admitted to our infectious diseases department over a 5-year period (2007–2011), for a MOE. The diagnosis was based on a combination of clinical finding, biological and radiological investigations.

**Results:** We identified one man and six women, all diabetics. The mean age was 63 years (50–73 years). The main symptoms were severe otalgia (seven cases), otorrhoea (seven cases), oedema of the external ear canal (six cases) and headache (three cases). Three patients developed facial paralysis during disease progression. The pathogens identified on culture or PCR of ear samples were *Aspergillus* (two cases), *Candida albicans* (two cases), *Candida parapsilosis* (one case), *Candida tropicalis* (one case) and *Candida krusei* (one case). All patients have bone erosion on computed tomography. After treatment by amphotericin or fluconazole or voriconazole, the evolution was favorable in five cases, one patient has persistent infection, and one patient died.

**Conclusion:** Fungal malignant external otitis should be suspected in cases where there is no response to antipseudomonal antibiotic therapy. Samples and deep biopsies from the external ear canal are needed to confirm the diagnosis.

**P793 Pulmonary aspergillosis in a burn patient**

A. Silva\*, G. Mota, F. Xambre, L. Krebs (Lisbon, PT)

**Introduction:** In acute phase of burn injury, thermal damage to the human skin barrier and local and systemic immune dysfunction predisposes patients to potentially serious fungal infections. The nature of burns injuries often result in complex intensive care and exposure to multiple risk factors for fungal infection. *Aspergillus* spp. is a fungus acquired by inhalation of airborne spores and may cause various clinical conditions. Pulmonary disease caused by *Aspergillus*, mainly *A. fumigatus*, can result in Invasive Pulmonary Aspergillosis (IPA), especially in immunocompromised patients, and has become the most important airborne pathogen in developed countries.

**Case report:** A 56 years old man was admitted with electrical burns to 23.5% total body surface area involving his neck, back and bilateral upper extremities. There was no evidence of inhalation injury and the patient had no other pathological condition. Resuscitation was started immediately, using the Parkland formula and the patient was ventilated and treated with piperacillin/tazobactam. Patient was taken several times to the operating room for amputation of upper extremity, escharectomy and skin grafting. Ten days after admission in burn unit, the patient developed high fever, expectoration and leukocytosis. A fiberoptic bronchoscopy with bronchoalveolar lavage was performed and 11 days later revealed *aspergillus fumigatus*. A therapy with amphotericin B was initiated and continued for 29 days, and he has discharged free from symptoms and signs. On 37<sup>o</sup> day did another fiberoptic bronchoscopy. On 50<sup>o</sup> day bronchoalveolar lavage culture and galactomannan detection were negative. After 39 day the patient was extubated and clinically he had no signs of infection.

**Conclusion:** Infections remain a primary cause of morbidity and mortality in burned patients, with fungal infections being among the main pathogens.

Advancements in burn care therapy have extended survival of seriously burned patients, exposing them to increased risk of infectious complications, notably fungal infections. Invasive Aspergillosis must be considered as an emerging and devastating infectious disease in intensive care unit (ICU) patients, even in the absence of an apparent predisposing immunodeficiency. Total body surface area (TBSA) burn and length of stay (survival after burn) were identified as

contributing factors for the incidence of fungal infection and mortality due to fungus.

**P794 Acremonium endophthalmitis after cataract extraction: a report of two rare cases**

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**Objectives:** Infective endophthalmitis is one of the most significant complications of cataract surgery. Typically it is caused by the perioperative introduction of microbial organisms (mainly gram-positive bacteria) into the eye from the patient's normal conjunctival and skin flora. On the contrary, fungal aetiology is considered an extremely rare occurrence.

We report two cases of *Acremonium endophthalmitis* after cataract surgery on the same day in the same operating room of the hospital's ophthalmology clinic.

Both patients, a 75-years-old man and a 64-years-old woman, developed endophthalmitis 2 and 3 weeks after cataract extraction, respectively, with decrease in visual acuity, loss of media clarity, ocular hyperemia followed by palpebral edema, conjunctival hyperemia and hypopyon.

**Methods:** The samples culture was performed using conventional methods for bacteria and fungi. Fungal DNA for molecular analysis was extracted from colonies grown on Sabouraud dextrose agar at 30°C using "HPPT Preparation Kit" (Roche Diagnostics) following the yeast and moulds extraction manufacturer's protocols. A restriction fragment length polymorphism (RFLP)-PCR of the nuclear 5.8S and internal transcribed spacer (ITS2) regions of the ribosomal DNA, amplified with the universal ITS3 and ITS4 fungal primers, was performed by the endonucleases *Hinf*I, *Msp*I, *Sau*3 AI and *Taq*I.

**Results:** Moulds with similar macroscopic and microscopic features, consistent with *Acremonium* genus, were isolated from all the samples (vitreous washing, aqueous fluid and intraocular lens from the first patient and aqueous fluid from the second). Bacterial cultures were negative. Amplification products, ranging from 360 to 380 bp, were obtained from all the cultures indicating the presence of *Acremonium* spp. DNA. RFLP of the amplicons showed the same restriction pattern for all the samples culture from both the patients.

**Conclusion:** Since filamentous fungi with similar morphologies and the same 5.8S and ITS2 rDNA restriction pattern (though it was not possible to identify the *Acremonium* species involved, it is likely that it was the same in the two cases) were isolated from all the analyzed samples and the two patients underwent surgery on the same day in the same operating room, it is possible to speculate that inadequate sterilization procedures of the instruments or contaminated irrigation fluids and disposables could have been involved in the transmission of the infection.

**P795 Penicilliosis, cryptococcosis, and histoplasmosis: a comparative study between epidemiology, clinical features, microbiology, treatment, and outcome**

P. Uppathannarakorn\*, A. Jindamporn, C. Suankratay (Bangkok, TH)

**Objectives:** Penicilliosis, cryptococcosis, and histoplasmosis are among the most common yeast infections in Thailand. It is very much difficult to distinguish disseminated infection caused by these three fungi based on clinical manifestations alone. The aim of this study was to compare epidemiology, clinical features, microbiology, treatment, and outcome between these three fungal infections.

**Methods:** Medical records of all patients with diagnosis of *Penicillium marneffe*, *Cryptococcus neoformans*, *Histoplasma capsulatum* attended at King Chulalongkorn Memorial Hospital, Bangkok, Thailand from January 2001 to December 2010 were analyzed.

**Results:** Of 122 patients, there were 28, 50, and 42 *P. marneffe*, *C. neoformans*, and *H. capsulatum* infections, respectively. HIV infection

was the most common preexisting condition, accounting for 82.1%, 46%, and 82.1% of penicilliosis, cryptococcosis, and histoplasmosis, respectively. Of 28 patients with penicilliosis, all had disseminated infections. Of 50 patients with cryptococcosis, there were 35 (70%) localized (33 and 2 patients with meningitis and lung infection) and 15 disseminated infections. Of 42 patients with histoplasmosis, there were 5 (11.9%) localized (four and one patients with adrenal glands infection and myelitis) and 37 disseminated infections. Interestingly, the characteristic umbilicated skin lesions were noted in 22 (78.5%), 2 (4%), and 17 (40.5%) patients with penicilliosis, cryptococcosis, and histoplasmosis, respectively. The most common diagnostic method was based on microscopic examination of clinical specimens in 25 (89.3%), 49 (98%), and 41 (97.6%) patients with penicilliosis, cryptococcosis, and histoplasmosis. A definite diagnosis was made based on positive cultures in 17 (58%), 12 (24%), and 6 (14%) patients with penicilliosis, cryptococcosis, and histoplasmosis, respectively. The mortality was 7 (24%), 9 (18%), and 10 (23%) in penicilliosis, cryptococcosis, and histoplasmosis, respectively.

Variable	Penicilliosis (N=25)	Cryptococcosis (N=50)	Histoplasmosis (N=42)
<b>I. Epidemiology</b>			
-HIV infection	23 (82.1)	23 (46)	32 (82.1)
<b>II. Clinics</b>			
-Localized infection	0	35 (70) <sup>1</sup>	5 (11.9) <sup>2</sup>
-Umbilicated skin	22 (78.5)	2 (4)	17 (40.5)
<b>III. Microbiology</b>			
-Positive culture	17 (58)	12 (24)	6 (14)
-Positive microscopic examination	25 (89.3)	49 (98)	41 (97.6)
<b>IV. Outcome</b>			
-Mortality	7 (24)	9 (18)	10 (23)

1. Localized infection in cryptococcosis :33 meningitis and 2 lung infection

2. Localized infection in histoplasmosis : 4 adrenal glands infection and 1 myelitis

All data are shown as number and percentage in parenthesis.

**Conclusion:** Most patients with penicilliosis and histoplasmosis present with disseminated infection. In contrast, subacute and chronic meningitis is the most common presentation in most patients with cryptococcosis. In addition, the characteristic umbilicated skin lesions are frequently noted in patients with penicilliosis and histoplasmosis, compared to those with cryptococcosis.

#### **P796** Cryptococcosis risk factors for HIV-infected patients in Latvia

P. Aldins\*, I. Eksteina, G. Sture (Riga, LV)

Latvia HIV incidence is 12.9/100000, it is higher than EU average. The first HIV cases in Latvia was detected in the end of 80ties, but after 10 years HIV epidemic in drug users population was started. The result is increasing number of patients with different opportunistic diseases, very common are invasive fungal diseases, including cryptococcosis.

**Objectives:** Identify risk factors for cryptococcosis in HIV-infected patients in Latvia.

**Methods:** Investigated all patients with confirmed extrapulmonary Cryptococcosis diagnosis from 2001 to 2008 in AIDS department in Infectology Center of Latvia.

**Results:** In total, 97 patients included; 70% males, 79% IVDU. Diagnose of cryptococcosis based on antigen detection in 69%, on morphological findings in 4%.

All patients had evidence of HIV infection. CD4 < 350 mm<sup>3</sup> 67%, on HAART 42%. More than in common population were found serious illnesses: HCV 85%, TB 26%, Syphilis 14%, Manganese Parkinsonian syndrome and other.

Initially treatment started for 91 patient, with Fluconazole (77%), Amphotericin B (7%), Itraconazole (6%), Voriconazole (4%). Prophylactic treatment was continued for 81%.

Twenty-two of the discussed patients died, 15 of them had not received prophylactic treatment.

Disease cured 79% with CD4 > 350 mm<sup>3</sup>, 60% with CD4 < 350 mm<sup>3</sup>. Relapse showed in 13%, mainly in patients with CD4 < 350 mm<sup>3</sup> (62%).

**Conclusions:** Increased risk of cryptococcosis in Latvia has HIV infected patients with other serious disease, with CD4 < 350 mm<sup>3</sup>, not receiving HAART. Mortality risk increased if the patient does not continue to receive prophylactic antifungal treatment.

#### **P797** Genital white piedra caused by *Trichosporon japonicum*

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**Introduction:** *Trichosporon* species have been reported as emerging pathogens in humans. *T. japonicum*, named by Sugita et al. (1998), was isolated from air but few cases isolated from clinical specimens have been reported. We believe this is the first reported genital white piedra case caused by *T. japonicum*.

**Clinical case:** A female patient, aged 83, with hypertension, osteoarthritis and osteoporosis, came to the dermatology consultation with 1-month pruriginous vulva lesions. Physical examination revealed two whitish-brownish overlapping plaques in the labia majora, with slight oedema and erythema. No alopecic plaques were seen in pubic hair. On friction, plaques came away with difficulty; she had no other body skin lesions.

**Methods:** A sample of lesion flakes was taken by scalpel scraping and sent to the microbiology laboratory for direct fluorescence microscopy with calcofluor and the corresponding culture in Sabouraud dextrose agar (SDA) with chloramphenicol, and SDA with chloramphenicol and cycloheximide. Culture plates were incubated at 30°C for up to 21 days. Colonies were examined in a lactophenol cotton blue wet mount for microscopic characteristics.

**Results:** Direct microscopy showed no compatible fungal cells. By day 10 (30°C), colonies were observed on SDA and were yeast-like in consistency, butyrous, cream, smooth with a mucoid texture, becoming irregular to warty in older cultures. Observation microscopy with lactophenol-blue showed budding yeast cells, hyphae and arthroconidia. Urease activity and growth at different temperatures (25, 37 and 42°C) were seen. The Vitek II yeast identification system (bioMérieux) misidentified the isolate as *T. asahii*. Molecular identification of the isolate was performed in the Microbiology National Centre of Spain. The patient was treated empirically with 2% topical Itraconazol every 12 hours after shaving pubic hair. After isolating *T. japonicum*, the patient had 2-monthly check-ups. After a 6-month follow-up, only slight clinical improvement was noted so oral Itraconazol treatment began (100 mg/12 hour; 4–8 weeks). Microbiological controls were negative after oral treatment.

**Conclusions:** *T. japonicum* emerges as a pathogen agent in humans. Difficulties in the identification imply the delay of an earlier usage of appropriate antifungal therapy. Moreover, the Vitek II yeast identification system is not reliable enough to correctly identify clinically relevant *Trichosporon* species and molecular analysis is required.

#### **P798** Evaluation of invasive fusariosis in haematological patients: a 10-year retrospective study

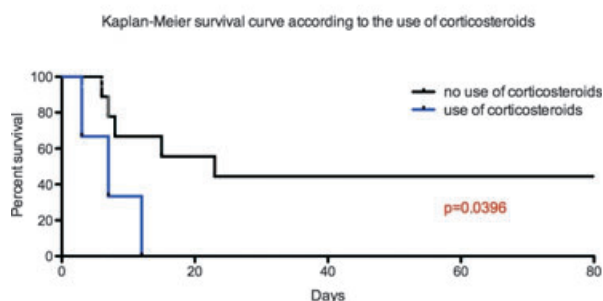
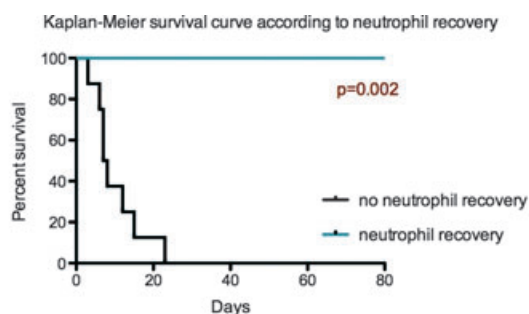
H.M. Morales\*, J. Nobrega, F. Dullely, M. Giudice, M.A. Shikanai-Yasuda, S.F. Costa (Sao Paulo, BR)

**Objectives:** The epidemiology of Invasive Fungal Infection in the hematological population has changed over the last two decades. *Fusarium* spp. represents the third most common cause of IFI among those patients. We aimed to evaluate the cases of Invasive Fusariosis (IF) in hematological patients from a Brazilian center.

**Methods:** IF patients were collected between 2001 and 2011. IF was diagnosed by EORTC/MSG criteria. The patient's charts were analyzed retrospectively for demographic, clinical and therapeutical

characteristics. The clinical outcome was obtained at 12 weeks and a Kaplan-Meier Survival Analysis with Logrank Significance Test performed using GraphPad Prism 5.0.

**Results:** Twelve patients with IF were identified. There were eight male and four female subjects. The mean age was 32 (16–52) years. The most common underlying diseases were Aplastic Anemia (4) and Acute Leukemia (4). Six patients were previously submitted to bone marrow transplant, five of them allogeneic. Five patients were classified as nosocomial fusariosis. The majority of cases (11) were neutropenic in the time of diagnosis and fever was the most common sign (11). Five patients had concomitant bacteremia. The median SOFA score in the time of diagnosis was 4 (3–11) and 27 days was the median time of neutropenia before the diagnosis. The diagnosis was made mainly by blood culture (5) and by cutaneous culture (4). Nine patients had disseminated fusariosis. No inflammatory response (2) and purpuric perivascular dermatitis (2) were the major findings in the cutaneous biopsy. Pulmonary fusariosis had radiographic manifestations of an angiotropic mold; nodules (6) and consolidation (6) were the most common findings on CT. Monotherapy was used in three patients. Eight received combined therapy, all of them consisting of amphotericin B plus voriconazole. The survival rate at 12 weeks was 33% and the median time of survival was 8 days. The use of high dose corticosteroids ( $p = 0.04$ ) and the neutrophil recovery ( $p = 0.002$ ) were statistically related with mortality at 12 weeks (figure 1).



**Conclusion:** Invasive Fusarial is a rare yet lethal disease with no defined therapy. The impact of combined therapy on the outcome of IF is still controversial. We couldn't demonstrate the potential benefit of this strategy; on the other hand, our data reinforced the importance of high dose corticosteroids and the neutrophil recovery on survival of our patients.

#### **P799** Efficacy of micafungin on *Geosmithia argillacea* infection in a cystic fibrosis patient

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Cystic fibrosis are at risk of colonization by a number of fungi, including *Geosmithia argillacea*. We report here a case of clinical and

microbiological efficacy of micafungin in a cystic fibrosis patient chronically colonized with *Geosmithia argillacea*.

O.D., a female F508Del-CFTR homozygous patient was diagnosed at birth with cystic fibrosis in January 1996. She was found chronically colonised with *Aspergillus fumigatus* from 2001 to 2006. She was treated alternatively with oral voriconazole and itraconazole from 2004 to 2008, and with posaconazole since february 2008. *Geosmithia argillacea* was first diagnosed in May 2007, and chronic colonisation was persistent from this date to August 2010 with 23/28 fungus positive sputum samples, in spite of posaconazole therapy. For an isolate obtained in October 2008, minimal inhibitory/effective concentrations (MIC/MEC, mg/mL) determined using the Eucast method were 2.0, 2.0, 16.0, 2.0, 0.25 and 0.015 for amphotericin B, itraconazole, voriconazole, posaconazole, caspofungin and micafungin, respectively. The FEV1 predicted value was 73% at the time of first fungus isolation and was decreased to 47% in October 2009. She then was given caspofungin for 21 days ((70 mg/day, later reduced to 50 mg) which resulted in clinical improvement (FEV1 = 64% in January 2010) without eradication of *G. argillacea*. O.D. was then treated from September, 23 to November 3, 2010 with micafungin (100 mg bid for 21 days and 100 mg/day for the following 21 days) which resulted in clinical and microbiological improvement. FEV1 predicted ranged 67–68% in October and December 2010, and February and May 2011, and from the end of treatment to December 2010, 5/6 sputum samples were found negative for *G. argillacea*. The positive sample contained fungus of the same genotype as previous isolates.

The present case is to our knowledge the first description of *G. argillacea* eradication in a chronically colonized cystic fibrosis patient. Since no change in bacterial colonization was observed before, during, and after *G. argillacea* colonization, the present case is consistent with a pathogenic role of the fungus in cystic fibrosis patients. In vitro antifungal susceptibility assays suggested that echinocandins are most effective agents against this fungus with a lowest MEC for micafungin (seven isolates studied, MEC range: 0.015–0.03), although eradication could only be obtained with high dose micafungin for a long time (6 weeks).

#### **P800** Central nervous system aspergillosis in Saint Petersburg, Russia

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**Objectives:** To define demographic parameters, underlying diseases, aetiology, clinical features and survival rate in patients with central nervous system (CNS) aspergillosis in Saint Petersburg, Russia.

**Methods:** Retrospective analysis. The diagnosis was established on the basis of EORTC/MSG 2008 criteria. Cases of proven or probable invasive aspergillosis (IA) were included.

**Results:** In 1998–2010 year. Two hundred and ninety-five cases of IA were registered in 19 hospitals in Saint Petersburg. CNS aspergillosis was revealed in 11 (3.7%) patients, three men and eight women, 4–67 years old (median – 30.5), seven adults and four children.

Primary host conditions were hematological disorders (55%) (including acute leukemia – 36%), non-hematological malignancy (9%), viral meningitis (9%), infectious hepatitis (9%).

Major risk factors were prolonged neutropenia (72%), lymphocytopenia (72%), cytostatic chemotherapy (64%), corticosteroid therapy (45%), stem-cell transplantation (9%), bacteremia (18%).

Extra-CNS sites of infection were revealed in 82% of patients and included: lungs (55%), myocardium (9%), more than two organs (18%). The most common symptoms were: fever, headache, mental status alterations, drowsiness, dysarthria, hemiparesis ans seizures.

Galactomannan test in serum and cerebrospinal fluid was positive in 72% of patients, positive histology – in 36%, *Aspergillus fumigatus* was isolated from cerebral abscess in 9% of patients.

Antifungal therapy was conducted in 64% of patients: amphotericin B – 45%, voriconazole – 36%, itraconazole – 36%, caspofungin – 18%, amphotericin B lipid complex – 9%. Surgical treatment was used in 9% of cases. Four patients (36%) died before initiation of treatment.

Twelve-week overall survival rate in patients who received treatment was 86%, 12 months – 57%. Overall survival rate in patients with CNS aspergillosis did not differ significantly from the survival rate in patients with invasive pulmonary aspergillosis.

**Conclusion:** CNS aspergillosis developed in 3.7% of patients with invasive aspergillosis. Main underlying diseases were hematological malignancies (55%). Extra-CNS sites of infection were revealed in 82% of patients. Twelve-week overall survival rate was 86%.

#### **P801** Considerable increase in frequency of *Saccharomyces* species in vaginitis

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**Objects:** In contrast with the tendencies seen for the distribution of fungal agents of invasive infections, where non-*C. albicans* *Candida* species and non-*Candida* species are becoming increasingly more frequent new opportunistic agents of infection, our statistical analysis did not reveal such a tendency for genital samples; recent literature sources confirm this [1].

**Methods:** Between 2003 and 2010, we processed 15 000–20 000 genital samples annually (vaginal, urethral, glans, etc.), mainly from women. The results were analysed statistically to examine the tendencies in frequency of fungal pathogenic agents.

**Results:** Fluconazole-resistant emerging invasive fungi such as *C. glabrata* and *C. krusei* decreased in frequency in genital samples, as did *C. inconspicua*. Fluconazole-sensitive non-*C. albicans* *Candida* species likewise decreased in frequency (*C. parapsilosis* and *C. kefyr*), or exhibited a constant low frequency (*C. tropicalis* and *C. guilliermondii*), but none of them became more frequent in the examined period. The incidence of the main pathogenic agent, *C. albicans*, rose slowly (85–90%). Most of the non-*Candida* fungi (*Rhodotorula*, *Kloeckera*, *Pichia*, *Cryptococcus*, *Geotrichum*, etc.) displayed only a low frequency. On the other hand, independently of the age groups, the rise in frequency of *Saccharomyces* species (mainly *S. cerevisiae*) in frequency was spectacular: in the examined 8-year interval, i.e. from 1.14% to 3.37%, a 2.95-fold increase in frequency.

**Conclusions:** The pathogenic processes in cases involving *Saccharomyces* sp. frequently lead to chronic vaginitis as *Saccharomyces* sp. often have intrinsic resistance to fluconazole and other antifungal agents (other azoles). Such circumstances are possibly responsible not only for the chronic pathogenic processes [2], but also for the considerable rise in frequency. 1. Hetticarachchi, N., Ashbee, HR., Wilson, JD. Prevalence and management of non-albicans vaginal candidiasis. Sex Transm Infect 2010; 86: 250. 2. Sobel, JD., Vazquez, J., Lynch, M., et. al. Vaginitis due to *Saccharomyces cerevisiae*: epidemiology, clinical aspects, and therapy. Clin Infect Dis 1993; 16 (1): 93–99.

#### **P802** Could a shorter incubation period be possible for dermatophytes?

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**Objective:** Dermatophytes are a group of fungi able to infect nails, skin or hair of a living host, causing dermatophytosis. Diagnosis is carried out by conventional culture in most hospitals. Most authors recommend to incubate the cultures for a minimum of 4 weeks if dermatophytes are suspected. In this study we try to analyze the necessity to incubate the samples over four weeks and the effectiveness of a shorter incubation time, evaluating if dermatophytes could grow in two weeks, in order to decrease the incubation time.

**Methods:** A total of 3573 samples were examined in a period of 27 months. The samples were cultured in Sabouraud dextrose agar, Potato Dextrose agar (PDA) and Dermatophyte Test Media (DTM) and were incubated at 30°C for four weeks. Culture plates were examined twice a week (on Monday and Thursday) and the day on which the fungal growth was first noted, was recorded.

**Results:** From the total of 3573 samples examined, 606 (16.94%) were positive for dermatophytes' growth and 2967 (83.04%) were negative. For 571 (94.22%) samples dermatophytes appeared earlier than two weeks of incubation. In 9 (1.46%) cases the time of detection was between 15 and 17 days. In 10 (1.65%) isolates growth took place after 18 days of incubation. Finally, the day of dermatophytes' growth couldn't be determined in 16 samples (2.64%).

Due to the limitation of examining plates twice a week, occasionally it was unable to perform the examination just at the fourteenth day so the examination time were in some cases between 15 and 17 days.

The negative predictive value (NPV) was calculated taking as false negative the number of samples in which dermatophytes' growth was after 18 days of incubation and considering true negative the number of samples in which the dermatophytes' growth was negative after a 4 weeks incubation. The NPV was 99.66%.

**Conclusion:** In the light of this study, to summarise we can conclude that after 15 days of incubation period the possibilities of dermatophyte grow are minimum. Thus, plates could be incubated only for this time with the aim of decreasing the laboratory workload and therefore costs.

#### **P803** *Candida* peritonitis after abdominal transplantation

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**Objectives:** To evaluate the characteristics, incidence rate and outcome of *Candida* peritonitis in abdominal transplantation.

**Methods:** All liver and pancreas transplant recipients from July 2003 to December 2009 were included. Pre-, peri- and post-transplant variables were prospectively collected. *Candida* peritonitis was diagnosed on the basis of macroscopic findings and positive culture of peritoneal fluid collected during operation or by needle aspirate in the early post-transplant period (first 30 days).

**Results:** During the study period we included 717 patients, 530 (74%) orthotopic liver, 130 (18%) simultaneous kidney-pancreas, 31 (4%) double liver-kidney, 24 (3%) isolated pancreas and 2 (1%) double liver-heart transplantation. We diagnosed five cases of *Candida* peritonitis: 3 in pancreas recipients (all *C. albicans*) and 2 in liver recipients (*C. tropicalis* and *C. krusei*). The incidence rate of *Candida* peritonitis during the first 30 days after transplantation was 6.5 cases/10 000 transplants days in pancreas recipients and 1.2 cases/10 000 transplant days in liver recipients ( $p = 0.035$ ). Fungal infection occurred in 38 patients in the cohort (19 aspergillosis, 15 candidiasis, 2 cryptococcal and 2 other fungi) of which 19 occurred in the first 30 days (*Candida* peritonitis represented 26% of cases). All five patients received oral nistatin previous to the development of *Candida* peritonitis. Four patients developed complications that required abdominal surgical reoperation. No cases with fungemia were diagnosed. Four cases had concurrent bacterial coinfection, three with peritonitis. Treatment included echinocandins plus fluconazole in three patients, fluconazole in monotherapy in one (*C. tropicalis*) and echinocandin plus amphotericin B in one (*C. krusei*). No cases of related mortality were registered and all patients were alive at 1 year follow-up.

**Conclusions:** *Candida* peritonitis risk was 5.4-folds more frequent in pancreas than in liver recipients. With appropriate treatment no fatal cases were observed. Although the number of patients is low, we must consider appropriate antifungal prophylaxis in patients with abdominal complications in the post-transplant period.



**P804 Invasive aspergillosis among heart transplant recipients: a 24-year perspective**

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**Background:** Invasive aspergillosis (IA) has very high mortality in heart transplant (HT) patients (pts). However, no recent series provide an updated, non-biased perspective of the problem.

**Methods:** Prospective follow-up of all HT pts from Aug 1988 to Aug 2011 (24-year study) with IA. Antifungal prophylaxis was started in Oct 1994.

**Results:** IA was diagnosed in 31/479 consecutive HT pts (6.5%): 25 proven (80.6%) and 6 probable. The incidence of IA decreased: 74% of the cases occurred in the first 12 years (1988–1999). Early IA (first 3 months after HT) accounted for 23 cases (median 34 days after Tx [19–58]) and eight cases (26%) were late (median 125.5 days after HT [100–237]). The main risk factors were other cases of IA in the program (58.1%), CMV disease (54.8%), re-operation (38.7%) and post-Tx hemodialysis (19.4%). IA emerged despite antifungal prophylaxis in eight cases with low levels (7 itra, 1 caspo). The most common symptoms were fever (45%), dyspnoea (35%) and cough (32%). Six pts were asymptomatic (19%). The predominant radiographic patterns were nodular (58%), cavitation (42%), pleural fluid (39%) and alveolar infiltrate (23%). IA affected the lungs (90.3%), central nervous system (CNS) (16%), mediastinum (9.7%), myocardium (6.5%) and skin, prostate and paranasal sinuses (3.2% each). Dissemination occurred in 26%. The efficacy of the diagnostic methods was as follows: culture 27/30 (90%) and PCR 4/5 (80%). Monotherapy was used in 77% (amphotericin B, 7; lipid amphotericin B, 12; voriconazole, 2), combined therapy in 7 pts (2 as rescue therapy) and surgery in 7 (22.6%). In four cases of early IA, diagnosis was postmortem. Related mortality was 32% (43.5% [10/23] in 1988–1999 cases and 0/8 in 2000–2011). Mortality was lower in early cases (16% vs 58%,  $p = 0.074$ ). Risk factors for mortality in the univariate analysis were long pre-Tx stay, pre-Tx mechanical ventilation (MV), emergency surgery, OKT3 induction, concomitant CMV disease, CNS involvement (mortality 100%), alveolar infiltrate, need of MV (mortality 50%) and thrombocytopenia. Multivariate analysis showed that CNS involvement and CMV during IA were independent risk factors for mortality.

**Conclusion:** The incidence of IA in HT has decreased, partially due to implementation of antifungal prophylaxis. Most cases occur in the first 3 months post-HT with a high frequency of disseminated disease and atypical sites of infection (heart, mediastinum, prostate). Mortality has decreased significantly in recent years.

**P805 Isolation of *Aspergillus* species from the respiratory tract of lung transplant recipients is associated with increased mortality**

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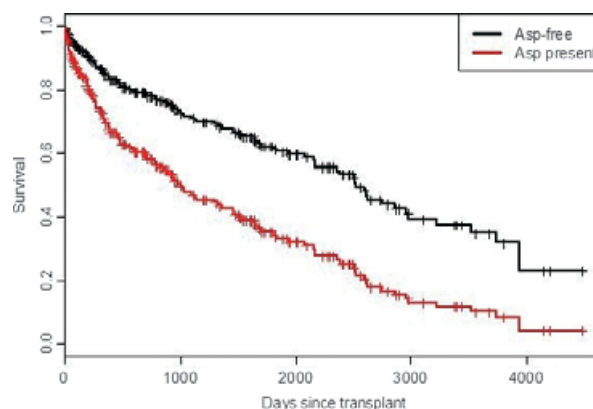
**Objectives:** *Aspergillus* spp. are the leading cause of invasive fungal infection in lung transplant patients. Invasive pulmonary aspergillosis (IPA) is responsible for an unacceptably high mortality despite optimal medical therapy. We investigated the relationship between the isolation of *Aspergillus* spp. from the respiratory tract of lung transplant recipients and their risk of mortality.

**Methods:** A retrospective, observational cohort study of all patients who received lung allografts between January 1999 and May 2011, at a single UK centre was performed. All patients received antifungal prophylaxis with fluconazole alone or in combination with nebulised amphotericin B deoxycholate. Patients were included in the *Aspergillus* group if they had at least one positive culture of *Aspergillus* spp. from the respiratory tract. The time from transplantation (Tx) to death was analysed using a Cox regression model. Covariates included gender, age, single vs. double lung Tx, ischaemic time, presence of airway

complication, antifungal prophylaxis regimen, episode of acute rejection, a diagnosis of cystic fibrosis vs. all other underlying diagnoses, donor and/or recipient cytomegalovirus (CMV) serostatus and whether *Aspergillus* was acquired before or after Tx.

**Results:** Two hundred-thirteen patients were included. The median follow-up time was 5 years during which 102 patients (47.9%) died. *Aspergillus* was isolated in 74 patients. In 15 patients (20.3%) *Aspergillus* was identified prior to Tx. Twenty patients (34%) had their first *Aspergillus* isolate in the first 60 days post Tx. Forty one patients (55.4%) in the *Aspergillus* group and 61 patients (43.9%) in the control group died during follow-up. Other than *Aspergillus*, CMV serostatus was the only covariate that had an impact upon mortality. A hazard ratio of 2.2 (95% CI 1.5–3.3;  $p < 0.001$ ) for death following a positive *Aspergillus* sample was observed from the regression model (See figure). Adjustment of the model for CMV status did not affect the hazard ratio. Isolation of *Aspergillus* prior to Tx was not associated with increased mortality.

**Conclusion:** Isolation of *Aspergillus* spp. from patients following lung



Tx is associated with a significant increase in mortality. Novel preventative strategies are required to minimise *Aspergillus* spp. colonisation in lung transplant recipients.

**P806 Mucormycosis in patients with haematological and oncological diseases in Saint Petersburg, Russia**

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**Background and Objectives:** Mucormycosis is an increasingly common infection in immunocompromised patients. The aim of the study – to evaluate clinical characteristics and results of treatment of haematological and oncological patients with mucormycosis in St. Petersburg, Russia.

**Methods:** The prospective study during the period 2005–2011. The diagnosis of mucormycosis was made according to EORTC/MSG criteria (2008).

**Results:** We observed 16 patients with underlying haematological and oncological diseases who developed proven (56%) or probable (44%) mucormycosis. The mean age of patients was 25 years (range 10–54), male and female ratio 1.2:1. Underlying diseases included: acute myeloid leukemia (44%), acute lymphoblastic leukemia (25%), neuroblastoma (6%), Hodgkin's lymphoma (6%), aplastic anemia (6%), myelodysplastic syndrome (6%), and myeloid sarcoma (6%).

In 50% of patients mucormycosis were diagnosed after or with invasive aspergillosis.

Main clinical forms of mucormycosis were: pulmonary (82%), subcutaneous (6%), osteomyelitis (6%), and gastrointestinal (6%). Two and more organs were involved in 44% of patients.

Diagnosis was established by histology and/or microscopy in all patients. In 56% of cases the diagnosis was confirmed by culture. Aetiologic agents included: *Lichtheimia corymbifera* (2), *Rhizopus microsporus* var. *oligosporus* (1), *Rhizopus* spp. (3), *Rhizomucor pusillus* (1), and *Rhizomucor* spp. (2).

Antifungal therapy was performed in 13 patients (three cases were diagnosed post-mortally). Posaconazole was used in 77% of patients, amphotericin B deoxycholate – 69%, caspofungin – 54%, amphotericin B lipid complex – 46%, and liposomal amphotericin B – 8%. Combination therapy was performed in 69% of patients (amphotericin B deoxycholate + caspofungin, posaconazole + amphotericin B deoxycholate). Twelve weeks overall survival was 38%.

**Conclusions:** 1 Main underlying diseases were acute myeloid leukemia and acute lymphoblastic leukemia;

2 Mucormycosis were diagnosed after or with invasive pulmonary aspergillosis in 50% of patients;

3 Pneumonia was most common clinical manifestation (82%); two and more organs were involved in 44% of patients;

4 Twelve weeks overall survival of haematological and oncological patients with mucormycosis was 38%.

#### P807 **Kodomaea ohmeri as an emerging pathogen in Southwestern China: three case reports and literature review**

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**Objectives:** *Kodomaea ohmeri* (*K. ohmeri*) is emerging as a new opportunistic fungal pathogen along with the increasing number of immunocompromised patients worldwide. It has recently been identified as an important etiology of fungemia, endocarditis, cellulitis, funguria and peritonitis in immunocompromised patients. However, risk factors, laboratory diagnoses, and treatments for *K. ohmeri* infection have been limited, and the optimal therapy for *K. ohmeri* infection has not been identified.

**Methods:** We report three new cases of infection caused by *Kodomaea ohmeri*. In the past 20 years 30 cases have been described in the English medical literatures. We review all cases described thus far to investigate the nature and treatment of *K. ohmeri* infections, and compare the new cases with those described in the literature.

**Results:** Case 1 presents a 61-year-old woman with *K. ohmeri* peritonitis. This is the second reported case of *K. ohmeri* peritonitis associated with CAPD and the first case of infection caused by *K. ohmeri* in China. Case 2 and Case 3 report two man suffered from *K. ohmeri* fungemia, which were the first reported two cases of *K. ohmeri* fungemia in Chinese mainland. We investigated 30 cases of *K. ohmeri* infection which have been reported in the English medical literatures (including the present three cases). Among these cases, 26 patients presented with fungemia, 2 with peritonitis, one with funguria and one with a wound infection. 73.3% (22 cases) of *K. ohmeri* cases were related to placement of central or peripheral venous catheters. Good outcomes (10/14 cases; 71.4%) were found following removal of indwelling catheters and implants. Amphotericin B and echinocandins, such as caspofungin and micafungin, also showed excellent minimum inhibitory concentrations against *K. ohmeri*. In addition, 40.0% (12/30) of the patients died as a result of overwhelming infection despite antifungal therapy.

**Conclusion:** *K. ohmeri* can cause systemic infections in a broad range of patients categories, particularly in immunocompromised patients with (without) catheter/prosthetic implants insertion. The increasing numbers of infection cases indicates that *K. ohmeri* would be added to the list of potential yeast pathogens. With high mortality of *K. ohmeri* fungemia, early identification and appropriate antifungal therapy in combination with removal of any implanted devices are of great significance to survival outcomes.

Priate antifungal therapy in combination with removal of any implanted devices are of great significance to survival outcomes.

#### P808 **Fungiscope – a Global Rare Fungal Infection Registry**

M. Vehreschild\*, A. Hamprecht, G. Fischer, S. de Hoog, J. Vehreschild, O.A. Cornely (Cologne, Stuttgart, DE; Utrecht, NL)

**Background:** We are coordinating a global registry for cases of rare invasive fungal diseases (IFD). Our objective is to broaden the knowledge on epidemiology, to determine the clinical pattern of disease, to describe and improve diagnostic procedures and therapeutic regimens, as well as to facilitate exchange of clinical isolates among the contributors.

**Methods:** Fungiscope™ – A Global Rare Fungal Infection Registry uses a web-based electronic case form accessible via [www.fungiscope.net](http://www.fungiscope.net). For inclusion in the registry we require positive cultures or histopathological, antigen or molecular genetic evidence of IFD and the associated clinical symptoms and signs of invasive infection. The data entered onto the registry include demographics, underlying conditions, neutrophil count, concomitant immunosuppressive medications, clinical signs and symptoms of IFD, site of infection, diagnostic tests performed, pathogen identification, antifungal treatment, surgical procedures performed, response to treatment, overall survival and attributable mortality. For an overview on the project's structure see figure 1.

**Results:** Overall, 244 cases have been completed. Zygomycetes (n = 95; 39%), yeasts (n = 40; 16%), *Fusarium* spp. (n = 37; 15%), and Dematiaceae (n = 31; 13%) were the most frequently registered pathogens.

Chemotherapy or allogeneic stem cell transplantation for a haematological malignancy was the most predominant risk factor (n = 118; 48%), as well as diabetes mellitus (n = 64; 26%), stay at an ICU (n = 42; 17%) and chronic renal disease (n = 34; 14%).

Sites of infection included the lung in 94 patients (39%), followed by blood stream infections (n = 45; 18%), the sino-nasal region (n = 35, 14%) and deep soft tissues (n = 33; 14%).

For 123 (50%) patients, a favourable outcome, defined as a complete or partial response to treatment of IFD was documented. Overall mortality and mortality attributable to IFD was 42% (n = 102) and 28% (n = 69), respectively.



**Conclusion:** The clinical relevance of rare IFD is increasing steadily. In a short period of time, a wide variety of cases from Europe, Asia and South America could be documented. Further investigators are cordially invited to contribute to Fungiscope.

**P809 Host factors for invasive fungal infection among patients with haematological malignancies: a case control study**

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**Objectives:** Host factors defined by revised EORTC/MSG criteria describe predisposing conditions of the individual for development of invasive fungal infection (IFI). Fulfilment of these factors is required for establishing diagnosis of possible or probable IFI which account for the majority of IFI in recent epidemiologic studies among patients with hematological malignancies. Host factors have, therefore, a major impact on IFI epidemiology when current criteria for defining IFI applied. This study evaluates host factors among patients with hematological malignancies.

**Methods:** This is a single center study. Fifty-eight patients with haematological malignancies who developed probable (n = 38) or proven (n = 20) IFI within a 5-year-period were retrospectively evaluated regarding host and risk factors for IFI such as neutropenia, use of corticosteroids or t-cell suppressants, stem cell transplantation (SCT), underlying diseases and demographic factors. *Aspergillus* spp. was the leading causative pathogen (n = 36), followed by *Candida* spp. (n = 12). Results obtained were compared to results of patients with hematological malignancies who did not develop IFI (120 patients who received systemic antifungal therapy and 197 patients who did not, all data collected in 2010). Patients with possible IFI were excluded from the study.

**Results:** Prolonged neutropenia, recent allogeneic SCT, steroid therapy and t-cell suppressive therapy were significantly associated with development of IFI and/or invasive mould infection (IMI) in our patient collective. In the case of prolonged corticosteroid use a cut-off of 14 days was highly significantly, while the currently proposed cut-off (21 days) was significantly associated with development of IFI. Results are depicted in Table 1.

	Group 1 Patients with Probable/Proven IFI	Group 2 Patients with Systemic Antifungal Therapy but without IFI	Group 3 Patients without Systemic Antifungal Therapy and without IFI	P Value if Significant
n	58	120	197	
Sex				
Male/female	40/18	72/47	100/97	
Age in Years (Median)	50.70	54.24	62.54	
Room of Care Hepa-filtered Room/Standard Room	14/44	19/101	9/188	
Underlying Disease				
AML	24	48	13*	P<0.001
MDS	4	12	3*	P<0.01
MM	2	4	3*	P<0.01
T-CLL	4	9	33*	P<0.01
T-ALL	10	8	3*	P<0.01
Others	7	11	18	
Polychemotherapy				
High Dose	30*	66	87	p<0.01
Low Dose	8	17	49	
None	11	37	61	
Bronchoscopy Rate	30/58 (52%)*	10/120 (8%)	54/197 (3%)	P<0.001
GWB	10	18	0	
1 or more Host Factors Present	19/58 (33%)*	15/120 (12.5%)	11/97 (11.5%)	p<0.01
Neutropenia (<0.05x10 <sup>9</sup> )				
No	17	62	175	
< 10 Days	6	39	15	
> 10 Days	35*	29	7	p<0.001
Median (Days) in Case of Neutropenia	17	10.5	6	
T-Cell-Suppressants within 90 Days	41	69	36*	P<0.001
Use of Corticosteroids (Mean Minimum Dose of 0.3 mg/kg/day of Prednisone Equivalent)				
No	32	80	121	
< 14 Days	0	31	12	
> 14 Days < 21 Days	5*	3	1	P<0.001
> 21 Days	13*	6	3	P<0.001
HSCt				
Allogeneic Current	11	12	0*	P<0.001
Allogeneic Prior	9	22	17	
Autologous Current	2	5	0	
Autologous Prior	3	2	2	

**Conclusion:** We conclude that host factors according to revised EORTC/MSG criteria were significantly associated with development of IFI/IMI in our collective of patients. In case of previous allogeneic

SCT not related to current onset of IFI we found, however, no association with IFI. Concerning prolonged corticosteroid treatment a cut-off of 14 days may seem favourable when compared to the currently proposed cut-off. Further and bigger studies are necessary to evaluate these issues.

**P810 Registry for systemic mycosis in Germany (ReSyMe) – role of EORTC/MSG diagnostic criteria in clinical practice**

M. Ruhnke\*, A. Mikojalewska, A. Groll, O.A. Cornely, C. Lass-Flörl on behalf of the ReSyMe working group

**Introduction:** The Registry of Systemic Mycoses (ReSyMe) was developed as a multi-center prospective study by the German-speaking Mycological Society (DMyG) and the Paul-Ehrlich-Society (PEG) to study the current use of antifungal therapy in Germany with regard to the EORTC/MSG diagnostic criteria (version from 2002).

**Material and methods:** In this prospective, non-comparative study, 543 patients treated with systemic antifungal therapy were documented within a 1 year period (5/2008–4/2009) via an online-documentation system. Primary questions of the registry addressed: (i) analyzing the current epidemiology for invasive fungal infections in Germany. (ii) analyzing the proposed definitions for invasive fungal diseases (possible, probable, and proven disease) on the bedside in everyday practice (EORTC/MSG). (iii) analyzing antifungal therapy in the ICU, as compared to non-ICU patients.

**Results:** Fifteen clinical units from 10 hospitals participated in the study. In total, 543 patients (3–101 patients per clinical unit) were documented, with a median age of 60 years (1–95 years). 61.5% were male (51.8–70%), 38.5% were female (48.2–30.4%). Two hundred and forty-two patients were treated at the ICU (44%) and 56% (n = 301) were non-ICU patients. Among the ICU patients, 67.8% underwent a surgical procedure (SD), 16.2% suffered from an internal disease (MD), 12.8% from haematological cancer (HC), and 3.3% had a solid tumor. According to the main underlying disease, all patients were divided into four groups: the largest group represented patients with a haematological malignancy (55.9%), followed by patients with surgical procedure (30.7%), internal disease (8.4%), and solid tumors (5%).

Patients with MD (mean 70 years) and SD (mean 64 years) were older as compared to the other two groups. Ninety-eight percent (SD) and 85% (MD) of patients were treated in an ICU as compared to 10% with HC. According to the EORTC criteria for the definition of an invasive fungal disease (IFD), antifungal therapy could be applied in ICU patients (SD) in 21% for proven IFD, 15% probable, and 36% possible IFD. In 21% treatment was given as empirical therapy in ICU patients as compared to 35% in non-ICU patients. The lungs (61%) were the most common site of IFD, 18% had a disseminated infection. The majority of patients received first-line treatment with azoles (33% fluconazole, 29% voriconazole). Echinocandins were given in 10% of patients. EORTC-MSG criteria could be applied in 72% of ICU patients, but in only 65% of non-ICU patients.

**P811 Pre-hospital risk factors for invasive fungal disease in patients with acute myeloid leukaemia at diagnosis: preliminary results from the SEIFEM 2010 Study**

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**Objectives:** To investigate the potential relationship between pre-hospital exposures to fungal sources and the development of invasive

fungal diseases (IFDs) in adult acute myeloid leukemia patients (AMLs).

**Methods:** From January 2010 to March 2011, in 31 Italian participating centers, all consecutive patients (pts) with newly diagnosed AMLs were registered. Information about personal habits and possible environmental exposures were investigated. In particular we collected data about: comorbidities, job, hygienic habits, work and living environment, voluptuary habits (i.e. smoking, alcohol, illicit substances abuse), hobbies, pets. We also included data on other well-known risk factors, such as age, neutropenia, mucosal damages, etc. In order to make our study population very homogeneous, we focuses on pts treated with conventional chemotherapy only. All cases of proven/probable IFDs occurred until the 30th day from the end of first induction were recorded.

**Results:** Five hundred and ninety-three pts were enrolled in the study; of them, 447 were included in the present analysis and 43 developed a proven/probable IFDs (30 molds and 13 yeasts) (incidence 9.6%). Median age was 61 (range 18–81). Main variables included in the risk analysis have been reported in the table. In particular, at preliminary analysis a significant association with IFDs development was found for performance status ( $p < 0.001$ ), chronic obstructive pulmonary diseases ( $p 0.04$ ), urinary catheter ( $p < 0.001$ ), neutropenia ( $<500$  neutrophils/ $\mu\text{L}$ ,  $>7$  days) ( $p 0.03$ ).

A not significant trend was noted for incidence by gender (males 12% vs females 7%), for diabetes (yes 18%, no 9%), construction sites in the last 3 months to  $<500$  m from home (yes 12%, no 8%), home restructuring in the last 6 months (yes 14%, no 9%). We did not find any association for weight, occupational exposure, geographical origin. For mold infections only, those patients living in a flat resulted to be at higher risk when compared to those living in house with garden ( $p 0.03$ ). Other variables showing a correlation with the onset of invasive yeast diseases were chronic kidney failure ( $p 0.006$ ) and liver diseases ( $p < 0.001$ ).

VARIABLES	# <sup>a</sup> pts	# <sup>b</sup> cases	p-value
Gender			
- male	232	28 (12%)	0.04
- female	215	15 (7%)	
Performance status			
- 0-1	342	22 (4%)	<0.001
- 2-3	97	18 (19%)	
- 4	8	3 (38%)	
Diabetes			
- No	489	34 (9%)	0.054
- Yes	38	7 (18%)	
Chronic kidney failure			
- No	434	40 (9%)	0.09
- Yes	13	3 (23%)	
COPD			
- No	434	35 (9%)	0.04
- Yes	23	5 (21%)	
Liver disease			
- No	428	39 (9%)	0.003
- Yes	19	4 (21%)	
cigarette smoking			
- No	327	31 (9%)	0.2
- Yes	120	12 (10%)	
Mucositis stage <sup>c</sup>			
- 0	267	17 (8%)	0.34
- >0	240	24 (11%)	
CVC			
- No	112	9 (8%)	0.5
- Yes	335	34 (10%)	
Urinary catheter			
- No	349	24 (7%)	<0.001
- Yes	78	19 (24%)	
Neutropeni <sup>d</sup>			
- No	37	0	0.03
- Yes	410	43	
Type of house			
- flat	248	18 (7%)	0.038
- house with garden	199	25 (13%)	
Home restructuring			
- No	398	34 (9%)	0.24
- Yes	49	7 (14%)	
Construction site near home			
- No	310	24 (8%)	0.18
- Yes	137	17 (12%)	

**Legend:**

CVC: central venous catheter; COPD: chronic obstructive pulmonary disease

<sup>a</sup>: it comprehend oral mucositis, esophagitis, vomiting, diarrhea. Zero means none of them.

<sup>b</sup>: Neutrophils  $<500/\mu\text{L}$ , duration  $> 7$  days.

<sup>c</sup>: In the last 6 months.

<sup>d</sup>: in the last 3 months, within 500 meters.

**Conclusions:** Several hospital-independent fungal sources emerged at univariate analysis to potentially influence IFDs onset. Investigation of

these factors at time of admission may be helpful in defining patients' risk category and in better targeting prophylactic strategies.

**P812** Prospective registry of invasive fungal diseases in acute myeloid leukaemia: preliminary results on 142 cases

M. Caira\*, A. Candoni, A. Busca, M. Delia, C. Caramatti, C. Cattaneo, L. Melillo, M.E. Mitra, L. Paris, L. Potenza, G. Leone, A. Nosari, F. Aversa, L. Pagano on behalf of the SEIFEM group

**Objectives:** To evaluate epidemiological characteristics, treatments and outcome of invasive fungal diseases (IFDs) in acute myeloid leukemia patients (AMLs).

**Methods:** From January 2010 to March 2011, 31 Italian participating centers registered all consecutive cases of IFDs in adult AMLs at first induction (until 30th day from the end of chemotherapy). The parameters we analyzed were: age, sex, severity and duration of neutropenia, antifungal prophylaxis, certainty of IFD diagnosis, empirical/pre-emptive therapy, target therapy, etiologic agent, outcome. Response rate to antifungal therapy and mortality rate were thus analyzed.

**Results:** over a 15 month period, 142 IFDs were collected in 593 newly diagnosed AMLs (incidence 23.6%). Median age was 60 (range 18–81), with a male/female ratio of 1.6/1. The most part of IFDs (128, 90%) occurred in pts who had received conventional chemotherapy (128/498, incidence 25.7%). As expected, IFDs incidence was lower in those receiving either supportive or low dose therapy (14/95, 14.7%). Probable and proven IFDs were 37 and 14, respectively; remaining cases were classified as possible IFDs (91, 64%). A deep neutropenia (PMN count  $<500/\mu\text{L}$ ) lasting for at least 7 days occurred in 129 of them (91%). Antifungal approaches are reported in the table. Most of pts had received systemic antifungal prophylaxis (120/142, 85%), more frequently with posaconazole. Liposomal AmB and caspofungin were the most frequently employed drugs, as empirical/pre-emptive therapies. Of 51 proven/probable IFDs, the majority were mold infections (36, 69%), with a mold/yeast ratio of 2.4/1. Among molds, aspergillosis (IA) were predominant (27, 75%). Four cases of rare fungal agents were identified (one *Fusarium*, one Blastoschizomices, one Geotrichum and one Trichosporon). At 30th day, 104 pts had achieved a favourable response; the overall response rate was 73%. IFD-attributable mortality rate (AMR) was 11.3%, ranging from 5.5% for possible to 21.6% for proven/probable cases.

	ALL CASES (142)	Possible IFDs (91)	Proven/probable IFDs		
		N° cases (91)	N° cases (51)	Molds (36)	Yeasts (15)
Systemic antifungal prophylaxis	120 (85%)	72	42 (82%)	30	
> Administered	22 (15%)	13	9 (18%)	6	12
> Not administered					3
Prophylactic systemic drug					
> Itraconazole	34 (28%)	22	12 (29%)	9	3
> Fluconazole	28 (23%)	14	14 (33%)	9	5
> Posaconazole	55 (46%)	40	15 (36%)	12	3
> Other	3	2	1 (2%)	0	1
Empirical/pre-emptive					
> Performed	124	85	39 (76%)	31	8
> Not performed	18	6	12 (24%)	5	7
Empirical/pre-emptive drug <sup>1</sup>					
> Caspofungin	25 (24%)	17	8 (23%)	6	2
> L-AmB	51 (48%)	37	16 (46%)	11	5
> Voriconazole	17 (16%)	8	6 (23%)	7	1
> Abirater	8 (7.5%)	7	1 (3%)	1	0
> Other	5 (4%)	3	2 (5%)	1	0
Drug in 1 <sup>st</sup> line target therapy <sup>2</sup>					
> L-AmB			11 (27%)	8	3
> Caspofungin			4 (14%)	0	5
> Voriconazole			20 (48%)	17	5
> Combined			3 (7%)	0	3
> Other			2 (5%)	1	1
Favourable responses (RR)	104 (73%)	72 (79%)	32 (63%)	25 (69%)	7 (47%)
IFD attributable deaths (AMR)	16 (11.3%)	5 (5.5%)	11 (21.6%)	5 (13.9%)	6 (40%)

**Conclusions:** IFDs continue to be a challenging complication in high risk patients. Our results confirm the recently reported trend in reduction of IFD-AMR. On the contrary, cases with unidentified origin continue to be the most frequent. This datum makes it necessary

to improve our diagnostic work-up to better target treatment and preventive strategies, and to reduce the risk of overtreatment.

## Lab news: from diagnosis, in vitro activity, pathomechanisms to antifungal resistance

### P813 Investigation of efflux pumps and ERG11 gene expression levels in fluconazole resistant *Candida albicans* isolates

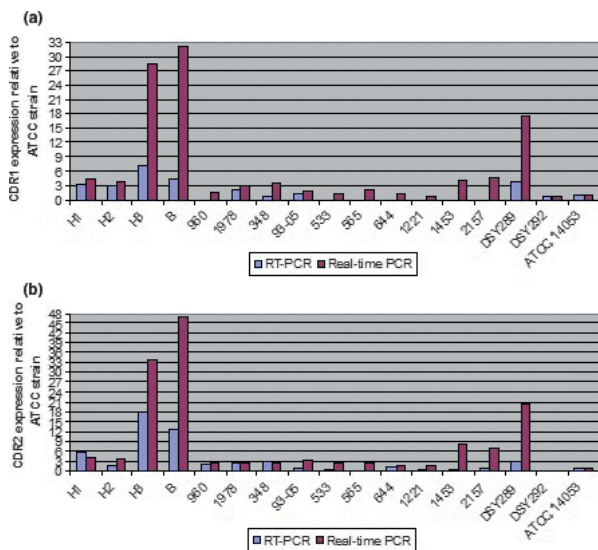
S. Gulat, O. Irmak, M. Doluca Yucesoy\* (Izmir, TR)

**Objectives:** Widespread and repeated use of fluconazole in the prophylaxis and therapy resulted in resistance among *Candida albicans* strains. In this study, investigation of the expression of efflux pump encoding genes, CDR1, CDR2, and MDR1 and lanosterol demethylase encoding gene, ERG11 by reverse transcription- and real-time polymerase chain reaction (PCR) was aimed in fluconazole susceptible (S)/resistant (R) *C. albicans* isolates in order to determine the role of these mechanisms in fluconazole resistance.

**Methods:** Five fluconazole R, six S and three trailing effect showing S *C. albicans* strains, isolated from clinical specimens in three hospitals in Turkey were included. MIC values of fluconazole were determined by microdilution method performed according to CLSI M27-A3 standards and E-test on yeast extract peptone dextrose agar with and without cyclosporin A.

The expression of CDR1, CDR2, MDR1 and ERG11 transcripts were determined by semi-quantitative reverse transcription (RT)-PCR and quantitative real-time PCR methods. The expression of these genes was normalized with their housekeeping gene (ACT1) levels and compared with the drug susceptible *C. albicans* ATCC 14053 strain. Gene expression data were analyzed by Kruskal–Wallis and Mann–Whitney *U* tests via using SPSS version 15.0 software.

**Results:** For the detection of efflux pump gene expressions, both methods were generally consistent with each other. Mean CDR1, CDR2, MDR1 and ERG11 gene expression levels were higher in the fluconazole R isolates than the trailing effect showing S and S ones. Among five fluconazole R isolates, two and one isolate overexpressed CDR1 and CDR2 (Figure 1), and MDR1, respectively but none of them overexpressed ERG11. Statistically significant differences were observed for CDR1 and CDR2 expression levels between fluconazole R, trailing and S isolates when tested with RT-PCR ( $p < 0.05$ ), whereas this was not true when tested with real-time PCR ( $p > 0.05$ ). On the other side, no statistically significant difference was detected for MDR1 and ERG11 expressions among the isolate groups ( $p > 0.05$ ).



**Conclusion:** It can be concluded that overexpression of efflux pump genes, but not of ERG11, can be important mechanisms of resistance in our fluconazole R *C. albicans* isolates.

### P814 Combination of voriconazole and anidulafungin for the treatment of triazole-resistant *Aspergillus fumigatus* in an in vitro model of invasive pulmonary aspergillosis

A.R. Jeans\*, S.J. Howard, Z. Al-Nakeeb, J. Goodwin, L. Gregson, P.A. Warn, W.W. Hop (Manchester, UK)

**Objectives:** Voriconazole (VCZ) is the preferred treatment for invasive pulmonary aspergillosis but isolates of *Aspergillus fumigatus* with elevated VCZ MICs are increasingly seen and carry a greater risk of treatment failure. We investigated whether the combination of VCZ with anidulafungin (AFG) may be beneficial for the treatment of *A. fumigatus* strains with elevated VCZ MICs.

**Methods:** We used an in vitro model of the human alveolus to define the exposure response relationships for wild-type strains and those with defined molecular mechanisms of triazole resistance. A wild-type isolate (VCZ MIC 0.5 mg/L) and two strains with amino acid substitutions in the VCZ target protein Cyp51A (L98H; MIC 4 mg/L, G138C; MIC 16 mg/L) were studied. All strains had AFG minimum effective concentrations (MECs) of 0.0078 mg/L. Twenty-five different combinations of VCZ and AFG were investigated for each strain. Exposure response relationships were estimated using galactomannan (GM) as a biomarker. Drugs were administered to the endothelial compartment 6 hours post inoculation. Concentrations of VCZ and AFG were measured using HPLC. The interaction of VCZ and AFG was described using the Greco model.

**Results:** Fungal growth was progressively inhibited with higher drug exposures of VCZ. Strains with elevated VCZ MICs required proportionally greater VCZ exposures to achieve a comparable antifungal effect, but GM levels could be suppressed for all strains. GM levels were reduced by AFG monotherapy but not fully suppressed, and no additional reduction was achieved by further increases in concentration above the MEC. An additive effect between VCZ and AFG was demonstrated.

**Conclusion:** The combination of VCZ and AFG may be beneficial in the treatment of invasive pulmonary aspergillosis for isolates of *A. fumigatus* with reduced susceptibility to VCZ.

### P815 Evaluation of antifungal activity of ZnO/Ag nanoparticles on *Candida albicans* biofilm

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**Objectives:** Biofilms represent the most common type of microbial growth in nature and are critical to development of many infections in human. Recently it has been reported that 65% of all human infections involve biofilms. Nowadays *Candida albicans* has more effective role among nosocomial pathogens due to proper potential for biofilm formation. Using a novel method in field of inhibitory formation of *C. albicans* biofilm and elimination of fungal mass over surfaces is valuable for controlling infections. In this study antifungal effect of ZnO/Ag nanoparticles were evaluated on standard strain of *C. albicans*.

**Methods:** ZnO nanoparticles and Ag were synthesized through the sol-gel method by Zinc Acetate Dehydrate [ $Zn(CH_3COOH)_2 \cdot 2H_2O$ ] and  $AgNO_3$  as precursor respectively. The morphology properties of the products were analyzed by using Scanning Electron Microscopy (SEM). Biofilm of standard strain of *C. albicans* (ATCC 10231) was developed on flat-bottomed 96-well microtiter plates and antifungal effects of ZnO/Ag nanoparticles were evaluated by using standard accurate method, CCK8 (Cell Counting Kit 8) as colorimetric assay for measuring metabolic activity of sessile cells within the biofilm as it relates to cellular viability. Data were analyzed using t-test statistical method and SPSS software.

**Results:** Different Concentrations of synthesized ZnO/Ag (0.01–0.5 µg/mL) were assessed on *C. albicans* biofilm. Evaluation of the Morphology properties of the nanoparticles with SEM showed that nanoparticles were spherical and regular. Optical density of survival cells in coated samples was less than control samples (not treated) and at the concentration of 0.317 µg/mL was determined  $0.091 \pm 0.04$  in comparison of control groups  $0.253 \pm 0.07$ . Data of treated samples showed significant difference with control samples ( $p < 0.05$ ).

**Conclusion:** According to findings of this study ZnO/Ag nanoparticles were synthesized by means of wet chemical were showed significant antifungal property. So in field of elimination of the nosocomial infection, ZnO/Ag nanoparticles could be proper candidates as self cleaning or disinfectant agents and also could be used as coating of surfaces, utensils, furniture and lots of other materials. They could even be used with other antimicrobial agents with greater efficiency than ever.

**P816** **In vitro activity of isavuconazole against 1488 *Aspergillus* isolates – a pooled analysis of nine studies**

J. Smart\*, M. Jones, N. Azie (Deerfield, US; Basel, CH)

**Background:** Invasive aspergillosis remains an important cause of morbidity and mortality. Isavuconazole (ISA) is a novel, intravenous and orally available, broad-spectrum, water-soluble triazole currently in Phase III clinical trials for treatment of patients with invasive *Aspergillus*, *Candida*, and rare mould infections, including patients with renal impairment. The active moiety of ISA is BAL4815, which has been shown to have in vitro antifungal activity against clinically relevant species of yeasts and moulds including *Aspergillus* as well as Zygomycetes.

**Methods and Results:** We examined the in vitro activity of ISA against 1488 isolates of *Aspergillus* (Table 1). These data represent a compilation of minimal inhibitory concentration (MIC) data from 7 published reports and 2 unpublished laboratory studies undertaken by various academic investigators in EU and US laboratories during the period 2004–2010. All MIC data in the pooled analysis were derived using CLSI (M38-A) or EUCAST standard methodologies, although there were differences in methodologies between the test centers related to incubation period, determination of MIC endpoint, and number of replicates.

**Results:** Overall, ISA was active against all clinically relevant species of *Aspergillus* tested. The MIC results were evaluated at either 24 hours or 48 hours. The ISA and voriconazole (VRC) MIC ranges, MIC50s, and MIC90s are listed in Table 1 for each of the *Aspergillus* species tested. For the majority of the *Aspergillus* species, the 24 and 48 hour

MIC results were within a two-fold dilution for both ISA and VRC. At 24 and 48 hours, the overall MIC90 for all *Aspergillus* species was 1.0 mg/L for both ISA and VRC.

**Conclusions:** This pooled analysis of multiple studies demonstrates the potent in vitro activity of ISA against diverse range of *Aspergillus* species, including species refractive to other therapies such as *A. terreus*. These MICs are within clinically relevant concentrations achievable with clinical dosing. ISA is a promising agent for the treatment of invasive aspergillosis.

**P817** **Antifungal susceptibility of clinical *Cryptococcus neoformans/C. gattii* complex isolates in Korea**

E.J. Kang\* (Suwon, KR)

**Objectives:** Cryptococcosis is an opportunistic infection caused by two species of the genus *Cryptococcus neoformans* and *Cryptococcus gattii*. We previously reported the molecular epidemiological analysis of 78 clinical strains of the *C. neoformans/C. gattii* species complex, but antifungal susceptibility test was not taken. The Purpose of this study is to demonstrate the pattern of antifungal susceptibility of *Cryptococcus neoformans/C. gattii* Complex of South Korea.

**Methods: Strains** The 78 strains were isolated from the clinical specimens recovered from cryptococcosis patients who had been hospitalized between 1999 and 2008 in seven medical centers in South Korea.

**Antifungal susceptibility 1** Microdilution test

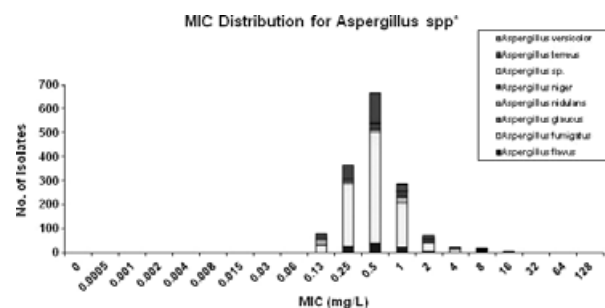
Microdilution test was carried out according to the Clinical Laboratory Standard Institute M27-A3 document. Amphotericin-B, voriconazole and fluconazole were tested against all isolates twice. *C. parapsilosis* ATCC 22019 was included as quality control.

**2** VITEK-2 system

VITEK-2 system was performed according to the manufacturer’s instructions.

**Statistics:** Statistical analysis was performed using the program SPSS version 18.0 software, and p-value < 0.05 was used to define significance. We compared the MIC of fluconazole in microdilution test according to the time period divided by 1999–2000 and 2001–2008. The Mann–Whitney test was used for comparison of MIC according to the time period.

**Results:** Among 78 strains, 75 strains (96%) were *C. neoformans* serotype A and three strains (4%) were *C. gattii*, serotype B. The antifungal susceptibility test results shows in Table 1. There is no resistant strain to amphotericin-B and voriconazole. Two strains are susceptible dose dependent for fluconazole in broth microdilution test. One strain in 73 strains appears to be resistant for flucytosine in VITEK-2 system. Eleven strains were recovered in 1999 to 2000 and 67 strains were in 2001 to 2008. Geometric means were 5.84 and 4.54 respectively. The difference of MIC between the two time periods was not statistically significant. ( $p = 0.698$ )



Species	No. of isolates	Isavuconazole (MIC mg/L)						Voriconazole (MIC mg/L)					
		24 hours			48 hours			24 hours			48 hours		
		Range	MIC <sub>50</sub>	MIC <sub>90</sub>	Range	MIC <sub>50</sub>	MIC <sub>90</sub>	Range	MIC <sub>50</sub>	MIC <sub>90</sub>	Range	MIC <sub>50</sub>	MIC <sub>90</sub>
<i>A. aspergillus</i>	1448	0.062-16	0.5	1	0.062-4	1	1	0.06-2	0.25	1	0.06-8	0.5	1
<i>A. fumigatus</i>	563	0.125-4	0.5	1	0.062-4	1	1	0.06-2	0.25	0.5	0.06-2	0.5	1
<i>A. terreus</i>	95	0.125-2	0.5	1	0.125-2	0.5	1	0.125-1	0.5	1	0.125-2	0.5	1
<i>A. nidulans</i>	75	0.062-4	0.25	1	0.063-4	NA	NA	0.06-2	0.25	2	0.125-8	NA	NA
<i>A. niger</i>	88	0.125-2	0.5	1	0.125-4	1	2	0.125-1	0.5	1	0.06-2	1	2
<i>A. terreus</i>	232	0.125-16	0.5	0.5	0.125-2	0.5	1	0.125-2	0.5	1	0.125-2	0.5	1

(a)

Antifungal agent	MIC(ug/ml)			Geometric mean	Number(%)	
	Range	MIC <sub>50</sub>	MIC <sub>90</sub>		S	SDD-I
Fluconazole	1.0-16.0	4.0	8.0	4.69	76(97.4)	2 (2.5)
Amphotericin-B	0.06-0.5	0.25	0.5	0.23	100	
Voriconazole	0.03-0.25	0.06	0.125	0.07	100	

(b)

Antifungal agent	MIC(ug/ml)			Number (%)	
	Range	MIC <sub>50</sub>	MIC <sub>90</sub>	S	R
Flucytosine	<=1.0-32	<=1.0	<=1.0	72(98.6)	1(1.4)

(a) Antifungal susceptibility test result by microdilution test<sup>†</sup>  
 (b) Antifungal susceptibility test result by VITEK-2<sup>‡</sup>

**Conclusion:** We measured antifungal susceptibility against 78 *Cryptococcus* isolates. Amphotericin-B and voriconazole showed good potency against all *Cryptococcus* strains. Reduced fluconazole susceptibility was seen in two strains in microdilution test. One strain

was resistant against flucytosine in VITEK-2 system. In vitro susceptibility of fluconazole was not significantly different according to the time period.

**P818** Inhibitory effect of aspirin and diclofenac treatment on some non-albicans *Candida* species

E. Rusu, C.D. Cristescu, A. Boiangiu\*, I. Avram, D. Pelinescu, T. Vassu (Bucharest, RO)

The most common organisms implicated in fungal infections are species of *Candida*, which are under normal circumstances in commensally relation with human host. While *Candida parapsilosis* is common in neonatal and pediatric age group, *C. glabrata* and *C. krusei* infections are becoming more frequent in the elderly population.

**Objectives:** The main goal of our study was to evaluate aspirin and diclofenac sodium antifungal activity and in vitro susceptibility of some non-albicans *Candida* strains to these non-steroidal anti-inflammatory drugs.

**Methods:** Eight yeast strains were isolated from pharyngeal and vaginal secretions. Strains were identified by conventional and biochemical test as: one strain *C. glabrata*, three strains *C. parapsilosis* and four strains *C. krusei*. Tests of viability cells in the presence of 2 mg/mL aspirin and diclofenac sodium were done by the microdilution method.

**Results:** Our results showed an antifungal activity of both non-steroidal anti-inflammatory drugs against tested strains. Viability reduction in presence of aspirin was 48% for *C. glabrata*, between 30–68% for *C. parapsilosis* strains and between 20–65% for *C. krusei* strains. For diclofenac sodium the results were similar, viability strains decreased with 50% for *C. glabrata* strain, 35–75% for *C. parapsilosis* strains and 23–60% for *C. krusei* strains. The greatest inhibitory effect that we observed was on *C. parapsilosis* strains in the presence of aspirin and *C. krusei* strains in the presence of diclofenac. Antifungal effect was strain depending.

**Conclusions:** According to our results, aspirin and diclofenac sodium have inhibitory activity against some non-albicans strains: *C. parapsilosis*, *C. krusei* and *C. glabrata*. These data underline the potential utility of these drugs in the management of fungal infections. The anti-inflammatory and analgesic properties of sodium diclofenac and aspirin might represent an additional advantage for their use in the management of infection with *Candida* species.

**Keywords:** viability, anti-inflammatory, antifungal effect

**Acknowledgements:** This work has been supported by the national research project CNCIS Human Resources TE66/2010.

**P819** Susceptibility profile of deep-seated yeasts isolates from a university hospital in the northern region of Portugal

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**Objectives:** An observational study was conducted at the biggest hospital in Portugal, aiming to evaluate the susceptibility profile of yeasts isolated from invasive fungal infections and relevant epidemiological data.

**Methods:** Between September 2010 and 2011, all yeasts isolated from invasive fungal infections admitted at the university hospital S. João, in Porto, were collected. All strains were identified using Vitek2 system and the antifungal susceptibility profile was determined according to CLSI M27-A3 protocol regarding eight antifungals. Clinical and demographic data were registered. Mortality attributed to fungal infections was calculated. Chi-square test and multivariable logistic regression were used to analyze data.

**Results:** The incidence of invasive infections was 3.2 per 1000 admissions. One hundred and forty six isolates were collected, from blood (61.5%), peritoneal liquid (29.5%), cerebrospinal fluid (6.4%) and pleural effusion (2.6%) cultures. Fifty-two percent corresponded to *Candida albicans*, followed by *C. glabrata* (15.2%), *C. parapsilosis*

(10.9%), *C. lusitaniae* and *Cryptococcus neoformans* (5.4% each), *C. krusei* (3.3%), *C. tropicalis* and *C. dubliniensis* (2.2% each) and *C. famata*, *C. kefyr* and *Trichosporon mucoides* (1.1% each). Regarding antifungal susceptibility profile, 16.3% of all strains were resistant to fluconazole; 4.7% were resistant to voriconazole and posaconazole and 2.3% to 5-flucytosine and caspofungin; no resistance was found to anidulafungin, micafungin or amphotericin B. Most isolates were collected from the Surgery department (38%) and Intensive Care Units (19.6%). Most patients were aged between 41 and 60 years (39%) and 67% were male. Most fungal infections were associated with surgical procedures (OR = 1.95, 95%CI [1.39–2.74]), gastrointestinal disease (OR = 5.33 [3.82–7.42]), neoplasia (OR = 5.22 [3.73–7.30]), and diabetes (OR = 1.56, [1.04–2.34]). Mortality rate attributed to fungal infections was 46%: 60% had gastrointestinal disease and 40% were oncological patients.

**Conclusion:** Epidemiological studies concerning fungal infections are scarce in Portugal, but extremely valuable. *C. albicans* was the most common isolate. Susceptibility profiles showed low antifungal resistance except to fluconazole; all isolates were susceptible to anidulafungin, micafungin and amphotericin B. Surgery and gastrointestinal disease were frequently associated with fungal infections being the outcome often fatal.

This work is supported by Pfizer Inc.

**P821** Activity of amphotericin B, caspofungin and anidulafungin on planktonic and biofilm *Candida* spp. by microcalorimetry

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**Objectives:** *Candida* biofilms are difficult to treat due to reduced susceptibility in biofilm. We compared the activity of amphotericin B (AMPB) and two echinocandins, caspofungin (CAS) and anidulafungin (AFG), against planktonic and biofilm *Candida* spp. using microcalorimetry, a real-time and highly sensitive assay for measurement of growth-related heat production.

**Methods:** *C. albicans* (ATCC 90028), *C. krusei* (ATCC 6258) and *C. glabrata* (DSY 562) were tested. Minimal inhibitory concentration (MIC) was determined by microbroth dilution according to the EUCAST guidelines (EDef 7.1) and confirmed by E-test. Microcalorimetry was performed using an isothermal calorimeter (TAM III, TA Instruments) at 37°C in sealed glass ampoules containing 3 mL RPMI. Planktonic *Candida* species ( $5 \times 10^5$  CFU) were added to RPMI containing serial dilution of AMPB, CAS or AFG (0.125–512 µg/mL). *Candida* biofilm was formed on porous glass beads (diameter 4 mm, pore size 60 µm) and incubated for 24 hour at 37°C in RPMI. Beads were washed and incubated for 24 hour in RPMI containing serial dilution of AMPB, CAS or AFG (0.5–1024 µg/mL). Beads were then washed and placed in 3 mL of RPMI into the calorimeter to quantify recovering yeasts. The minimal heat inhibitory concentration (MHIC) was defined as the lowest antifungal concentration reducing the heat-flow peak by ≥50%.

**Results:** The MICs for tested *Candida* spp. ranged from 0.5–2 µg/mL for AMPB, 0.125–0.5 µg/mL for CAS and 0.03–0.015 µg/mL for AFG. Table shows MHICs (in µg/mL) for planktonic and biofilm *Candida*. Compared to planktonic counterparts, the activity of AMPB was significantly reduced against *Candida* biofilms (MHIC ≥32 µg/mL). CAS showed antibiofilm activity against *C. albicans* and *C.*

Species	AMPB (µg/ml)		CAS (µg/ml)		AFG (µg/ml)	
	Planktonic	Biofilm	Planktonic	Biofilm	Planktonic	Biofilm
<i>C. albicans</i>	0.5	64	0.125	4	0.03	0.5
<i>C. glabrata</i>	2	32	0.125	2	0.015	1
<i>C. krusei</i>	1	32	0.5	32	0.03	1

*glabrata* (MHIC = 2–4 µg/mL), but not against *C. krusei* (MHIC = 32 µg/mL). AFG showed good activity against all *Candida* biofilms (MHIC ≤1 µg/mL). At high concentrations (>8 µg/mL), CAS and AFG induced paradoxical growth of planktonic *C. albicans* and CAS induced the same effect on *C. glabrata*.

**Conclusions:** Microcalorimetry allows real-time evaluation of antifungal activity on planktonic and biofilm *Candida* spp. AFG showed better activity against *Candida* biofilms than AMPB, whereas CAS showed variable results. In future, microcalorimetry might be used to study novel antifungals (alone or in combination) to determine the optimal treatment strategies.

**P822** **Loss-of-heterozygosity of FCY2 leading to the development of flucytosine resistance in *Candida tropicalis***

Y. Chen, H. Lo, C. Wu, H. Ko, T. Chang, Y. Yang\* (Hsinchu, Miaoli, TW)

**Objective:** We have found that certain clinical isolates of *Candida tropicalis* were able to produce drug-resistant progeny upon exposures to flucytosine (5FC) on agar media. Hence, we were interested in unveiling the molecular mechanism of the resistance.

**Methods:** We have collected the 5FC-resistant progeny within the inhibitory ellipses on the agar media and subjected them to sequence analyses on the genes known to be involved in 5FC metabolism. After identification of the potential mutations responsible, we then conducted site-directed mutagenesis on the genomic copy of the genes of a susceptible strain to assess the effect.

**Results:** Thirty susceptible clinical isolates could produce resistant progeny after exposure to the drug. Twenty-two of them had heterozygous G/T at the 145th position on FCY2, encoding purine-cytosine permease, whereas their progeny recovered from the inhibitory ellipses had homozygous T/T, resulting in null alleles for both copies of the gene and produced only truncated proteins, effecting the 5FC resistance. Furthermore, we found that two major fluconazole-resistant clinical clones, diploid sequence type 98 (DST98) and DST140, had homozygous G/G at the 145th position and neither was able to produce 5FC resistant progeny within the inhibitory ellipses.

**Conclusion:** *Candida tropicalis* strains containing heterozygous alleles may develop 5FC resistance readily whereas those with homozygous G/G wild-type alleles can be treated with 5FC. Therefore, a combination of 5FC and another antifungal drug is applicable for treating infections of *C. tropicalis*.

**P823** **Antifungal susceptibility from the Spanish fungaemia surveillance multicentre study (FUNGEMYCA): are there any differences among ICU and non-ICU patients?**

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**Objectives:** The introduction of new antifungal agents together with the emergence of new fungal species with unknown susceptibility requires surveillance for the detection of isolates with decreased susceptibility to antifungal agents, especially in critical care setting, in order to optimize the antifungal treatment. The aim of this study was to determine whether there are any differences in the antifungal susceptibility among ICU and non ICU adult patients in the age of new agents.

**Methods:** Isolates from a recent bloodstream nosocomial fungemia Spanish surveillance study were tested by the SensititreYeastOne (Y09) method in each participating centre (Forty-three institutions participated in the study) for susceptibility to eight antifungal agents. All isolates identified and results of the Y09 test were sent to the central laboratory. Clinical and microbiological variables were recorded. An univariate analysis as performed using SPSS 15.00 package. A p value < 0.05 was considered significant.

**Results:** A total of 1174 isolates (543 *C. albicans*, 306 *C. parapsilosis*, 150 *C. glabrata*, 101 *C. tropicalis*, 25 *C. krusei* and 49 other species) were studied, 367 from ICU patients. The overall rate of non susceptible isolates (applying M27-S3 clinical breakpoints) to fluconazole (MIC >8 mg/L), itraconazole (>0.12 mg/L), voriconazole (>1 mg/L), and posaconazole (>1 mg/L) were: 8.8%, 33%, 2.4%, and 3.6%, respectively; anidulafungin, caspofungin, and micafungin (>2 mg/L), 2.4%, 2.2% and 2.9%, respectively, and that to amphotericin B (>1 mg/L) was 0.1%. There were no statistical differences among ICU and non ICU patients in resistance rates for the three echinocandins (anidulafungin 1.6% vs. 2.7%; caspofungin 1.6% vs 2.4%; micafungin 2.4% vs 3.1%), for Amphotericin B (0.02% vs 0%) and for itraconazole (29.5% vs. 34.4%) However the resistance rates for fluconazole (4.6% vs 10.7%; p = 0.001), voriconazole (0.5% vs.3.3%; p = 0.005) and posaconazole (1.3% vs. 4.7% p = 0.005) were statistically lower in ICU patients.

**Conclusions:** The rate of azole resistance is lower in ICU patients than other hospitalization patients in the age of new antifungal agents whereas the rate of echinocandins resistance remains lower than azole agents in all episodes included. However Amphotericin B is still the agent with the lowest global rate of resistance.

**P824** ***Scopulariopsis* spp: epidemiology and in vitro antifungal susceptibility in a general hospital**

T. Peláez\*, B. Gama, L. Alcalá, E. Bunsow, M. Valerio, A. Fernández-Cruz, J. Guinea, E. Bouza (Madrid, ES)

**Background:** The genus *Scopulariopsis* contains hyaline and dematiaceous species, some of which are considered infectious agents in humans, mainly immunocompromised patients. Limited data are available on the susceptibility of *Scopulariopsis* species. We evaluated the epidemiology and antifungal susceptibility of clinical *Scopulariopsis* spp. isolates in our hospital.

**Methods:** A total 30 isolates of *Scopulariopsis* (collection of Hospital General Universitario Gregorio Marañón) were found between 1993 and 2010. The minimum inhibitory concentrations (MICs) were determined using the CLSI M38-A2 microdilution method with amphotericin B (AMB), itraconazole (IZ), voriconazole (VZ), posaconazole (POS), caspofungin (CAS), micafungin (MF), and anidulafungin (AND).

**Results:** We obtained 30 *Scopulariopsis brevicaulis* isolates (30 patients) from the following sites: nails (21), respiratory tract (3), sterile fluids (3), and other sites (3). The MICs and MECs ranges of *Scopulariopsis* spp. were as follows: AMB, 2–16; IZ, 4–32; VOR, 8–32; POS, 2–32; CAS, 32–64; MF, 16; and AND, 16. The geometric mean/MIC90 and MEC in µg/mL for *Scopulariopsis* spp. were as follows: AMB, 4.9/16; IZ, 23.1/32; VOR, 15.3/32; POS, 18.8/32; CAS, 36.1/64; MF, 16/16; and AND, 16/16.

**Conclusions:** During the study period, the number of *Scopulariopsis brevicaulis* isolates in our institution increased. The MICs of all antifungal agents tested against *Scopulariopsis* isolates were very high. Azoles and candins have no or very limited in vitro activity. Only amphotericin showed variable activity against *Scopulariopsis* isolates.

**P825** **Investigation for azole resistance mediating *Aspergillus fumigatus* cyp51A gene mutations using improved PCR assays and consecutive DNA sequence analysis in clinical samples of immunocompromised patients**

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**Objectives:** The incidence of azole resistance in *Aspergillus fumigatus*, the major fungal pathogen causing invasive aspergillosis (IA) in immunocompromised patients, is rising. As the diagnosis of IA is rarely based on positive culture yield in this group of patients, detection of azole resistance directly from clinical samples is urgently needed.



**Methods:** We established three sensitive and specific polymerase chain reaction (PCR) assays followed by DNA sequence analysis to detect the most frequent mutations in the *A. fumigatus* *cyp51A* gene conferring azole resistance (TR (tandem repeat) alteration in the promoter region, L98H and M220 alterations) directly from clinical bronchoalveolar lavage (BAL) and tissue samples. Furthermore the sensitivity of the original L98H PCR assay was optimised.

Up to now we screened 40 samples from 38 immunocompromised patients that had been tested positive for *Aspergillus* DNA previously in our diagnostic nested *Aspergillus* PCR assay.

**Results:** The detection threshold for the optimised L98H PCR assay was 200 fg of *A. fumigatus* DNA.

Using primarily this most sensitive assay, 23 of 40 samples yielded a positive signal, 17 samples were found to be PCR-negative.

The positive-tested samples were further submitted to the TR and M220 PCR assays. For the TR assay 16 samples resulted in positive and seven samples in negative signals, whereas 17 positive signals and six negative signals were found for M220 PCR assay.

DNA sequence analysis revealed a single L98H mutation in a lung tissue specimen of a steroid treated COPD patient and a L98H alteration in combination with the TR in a brain tissue sample of a patient with acute lymphoblastic leukemia and IA.

**Conclusions:** In order to detect azole resistance mediating mutations of the *A. fumigatus* *cyp51A* gene directly from clinical samples, we optimised our PCR assays. Positive PCR signals suggest the feasibility of the approach, however; DNA sequence analysis is mandatory to detect the gene mutations.

We consider our assay of high epidemiological and clinical relevance to detect azole resistance and to optimise antifungal therapy in patients with IA.

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We acknowledge the support of Susan J. Howard, Manchester, and Anne Dietz, Mannheim.

**P826 Unique step-wise development of a homozygous FKS mutation (S80P) in *Candida tropicalis* in vivo**

R.H. Jensen\*, H.K. Johansen, M.C. Arendrup (Copenhagen, DK)

**Objectives:** An increasing number of reports demonstrate acquired echinocandin resistance associated with both hetero- and homozygous mutations in the *fks* target gene. Here we analyse three sequential *C. tropicalis* isolates received from a leukemic patient over a 4-month period in which echinocandin resistance developed despite treatment with alternating drug classes.

**Methods:** Susceptibility testing was done according to EUCAST (azoles and anidulafungin) and by Etest (amphotericin B and caspofungin). The *fks1* gene was sequenced for resistant isolates and compared to a reference sequence for the ATCC 750 *C. tropicalis* strain. Genotyping was done by multilocus sequence typing (MLST) applying the pubMLST database covering 205 different sequence types from 260 isolates [http://pubmlst.org/perl/mlstdbnet/mlstdbnet.pl?file=ct\\_profiles.xml](http://pubmlst.org/perl/mlstdbnet/mlstdbnet.pl?file=ct_profiles.xml). Two unrelated isolates of *C. tropicalis* were included for comparison.

**Results:** The patient was initially blood culture (BC) positive 19.12.10. Five BCs drawn from 27.01.11–01.03.11 were all negative. *C. tropicalis* was again detected 05.03.11–16.03.11 in five subsequent BCs. Systemic antifungal treatment was as follows (daily dose): 19.12.10–24.01.11: caspofungin (70/50 mg), 24.01.11–11.02.11 fluconazole (800 mg), 11.02.11–09.03.11 caspofungin (70.50 mg), 09.03.11–31.03.11 ambisome (3 mg/kg), 31.03.11–04.05.11 posaconazole (800 mg). A Hickman catheter was kept in place but attempted sterilised with acid and fluconazole lock. Date and origin of study isolates are shown in the Table.

Susceptibility testing showed isolate #1 was susceptible whereas isolate #2 and #3 were echinocandin resistant (Table). Isolate #2 had a heterozygous S80S/P mutation in the *fks* gene (corresponding to S645P in *C. albicans*), whereas this alteration was demonstrated at both alleles

in isolate #3 (S80P). The MLST data suggested isolate #2 and #3 were clonally related since DNA sequences in six housekeeping genes were 100% identical and shared a unique pubMLST sequence type.

Isolate	Species	Date	Resistance mechanism	Allele profiles according to the pubMLST database							EUCAST (EU 7.1) (µg/ml)						Etest (µg/ml)	
				FKS1	FKS1	MRR1	SMFT2	SMFT3	SXR	ZNF	POS	AMB	VOR	TER	FLU	AMB	CAS	
#1	BC <sup>*</sup>	19.12.10	NA	-	-	-	-	-	-	-	-	-	-	-	-	-	0.50	0.125
#2	BC <sup>**</sup>	05.03.11	S80P	16	20	4	20	27	7	0.003	0.25	0.003	0.01	0.2	0.5	0.125		
#3	Cerebro	05.03.11	S80P	16	20	4	20	27	7	0.27	0.7	0.127	0.027	2	4	0.125		
REF1***	BC <sup>††</sup>	06.07.10	NA	1	7	4	6	2	8	0.003	0.003	0.003	0.003	0.0125	1	NA		
REF2***	BC <sup>††</sup>	23.01.11	NA	1	5	5	7	2097*46	1	0.003	0.003	0.003	0.027	0.5	0.5	0.125		

**Conclusion:** To our knowledge, this is the first report of a stepwise development in vivo of a heterozygous to homozygous S80P mutation in *C. tropicalis* after caspofungin treatment and despite 1 month of suppressive fluconazole therapy. Even the heterozygous mutant isolate was classified as echinocandin resistant illustrating the importance of the codon S80 of *C. tropicalis*. This study may contribute to our understanding of resistance development in vivo as a consequence of antifungal treatment.

**P827 In vivo activity of anidulafungin, caspofungin and micafungin against *C. glabrata* with and without FKS mutations**

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**Objectives:** We have recently observed that for some *C. glabrata* isolates with *fks* hot spot mutations, the MIC elevation for micafungin may be less pronounced than for the other echinocandins. In this study we investigated whether the efficacy of micafungin may be differentially dependent on certain *fks* mutations in the hot spot regions of *C. glabrata*.

**Methods:** Three clinical *C. glabrata* isolates with or without (S#3) *fks* hotspot mutations [R#83 (*fks2p-S663F*) and the RR#24 (*fks1p-S629P*)] were used. In vitro susceptibility (EUCAST and CLSI ± bovine serum albumin supplemented medium and disk testing) and in vitro growth rate in RPMI-2G broth were determined. In vivo efficacy in a haematogenous immunocompetent mouse model using three doses of each of the echinocandins was investigated. Drug concentrations were determined in plasma and kidney tissue and PK/PD relationships evaluated.

**Results:** In vitro testing confirmed differential susceptibility S#3: susceptible, R#83 moderately resistant and RR#24 most resistant. Compared to the S#3 isolate, the R#83 isolate showed slightly reduced maximal growth and the RR#24 isolate prolonged lag phase and reduced maximal growth. None of the echinocandins were efficacious in mice challenged with the RR#24 isolate even at doses associated with AUCs approximately three to eight times greater than in humans (Table). Micafungin was equally efficacious against the S#3 and the R#83 isolate with a ≥1 log reduction at doses associated with an AUC of ≥187. In contrast, for mice challenged with the R#83 isolate a ≥1 log reduction was achieved for caspofungin at the medium and high doses (AUC 393 and 787, respectively) but not the low dose (AUC 197). Anidulafungin failed to induce a ≥1 log reduction for any of the isolates at any of the doses (AUC range 139–557).

Agent	First experiment			Second experiment			Mean			
	AUC	S#3	R#83	RR#24	S#3	R#83	RR#24	S#3	R#83	RR#24
Anidulafungin	556.5	0.877	0.273	-0.418	0.822	0.756	ND	0.859	0.515	ND
High dose (2 mg/kg)	278.1	1.062	0.865	-0.064	0.463	0.417	ND	0.763	0.635	ND
Medium dose (0.6 mg/kg)	139.0	-0.270	-0.078	0.375	-0.006	-0.513	ND	-0.139	-0.296	ND
Caspofungin	787.0	3.121	2.186	-0.968	3.135	2.203	ND	3.129	1.695	ND
High dose (8 mg/kg)	393.1	2.964	1.224	-0.823	2.969	2.104	ND	2.966	1.684	ND
Medium dose (3 mg/kg)	196.6	2.143	0.669	-1.306	1.739	0.599	ND	1.941	0.939	ND
Micafungin	373.7	1.321	1.724	-0.577	1.694	1.726	ND	1.509	1.726	ND
High dose (2 mg/kg)	186.9	0.921	1.581	-0.863	1.093	1.599	ND	1.012	1.596	ND
Medium dose (0.6 mg/kg)	93.4	0.717	1.084	0.178	0.234	0.678	ND	0.476	1.386	ND
Low dose (0.2 mg/kg)										

\* Six animals per group.  
†† Three animals per group.

**Conclusion:** Differences in echinocandin MIC values associated with individual fks mutations appear to be associated with differential antifungal activity in an immunocompetent animal model. The mutant with the most clearly elevated MIC above the standard range for wild type isolates was not treatable with any of the echinocandins, whereas the isolate for which the micafungin MIC was only marginally elevated, and which may be difficult to discriminate from wild type isolates in routine testing, responded equally well to micafungin as the wild type. This study has implications for clinical practice and echinocandin breakpoint determination.

**P828** Emergence in vivo of *Aspergillus terreus* with reduced azole susceptibility and a CYP51A M220I alteration in a cystic fibrosis patient

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**Objectives:** Aspergilli are frequently found in the airways of CF patients. *A. fumigatus* is the predominant species and 4.5% of these have recently been shown to be azole resistant in a Danish cohort. Azole resistance in *A. terreus* is rarely reported but of clinical significance due to this species being intrinsically resistant to amphotericin B. We here report the emergence of *A. terreus* isolates with reduced azole susceptibility and investigate the underlying mechanism and origin.

**Methods:** Twenty sequential *A. terreus* isolates from a single CF patient (MB) obtained over a 7.5 year period and six unrelated *A. terreus* isolates were included. Susceptibility testing was performed (EUCAST EDef 9.1 methodology). Breakpoints have not yet been established but *A. terreus* isolates with itraconazole (ITR) MIC ≤0.5 mg/L were regarded as susceptible based on MIC ranges for wild type isolates. The CYP51A gene and promoter was sequenced (primers designed based on the reference strain NIH2624 ATEG05917). The isolates genotyped using RAPD-PCR and five different primers for maximal discriminatory power: A-ter2 (5-GCTGGTGG-3), NS7 (5-GAGGCAATAACAGGCTGTGATGC-3), (R108 (5-GTATTGCC CT-3), CII (5-GCGCACGG-3) and P4 (5-GATAGATAGATAGAT-3).

**Results:** Susceptibility testing of the isolates from MB identified five isolates as ITR S (MICs 0.25–0.5 mg/L): 2/2 from 2003 to 4, 2/7 from 2007 and 1/9 isolates from 2009. ITR MICs for the remaining 15 isolates were 1–4 mg/L. In contrast the ITR MICs of the six control isolates were 0.06–0.125 mg/L. Voriconazole (VOR) MICs were 1–4 mg/L for the MB isolates but 0.25–0.5 mg/L for the control isolates. The posaconazole (PSC) MICs were 0.06–0.5 mg/L for the MB isolates and <0.03–0.06 mg/L for control isolates. Genotyping showed 18/20 MB isolates to be identical. The remaining 2 MB isolates differed for one (A.terr-2) or two primers (Aterr-2 and NS7), respectively. Cyp51A sequencing identified a M217I alteration (corresponding to M220I in *A. fumigatus*) in the 15 isolates with elevated ITR MICs.

One control isolate with wild type susceptibility had an A221V alteration.

**Conclusion:** To our knowledge this is the first report on acquired azole resistance in *A. terreus* involving an M217I alteration. This alteration was found in all ITR resistant isolates and genotyping suggested this alteration was of endogenous origin. VOR and PSC MICs were elevated also in isolates without CYP51A mutations suggesting several mechanisms may act in concert in these isolates.

**P829** A unique amino acid substitution in the ERG11 gene mediated azole cross-resistance in *Candida tropicalis* bloodstream isolates

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**Objectives:** Compared with *C. albicans* there are relatively few studies examining the virulence factors of *C. tropicalis*, and its mechanisms of drug-resistance.

*C. tropicalis* accounts for a significant proportion of *Candida* bloodstream infection, and traditionally is considered as second to *C. albicans* in terms of virulence and clinical importance. Here we investigated the molecular mechanism responsible for cross-resistance to fluconazole and voriconazole in *C. tropicalis* clinical strains isolated from bloodstream infections.

**Materials and methods:** During a 10-year retrospective surveillance of candidemia we collected ten *C. tropicalis* isolates, showing resistance to fluconazole and voriconazole. For comparative purpose an equal number of azole-susceptible strains were studied. Isogenicity of the isolates was investigated by RAPD (primers OPE03, GC70 and UBC703) and Maldi-TOF analysis.

Antifungal susceptibility testing was performed by Sensititre procedure and broth microdilution method. Quantification of the expression of the CtMDR1, CtCDR1 and CtERG11 genes was performed by real-time PCR, using SYBR Green chemistry. To stimulate the expression of efflux pumps genes, all the isolates were cultured in presence and absence of various fluconazole concentrations. The primers have been designed with the primer express 3.0 software (Applied Biosystems). For ERG11 sequencing, five pairs of oligonucleotide primers (Bouchara et al., 2005) were used.

**Results:** The resistant isolates, coming from four different hospitals, clustered in three groups. No significant differences were found in the expression levels of the resistant isolates compared to the susceptible ones, even if cultured in the presence of sub-MIC concentration of fluconazole.

Comparison of the CtERG11 gene sequences of the ten *C. tropicalis* resistant isolates with the available corresponding sequence in the GenBank database (accession number M23673) revealed the point mutation Y132F in the coding region. This mutation is located in the region between the B' and C helices that have been postulated to be involved in inhibitor- or substrate-induced structural changes.

**Conclusions:** In contrast to what observed in *C. albicans*, where azole resistance is usually the result of a combination of different mechanisms, in *C. tropicalis* we found a unique single point mutation sufficient to induce a decreased affinity of fluconazole and its derivative voriconazole.

**P830** Posaconazole concentrates within the endoplasmic reticulum of host and fungal cells

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**Objectives:** Infections due to *Aspergillus fumigatus* remain a critical concern in high risk hematology patients. Posaconazole (PCZ) prophylaxis has proven highly effective in preventing these infections, despite relatively low serum concentrations. We have previously shown that PCZ concentrates within host cell membranes to a high enough concentration to both inhibit growth and prevent

Sample-ID	Primer					Genotype	Cyp51A	Date
	A-ter-2	NS7	P4	R108	CII			
MB-03-1	11	n1	p1	r1	c1	A	wt	17/11/2003
MB-04-1	11	n1	p1	r1	c1	A	wt	12/01/2004
MB-07-1	11	n1	p1	r1	c1	A	wt	09/01/2007
MB-07-2	11	n1	p1	r1	c1	A	M217I	27/02/2007
MB-07-3	11	n1	p1	r1	c1	A	M217I	06/06/2007
MB-07-4	11	n1	p1	r1	c1	A	wt	03/09/2007
MB-07-5	11	n1	p1	r1	c1	A	M217I	09/10/2007
MB-07-6	11	n1	p1	r1	c1	A	M217I	30/10/2007
MB-07-7	11	n1	p1	r1	c1	A	M217I	30/12/2007
MB-09-1	11	n1	p1	r1	c1	A	M217I	13/07/2009
MB-09-2	12	n2	p2	r2	c2	B	wt	04/09/2009
MB-09-3	11	n1	p1	r1	c1	A	M217I	26/09/2009
MB-09-4	11	n1	p1	r1	c1	A	M217I	30/09/2009
MB-09-5	19	n1	p1	r1	c1	I	M217I	30/09/2009
MB-09-6	11	n1	p1	r1	c1	A	M217I	07/10/2009
MB-09-7	11	n1	p1	r1	c1	A	M217I	13/10/2009
MB-09-8	11	n1	p1	r1	c1	A	M217I	26/10/2009
MB-09-9	11	n1	p1	r1	c1	A	M217I	11/11/2009
MB-11-1	11	n1	p1	r1	c1	A	M217I	10/03/2011
MB-11-2	11	n1	p1	r1	c1	A	M217I	25/03/2011
UKNEGUAS	13	n3	p3	r3	c3	C	wt	1989
UKNEGUAS	13	n3	p3	r3	c3	C	wt	1997
OUH	16	n4	p2	r2	c2	H	Not done	2004
Estbjerg	16	n1	p5	r4	c1	F	wt	2005
Shady	17	n1	p1	r1	c1	G	wt	2005
RH	15	n5	p1	r5	c1	E	A221V	2008
RH	14	n6	p4	r3	c1	D	wt	2011

fungal damage. However, the specific subcellular location of membrane associated PCZ within host and fungal cells is unknown. The objectives of this study are to determine where PCZ localizes in both epithelial cells and fungi in order to better understand its mechanism of action.

**Methods:** Fluorescent PCZ was synthesized by conjugation with the fluorophore BOPIDY (BOPIDY-PCZ). A549 epithelial cells were exposed to varying concentrations of BOPIDY-PCZ and examined using confocal microscopy. Cells were costained with DAPI and an endoplasmic reticulum (ER) specific anti-ERP57 antibody to facilitate visualization of cell microstructures. In parallel, these experiments were also conducted with *A. fumigatus* hyphae. Finally, to confirm the specificity of these findings, competitive inhibition assays using unlabelled PCZ and voriconazole (VCZ) were performed.

**Results:** A549 epithelial cells exposed to BOPIDY-PCZ exhibited increased total cell fluorescence, centred around the peri-nuclear area. Similar findings were observed with hyphae of *A. fumigatus*, with increased fluorescence localizing to the perinuclear area, corresponding to the location of the ER. To confirm this finding, A549 cells were immunostained for ER protein 57 (ERP57). Staining for this protein demonstrated co-localization with BOPIDY-PCZ in the perinuclear region, suggesting that posaconazole concentrates within the membrane rich endoplasmic reticulum. Co-culture of host and fungal cells with BOPIDY-PCZ and unlabelled PCZ resulted in a decreased in fluorescence of the cells, while the addition of VCZ had little effect on ER concentration of PCZ, suggesting that the concentration of PCZ within the ER membranes is specific to this azole.

**Conclusion:** These results suggest that PCZ concentrates specifically to the endoplasmic reticulum within host and fungal cells. Since the target of PCZ, CYP51A, is found within the ER, this finding suggests that the ability of PCZ to concentrate to high concentrations in the endoplasmic reticulum may contribute to its antifungal activity.

**P831** Performance of a commercial real-time PCR assay using the ABI 7300 instrument for the detection of *Aspergillus* and *Pneumocystis* DNA in bronchoalveolar lavage fluid samples from critical patients

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**Objectives:** To investigate the performance of the MycAssay™ *Aspergillus* (MycAspAssay) and of the MycAssay™ *Pneumocystis* (MycPCPAssay) in detecting *Aspergillus* (Asp) or *P. jirovecii* (Pj) DNA in BAL samples from critical patients, using ABI 7300 instrument.

**Methods:** We enrolled 20 patients with or without fungal infections, according to the EORTC classification. In detail, seven patients were diagnosed with invasive aspergillosis (IA), eight patients with pulmonary infection by Pj (PCP) and five hospitalized patients with non-fungal infections (CTRL). DNA extracted by MycXtra™ (Myconostica) from BAL samples was amplified by real-time PCR by MycAspAssay and MycPCPAssay kits (Myconostica) using ABI 7300 instrument (Applied Biosystem). The real-time PCR data were compared with the results obtained with conventional diagnostic tests, namely culture, galactomannan (GM), immunofluorescence (IF), sequencing and *Aspergillus* spp. Q-PCR Alert Kit or *P. jirovecii* Alert kit (Nanogen).

**Results:** All the seven patients with IA as well as one of eight patients belonging to the PCP group were MycAspAssay positive whereas all the CTRL-group patients (n = 5) provided negative PCR results. All the MycAspAssay positive results were confirmed either by GM-assay or *Aspergillus* spp. Q-PCR Alert Kit.

Seven of the eight patients with PCP as well as four of the seven patients belonging to the IA group were MycPCPAssay positive, whereas all the CTRL-group patients provided negative PCR results. Six of the 7 MycPCPAssay positive results were in line with the IF, nested PCR and sequencing data.

**Conclusion:** Although being considered as a preliminary step towards a validation of the MycAssay™ *Aspergillus* and MycAssay™

*Pneumocystis* kits on ABI 7300, these data provide initial information on the performance of this platforms for the detection of DNA of either Asp or Pj in BAL of patients with suspected invasive mycosis. Work partially supported by GRANT MIUR-PRIN 2009.

**P832** Clinical validation of multiplex quantitative real-time PCR in critically ill patients for invasive candidiasis diagnosis: preliminary analysis

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**Background:** Mortality associated with invasive candidiasis in critically ill patients may reach 70%. Diagnostic limitations makes empirical treatment a common practice nowadays. New techniques like real-time quantitative PCR (RT-PCR) could help in daily management of these patients.

**Objectives:** Sensitivity (S), specificity (E), positive and negative predictive value (PPV and NPV) of RT-PCR in comparison to blood culture were calculated in a prospective study in patients at high risk of invasive candidiasis that have been admitted to an intensive care unit. Blood cultures and RT-PCR samples (serum and total blood) were periodically obtained at baseline, day +2, day +7, day +14 and day +21. Techniques based on PCR multiplex with molecular beacons were used. They detect the six most common species of *Candida* (*C. albicans*, *C. parapsilosis*, *C. tropicalis*, *C. glabrata*, *C. krusei* and *C. guilliermondii*). We also studied 20 healthy controls.

**Results:** The first eight patients were excluded because of blood cultures and RT-PCR samples were obtained through a catheter central-line and all of them showed a false positive RT-PCR result, probably related to central-line contamination. In the other 38 patients, samples were obtained from venipuncture. Results are shown in table 1.

All healthy controls showed negative blood cultures a negative RT-PCR. S, E, PPV and NPV of RT-PCR were: 59%, 90%, 71% and 84%, respectively. In three of four patients with a false-positive RT-PCR *Candida* spp were obtained from abdominal collections. All false-negative RT-PCR results were obtained in patients with catheter-related candidemia. When we analysed all samples obtained, the results were as followed: 15 true-positive, 116 true-negative, 14 false-positive and 15 false-negative RT-PCR results in comparison to blood cultures. To evaluate the best sample for RT-PCR, we observed a positive rate of 86% and 67% of serum and total blood, respectively, among the total positive RT-PCR samples.

In candidemic patients the most frequent *Candida* species isolated were *C. albicans* (59%) followed by *C. parapsilosis* (35%).

	Positive RT-PCR	Negative RT-PCR	
Positive blood culture	10	7	17
Negative blood culture	4	17 (+20 healthy controls)	41
	14	44	58

**Conclusions:** The quantitative real-time PCR has been positioned as a useful method for the diagnosis of invasive candidiasis and for monitoring anti fungal therapy response in critically ill patients. A special consideration can be obtained in patients with abdominal collections and negative blood cultures.

**P833** Molecular characterisation of fungal cultures by PLEX-ID broad fungal assay

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**Objectives:** We have developed and evaluated an assay for the characterization of fungal pathogens using the PLEX-ID technology, where PCR amplicons are analyzed by ESI-MS. The assay provides

broad fungal coverage across Ascomycota, Basidiomycota and Zygomycota, with an emphasis towards the individual characterization of 50 clinically important yet phylogenically diverse pathogens (e.g. *Aspergillus fumigatus*, *Candida albicans*, *Fusarium* spp., *Cochliobolus* spp., *Pneumocystis jirovecii*, *Cryptococcus neoformans*, *Mucor* spp).

**Methods:** We assembled a 16-primer pair assay that provides a balance between universal fungal detection and fine characterization of the most common fungal pathogens. For each sample, the resulting 4- to 10-loci signatures are compared to an in-house signature database built using in silico determination from publicly available fungal sequences and direct determination from over 70 reference isolates from ATCC. The assay was tested against a diverse collection of 305 culture samples from clinical isolates.

**Results:** The PLEX-ID detected fungi in 299/305 positive culture samples (98.0%), specifically retrieving culture results in 267 samples (87.5%). Fungi were also detected in 25 samples (8.2%) but were not fully characterized as the determined signatures did not have a decisive database match at the time of analysis. Seven samples with divergent culture and PLEX-ID characterization were further investigated by ITS1/ITS2 sequencing, confirming the PLEX-ID call in 4/7 instances. Fungal detection were missed in 6/305 samples (2.0%).

Correlation between PLEX-ID and culture ID results	Samples	%
Full agreement	267	87.5%
Agreement: Fungus detected, species not represented in DB	25	8.2%
Disagreement; ITS1/ITS2 sequencing confirms PLEX-ID result	4	1.3%
Disagreement; ITS1/ITS2 sequencing confirms culture ID result	3	1.0%
No detection by PLEX-ID	6	2.0%
<b>All samples from clinical isolates</b>	<b>305</b>	<b>100.0%</b>

**Conclusions:** The PLEX-ID fungal assay provides broad detection and identification of clinically relevant fungi. Testing with ~300 specimens showed 97% accuracy of identification. The instances of partially or incorrectly characterized isolates resulted primarily from gaps in the initial signature database that the present study helped to identify and eliminate. The assay can screen up to six samples per plate, allowing the analysis of up to 90 samples in 12 hour on a single instrument.

#### **P834** Molecular identification of yeasts causing fungaemia: are cryptic species frequent?

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**Objectives:** The study of the epidemiology of fungaemia is necessary to optimize empirical and proven treatment. However, morphological and biochemical procedures are unable to uncover the presence of closely related species (cryptic species) in complexes of *Candida parapsilosis*, *Candida guilliermondii*, and *Candida glabrata*. We used molecular techniques to provide definitive identification of species causing fungaemia in patients admitted to a tertiary hospital.

**Methods:** We studied 445 fungal isolates from the blood cultures of 401 patients with fungaemia admitted to our hospital between January 2007 and August 2011. Each isolate represented one fungaemia episode. Multiple episodes were defined as isolation of the same fungal species in further blood cultures taken  $\geq 7$  days after the last isolation in blood culture. Isolates were identified after amplification and sequencing of the ITS1-5.8S-ITS2 region. A phylogenetic tree based on the sequenced ITS1-5.8S-ITS2 region was constructed to detect the presence of cryptic species within the *C. parapsilosis*, *C. guilliermondii*, and *C. glabrata* complexes.

**Results:** Only 9 (2%) fungaemia episodes were caused by a mixture of two different yeast species. The distribution of species involved in the fungaemia episodes was as follows: *Candida albicans* (n = 217, 48.7%), *C. parapsilosis* (n = 129, 29%), *C. glabrata* (n = 45, 10.1%), *Candida tropicalis* (n = 28, 6.3%), *C. guilliermondii* (n = 6, 1.3%), *Candida krusei* (n = 6, 1.3%), *Candida dubliniensis* (n = 4, 0.9%), *Candida kefyr* (n = 2, 0.5%), *Candida lusitanae* (n = 2, 0.5%),

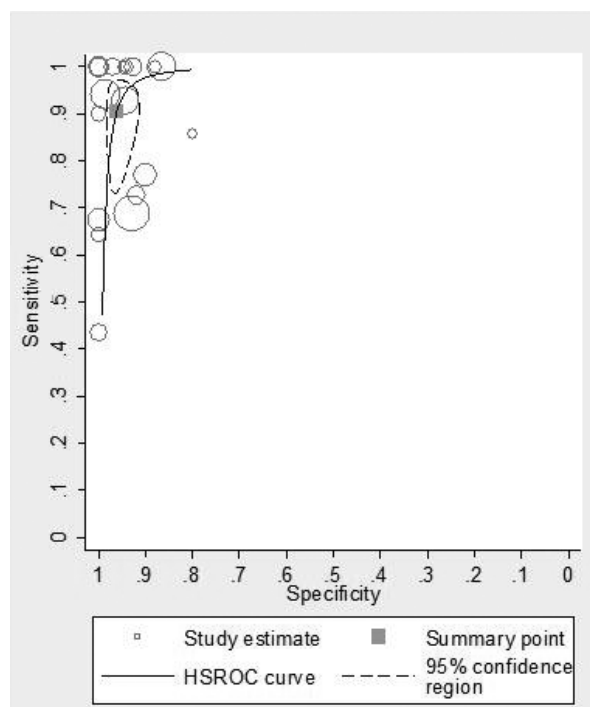
*Candida pelliculosa* (n = 1, 0.25%), other non-*Candida* yeasts (n = 14, 3.1%). Only three isolates from the *C. parapsilosis* complex were cryptic species. One adult patient who underwent cardiac surgery developed candidemia by *Candida metapsilosis*. Two adult patients with digestive cancer developed candidemia by *Candida orthopsilosis*. Only one of the isolates identified as *C. guilliermondii* was confirmed as *Pichia caribbica* (the isolated was from a patient with digestive cancer). No cryptic species were found in the isolates identified as *C. glabrata*. **Conclusion:** In our hospital, most episodes of fungaemia were caused by *C. albicans*, followed by *C. parapsilosis*. The presence of cryptic species within the *C. parapsilosis*, *C. guilliermondii*, and *C. glabrata* complexes was infrequent and represented only 2% of their isolates. Jesús Guinea (CP09/00055) and Pilar Escribano (CD09/00230) are supported by a contract from FIS.

#### **P835** PCR for the detection of invasive aspergillosis in bronchoalveolar lavage – systematic review and meta-analysis

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**Objectives:** To assess the sensitivity and specificity of PCR in bronchoalveolar lavage (BAL) for the diagnosis of invasive aspergillosis (IA), and to examine the standards of PCR performance and their effects on the diagnostic accuracy of PCR.

**Methods:** Systematic review. We included prospective, retrospective or case-control studies using PCR on BAL or bronchial secretions for the diagnosis of IA. We included only patients at risk for IA as defined by the EORTC host criteria (both cases and controls). Any PCR test was acceptable, including standard PCR, real-time, nested, multiplex or other PCR, using any primer gene and targeting all or specific *aspergillus* spp. We used only PCR tests taken within 14 days of infection onset. In the primary analysis we considered only proven and probable IA, defined by EORTC criteria. In a secondary analysis we also considered possible IA as disease. Risk of bias assessment was conducted using the QUADAS-2 tool. Coupled sensitivity and specificities were summarized using the bivariate model, from which hierarchical summary receiver operating curves (HSROC) were drawn. Covariates effects were investigated through meta-regression of diagnostic odds-ratios (DORs)



**Results:** Eighteen studies (1499 patients) were included. Ten used real-time PCR. Most studies used the 18S rRNA gene for primers. For the primary analysis (probable or proven IA), the summary sensitivity and specificity values were 90.5% (95% CI 79.2–95.9%) and 96.1% (95% CI 93–97.9%), respectively (figure). The DOR was 235.1 (95% CI 83.3–663.6). An analysis restricted to eight studies with an appropriate reference standard yielded summary sensitivity, specificity and DOR values of 80.7% (95% CI 63.6–90.9%), 92.9% (95% CI 90–94.9%) and 54.5 (95% CI 22.5–131.8), respectively. The pooled sensitivity and specificity for possible, probable or proven IA was 86.8% (95% CI 68.4–95.3%) and 94.9% (91.5–96.4%), respectively. Prior antifungal treatment decreased sensitivity. PCR methods did not affect results.

**Conclusions:** PCR from BAL in patients at risk for IA has >90% specificity. Considering the high mortality rate of IA, positive BAL PCR mandates appropriate antifungal therapy. Negative PCR cannot rule out IA, especially amongst previously treated patients.

**P836** Comparative sequence analysis of *Fusarium* spp. isolated from fungal keratitis specimens

X. Lu\*, M. Zhu, X. Shang, Z. Wang, X. Sun, M. Wang, Y. Sun, Y. Huang (Beijing, CN)

**Objectives:** To find out the exact pathogen of the fungal keratitis caused by *Fusarium* spp. that was one of the most common pathogen of fungal keratitis in China.

**Method:** One hundred and eighty-seven *Fusarium* strains were isolated from the corneal scraps from 2007 to 2010 in Beijing Tongren hospital, Capital medical University. All the isolates were identified on the basis of colony and microscopic features. All the

Table Results of identification of the 187 fusarium isolates

Fusarium	Morphology	ITS	EF-1 $\alpha$	MtSSU
<i>F. solani</i>	105	103	103	103
<i>F. moniliforme</i>	81	81	80	80
<i>F. delphinoides</i>	0	3	3	3
<i>F. thapsinum</i>	0	0	1	1
<i>F. oxysporum</i>	1	0	0	0
Total	187	187	187	187

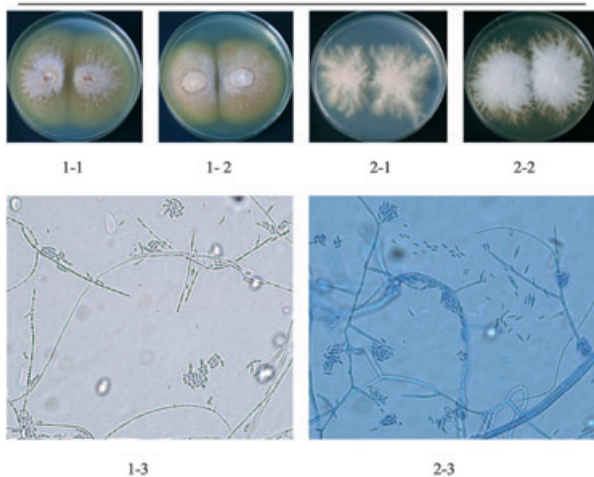


Figure 1-1 *F. delphinoides* (PDA)

Figure 1-2 *F. delphinoides* (SDA)

Figure 2-1 *Gibberella thapsina* (PDA)

Figure 2-2 *Gibberella thapsina* (SDA)

Figure 1-3 *Fusarium delphinoides* (10% potassium hydroxide (KOH) stain,  $\times 400$ )

Figure 2-3 *Gibberella thapsina* (lactophenol cotton blue stain,  $\times 400$ )

isolates were also identified by comparative sequence analysis using the internal transcribed spacer (ITS), the translation elongation factor 1 alpha (EF-1alpha), and mitochondrial small subunit rDNA (MtSSU) regions.

**Results:** By EF-1alpha and MtSSU regions sequence-based identification, 55.1% (103/187) isolates were *Fusarium solani* and 42.8% (80/187) were *Fusarium moniliforme* (table). The *Fusarium oxysporum* identified by morphological method was *F. solani* confirmed by the three regions sequence analysis. Three isolates were identified as *Fusarium delphinoides* that were the member of the *Fusarium dimerum* species group by comparative sequence analysis using the three regions, but they were *F. solani* by morphological features (figure 1-1, 1-2 and 1-3). One isolate was *Gibberella thapsina* (*Fusarium thapsinum*) by EF-1alpha and MtSSU regions sequence-based identification, but it was identified as *F. moniliforme* species complex using the ITS sequence analysis and its morphological feature was similar to *F. moniliforme* (figure 2-1, 2-2 and 2-3).

**Conclusion:** *F. solani* was the major pathogen of the fungal keratitis caused by *Fusarium* spp., followed by *F. moniliforme*. Other species of *Fusarium* spp. was rarely. The comparative sequence analysis using EF-1alpha and MS regions was better than morphological method and the sequence-based analysis using the ITS region.

**P837** Inter-laboratory comparison of treatment response in a murine model of invasive candidiasis using two *Candida albicans* isolates

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**Objective:** Murine models of invasive candidiasis are frequently used in the preclinical evaluation of investigational antifungals as these models are typically robust and inexpensive. However, there have been few inter-laboratory studies of outcome variability with the same model. Our objective was to conduct an inter-laboratory comparison of treatment response between two laboratories (UTHSCSA and University of Manchester) using a murine model of invasive candidiasis with two different *C. albicans* clinical isolates.

**Methods:** Immunocompetent 30 g outbred ICR or CD1 mice were inoculated intravenously with *C. albicans* SC5314 or ATCC 90028 (target starting inoculum  $1.5 \times 10^5$  and  $1.5 \times 10^6$  cells/mouse, respectively). Antifungal therapy began 1 day later and continued for 5 days. Treatment groups consisted of control, fluconazole (FLC) 10 mg/kg PO QD, and caspofungin (CFG) 1 mg/kg IP QD. Treatment continued until day 5 and mice were followed off therapy until day 21 to assess survival. Kidneys and brains were collected on day 8 in the fungal burden arm. Fungal burden was assessed by colony-forming units (CFU), and survival was assessed by Kaplan–Meier analysis. Each laboratory evaluated both isolates and conducted the experiments independently.

**Results:** Antifungal response, as measured by reductions in kidney fungal burden and improvements in survival, was very similar between the two laboratories (Table). CFG significantly improved survival and

Isolate	SC5314 (target starting inoculum $1.5 \times 10^5$ cells/mouse)							
	Kidney Tissue Log <sub>10</sub> CFU/g (mean SD)		Brain Tissue Log <sub>10</sub> CFU/g (mean SD)		Median Survival		Percent Survival	
Outcome	Manchester	UTHSCSA	Manchester	UTHSCSA	Manchester	UTHSCSA	Manchester	UTHSCSA
Control	4.71 (0.59)	5.46 (0.62)	1.52 (0.10)	1.50 (0.14)	9.5 days	7.5 days (p = 0.033)	20%	0%
FLC	2.13 (0.60)	2.73 (0.65)	1.48 (0.04)	1.48 (0.13)	>21 days	20 days	65%	30%
CFG	2.13 (0.60)	2.01 (1.21)	1.52 (0.08)	1.42 (0.04)	>21 days	>21 days	95%	100%
Isolate	ATCC 90028 (target starting inoculum $1.5 \times 10^6$ cells/mouse)							
	Kidney Tissue Log <sub>10</sub> CFU/g (mean SD)		Brain Tissue Log <sub>10</sub> CFU/g (mean SD)		Median Survival		Percent Survival	
Outcome	Manchester	UTHSCSA	Manchester	UTHSCSA	Manchester	UTHSCSA	Manchester	UTHSCSA
Control	5.01 (0.41)	5.85 (0.50)	2.00 (0.53)	3.66 (0.32) (p < 0.05)	4 days	10 days (p < 0.001)	5%	20%
FLC	3.28 (0.85)	3.59 (0.57)	1.97 (0.79)	2.95 (0.62) (p < 0.05)	14 days	16 days	10%	30%
CFG	1.58 (0.47)	2.69 (0.64)	1.69 (0.31)	2.71 (0.67) (p < 0.05)	15 days	>21 days	30%	60%

p-value UTHSCSA vs. Manchester data

reduced fungal burden in mice infected with SC5314, while modest reductions in fungal burden and survival improvements were observed with FLC. Similarly, both laboratories also reported significant improvements in survival and reductions in fungal burden for each antifungal in mice challenged with ATCC 90028. Interestingly, there were significant differences in median survival and fungal burden in the brains of untreated mice infected with ATCC 90028. These may have been due to differences between laboratories in the number of viable *C. albicans* cells used to challenge the mice or the subjective criteria used to judge moribund animals.

**Conclusions:** Antifungal treatment response was highly reproducible between these two laboratories. Both CFG and FLC were effective at improving survival and reducing fungal burden against two *C. albicans* clinical isolates. These results demonstrate that our murine model of invasive candidiasis provides a useful and reproducible tool for evaluating therapeutic agents.

**P838** Species distribution, biofilm production, and antifungal susceptibility of *Candida* bloodstream isolates at a university hospital

J. Pongracz\*, K. Kristóf (Budapest, HU)

**Objectives:** The aim of our study was to determine the incidence of candidaemia at our hospital with data on species distribution, biofilm production and in vitro antifungal susceptibility (planctonic and sessile) of the isolates.

**Methods:** All *Candida* bloodstream isolates were obtained at Semmelweis University in 2010–2011. The isolates were identified by their carbohydrate assimilation profile (API 20 C AUX) and morphology on malt agar. Biofilms were formed in 96-well polystyrene microtiter plates, in RPMI 1640 medium substituted with glutamate and MOPS, incubated at 37°C on a rocker. Following 72 hours of incubation, biofilms were washed three times with sterile saline, and biofilm mass was quantified by measuring the fluorometric emission of calcofluor-white stain added to the biofilms. Planctonic MIC (pMIC) values for fluconazole and anidulafungin were determined using the Eucast standard broth dilution method. For determination of sessile MIC (sMIC) values for fluconazole and anidulafungin, 72 hour biofilms were washed, and reincubated for 48 hours in serial dilutions of the antifungal agents. SMIC50 (defined as the antifungal concentration that results in 50% growth inhibition compared to the control well with no antifungal agent) was determined by Alamar blue assay.

**Results:** *Candida* species were isolated from the bloodstream in 40 cases over a 2-year time period (3% of all bloodstream isolates). *Candida albicans* was the predominant species (52.5%), followed by *C. parapsilosis* (17.5%), *C. glabrata* (12.5%), *C. tropicalis* (10%), *C. krusei* (2.5%), *C. lusitanae* (1%) and *C. kefyr* (1%). High biofilm producers were found among the *C. albicans* (33%), *C. tropicalis* (75%), and *C. parapsilosis* (33%) isolates. Fluconazole sMIC values were significantly elevated compared to pMIC for every high biofilm producer isolate, while anidulafungin remained active against the biofilms.

**Conclusion:** Thirty percent of all *Candida* bloodstream isolates were high biofilm producers. While all the high biofilm producers were susceptible to fluconazole in vitro by the EUCAST standard susceptibility testing method, fluconazole had no effect on mature biofilms. Anidulafungin was active against planctonic cells and biofilms as well.

**P839** A 3-year study of in vitro activity of new antifungal agents against *Candida* isolates causing candidaemias

A. Stylianakis\*, V. Papaioannou, S. Tsiplakou, P. Thomaidis, K. Tsopelas, D. Argyris, S. Mantzari, K. Mouta (Athens, GR)

**Objectives:** to evaluate the in vitro antifungal activity of caspofungin (CSF), anidulafungin (ANG), micafungin (MCF) and posaconazole

(PSC) against *Candida* clinical isolates not undergone selective pressure by the examined agents previously.

**Methods:** We examined n = 104 *Candida* isolates which caused candidaemias to equal numbered patients of our hospital during October 2008 till September 2011. These patients were hospitalized in the medical ward (n = 16), in the surgical ward (n = 48) and in the ICUs (n = 40) of our hospital. The fungal isolations were the first recovered ones from patients' blood samples. The examined agents were not administered to the included patients previously, so the examined isolates were considered as selection pressure free ones. The identification was performed by using germ tube test and the automated system VITEK II (Biomérieux). The determination of MIC levels of the CSF, ANG, MCF and PSC was performed using the E-test strips (Biomérieux, ABI-Biodisk) according to the manufacturer's instructions. The interpretation of results for echinocandines was done according to CLSI guidelines and for posaconazole referred to *C. albicans* and *C. parapsilosis* according to EUCAST guidelines.

**Results:** The identification of the isolates was *C. albicans* (n = 40), *C. parapsilosis* (n = 48), *C. glabrata* (n = 8), *C. dubliniensis* (n = 4) and *C. lusitanae* (n = 4). The number of susceptible isolates against the antifungal agents was shown in the following Table.

<i>Candida</i> spp (n)	CSF	ANG	MCF	PSC
	Susc. (n)	Susc. (n)	Susc. (n)	Susc. (n)
<i>C. albicans</i> (40)	40	40	40	28
<i>C. parapsilosis</i> (48)	40	24	48	48
<i>C. glabrata</i> (8)	8	8	8	-
<i>C. dubliniensis</i> (4)	4	4	2	-
<i>C. lusitanae</i> (4)	4	4	4	-

**Conclusions:** The new antifungal agents were in vitro active against the examined *Candida* clinical isolates and they may be good alternate solutions for treating invasive candidiasis. However the decreased susceptibility to posaconazole of *C. albicans* and, as expected, to caspofungin and anidulafungin of *C. parapsilosis* clinical isolates underlines the necessity to perform antifungal susceptibility testing of clinically relevant yeasts.

**P840** Ndt80p :a transcriptional regulator involved in stress response in human fungal pathogen *Candida albicans*

Y. Yang\*, S. Wu, C. Hsiung, C. Chen, C. Hsiao, H. Lo (Hsinchu, Miaoli, Taipei, TW)

**Objective:** The increased use of antifungal agents has led to an increase in incidences of drug resistance. We have found that Ndt80p, a transcription factor, was involved in drug resistance in *Candida albicans*, the most common human fungal pathogens for systemic infection. This study was to understand how Ndt80p regulated its target genes and its roles in the physiology of *C. albicans*.

**Methods:** We have applied genetic and functional studies to characterize the cellular functions of Ndt80p as well as site-directed mutagenesis to identify the potential binding site of Ndt80p on one of its target genes YHB1. We have also performed electrophoretic mobility shift assays (EMSA) to assess the direct interactions between the Ndt80p and the binding site.

**Results:** We found that Ndt80p directly regulated its target genes, such as YHB1, via the mid-sporulation element (MSE). Furthermore, the ndt80R432A allele, with a reduced capability to bind MSE, failed to complement the defects caused by null mutations of NDT80. In fact, the R432 residue in the Ndt80p DNA-binding domain was involved in all tested functions, including drug resistance, nitric oxide inactivation, germ tube formation, hyphal growth, and virulence.

**Conclusion:** Ndt80p is an important transcription modulator to various stress-response genes in *Candida albicans*. It directly regulated its

target genes via MSE. In addition, the importance of the R432 residue suggests a novel approach to design new antifungal drugs through screening compounds blocking the interaction between Ndt80p and its targets.

**P841** *Cryptococcus gattii* induces a distinct pro-inflammatory cytokine pattern compared to other cryptococcal species

T. Schoffelen\*, J. Meis, L.A.B. Joosten, M.G. Netea, T. Boekhout, T. Sprong (Nijmegen, Utrecht, NL)

**Objectives:** *Cryptococcus gattii* is an emerging pathogen. It can cause serious infections in immunocompetent patients, whereas infection caused by other cryptococcal spp. is only seen in immunocompromised patients. We investigated whether this distinction in virulence also relates to differences in cytokine production by host immune cells. Furthermore, we investigated which pattern recognition receptors (PRR) are involved in eliciting a cytokine response to *C. gattii*.

**Methods:** Human blood mononuclear cells were incubated in-vitro with *Cryptococcus gattii*, *C. neoformans* var. *neoformans* and *C. neoformans* var. *grubii* strains and with hybrid strains. A total of 40 cryptococcal isolates were used, comprising clinical and environmental isolates as well as laboratory strains. Cytokines were measured in culture supernatant using ELISA. Subsequently, cells were pre-incubated with receptor antibodies and inhibitory ligands to investigate the involvement of specific PRRs.

**Results:** We found that proinflammatory cytokines, including IL-1beta, IL-6 and IL-17, were induced more strongly by *C. gattii* isolates compared to the other cryptococcal species. Moreover, the hybrid strains containing *C. gattii* as a partner of the mating pair also induced higher levels of proinflammatory cytokines than the hybrid strains which were the result of mating between both *C. neoformans* varieties. In addition, stimulation with clinical *C. gattii* isolates resulted in significantly higher proinflammatory cytokine levels compared to stimulation with *C. gattii* environmental isolates.

*C. gattii* induced IL-1beta and TNF-alfa were substantially inhibited by blocking TLR4, while no effect was seen by blocking TLR2. Furthermore, blocking of TLR9 inhibited IL-17, but not IL-22 production, indicating that there was an IL-17 independent IL-22 response. In addition, inhibition of TLR9 tended to result in more IL-1beta and TNF-alfa production by *C. gattii*.

**Conclusion:** *C. gattii* elicited a more powerful proinflammatory cytokine response than other cryptococcal species. Moreover, clinical isolates induced higher levels of proinflammatory cytokines than environmental isolates. Furthermore, *C. gattii* induced a Th22 response independent of IL-17 production. Both TLR4 and TLR9, but not TLR2, seemed to be involved in *C. gattii* induced cytokine production.

**P842** Investigating clinical cryptococcosis in the context of phagocyte-Cryptococcus interactions

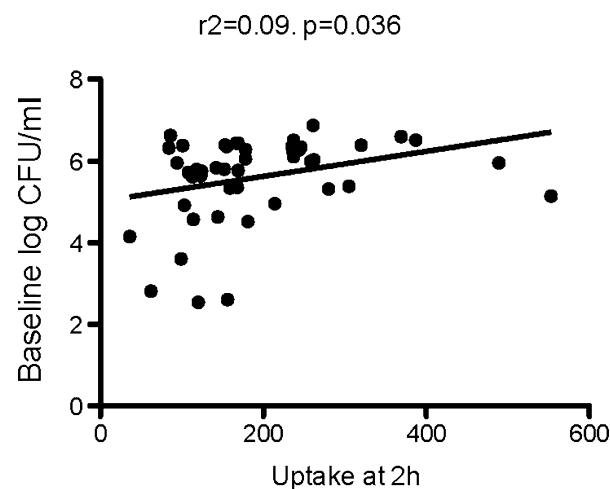
W. Sabiiti\*, T. Bicanic, R.C. May (Birmingham, London, UK)

**Background and objective:** Cryptococcal meningoencephalitis (CM) is a leading cause of death in HIV/AIDS patients in Sub-Saharan Africa. Intracellular parasitism of *Cryptococcus neoformans* in macrophages is an important factor in disease dissemination. Our previous work has demonstrated that *Cryptococcus gattii* isolates with high intracellular proliferation rates (IPR) within macrophages were more virulent and caused fatal disease in a murine model of cryptococcosis. In this study, we explore the association of rate of phagocytosis and IPR in clinical *C. neoformans* isolates with clinical parameters and outcome in patients with HIV-associated CM.

**Methods:** Forty-seven *Cryptococcus neoformans* isolates from patients enrolled in clinical trials were analysed in vitro for (i) Rate of phagocytosis (number of cryptococci engulfed by macrophages within 2 hour); (ii) IPR inside murine J774 macrophages at time points, zero (2 hour), one (18 hour), two (24 hour) and three (48 hour); and

(iii) Melanisation rate on L-2,3-dihydroxyphenylalanine agar. Intracellular proliferation rate was determined as a ratio of number of intracellular cryptococci at time point one to number of cryptococci at time point zero. Association with baseline CSF quantitative cryptococcal cultures, cryptococcal antigen titre, white blood count, glucose, opening pressure; rate of clearance of cryptococci and 2-week mortality was explored using linear and logistic regression.

**Results:** IPR was negatively correlated with the rate of phagocytosis at time point zero ( $r^2 = 0.2$ ,  $p \leq 0.01$ ). Rate of melanisation positively correlated with uptake ( $r^2 = 0.1$ ,  $p = 0.03$ ). Clinical parameter analysis showed positive correlation between CSF quantitative cryptococcal culture and rate of phagocytosis ( $r^2 = 0.1$ ,  $p = 0.03$ , see graph) while cryptococcal antigen titre was negatively correlated with IPR ( $r^2 = 0.13$ ,  $p = 0.01$ ).



**Conclusion:** Macrophages from HIV/AIDS patients are reported to have dysfunctional phagocytosis machinery, implying that highly phagocytosed cryptococcal strains are more likely to systemically disseminate to the central nervous system. The association of melanisation and high phagocytosis rate suggests that *Cryptococcus* require melanin for protection and survival inside the hostile intracellular environment. We hypothesize that high cryptococcal uptake by macrophages results in macrophage-cryptococcal overload, lysis and subsequent release of cryptococci, which accounts for high CSF fungal burden and cryptococcal antigen titre.

**P843** Thrombocyte activation in invasive fungal infections

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**Objectives:** Invasive fungal infections are associated with thrombocytopenia or thrombosis, but the mechanism by which fungi affect the thrombocytes is as yet unknown. The influence of *Aspergillus* and Mucormycetes on platelets is of particular relevance since the platelets represent a relevant part of the innate immunity and to participate in the antifungal immune defense. Therefore we studied whether *Aspergillus* and Mucormycetes secrete factors that modify activity and functionality of thrombocytes.

**Methods:** Fungi were grown for 2 days; the supernatant was harvested and given to human thrombocytes. Activation of the platelets was quantified by aggregometry and by FACS analysis quantifying specific markers.

**Results:** Even minimal volumes of the *Aspergillus* culture supernatant were able to potently stimulate the platelets, inducing high expression of the activation markers on the surface, annexin binding to the platelet

membrane and significant thrombocyte aggregation, even after few minutes of incubation. *Aspergillus*-derived compounds also harbored the capacity to stimulate internalization of labeled beads by the thrombocytes. In contrast, supernatants of different Mucormycetes harbored no or only minimal platelet-activating activity.

Two active components in the *Aspergillus* culture supernatant could be identified. First, the role of a fungal serine protease was confirmed by usage of serine protease inhibitors, which partly eliminated the thrombocyte-stimulating capacity of the *A. fumigatus* supernatant. Second, the mycotoxin gliotoxin seems to play a role, since an *A. fumigatus* mutant unable to synthesize this mycotoxin does not stimulate the thrombocytes to an large extent. Furthermore, the effect of the fungal supernatant could be mimicked by purified gliotoxin. Preliminary experiments with glutathione, a reducing compound that inactivates gliotoxin, suggest the possibility to counteract the action of the mycotoxin and thus to reduce the danger of excessive platelet activation during invasive aspergillosis.

**Conclusions:** Secreted fungal factors such as proteases and mycotoxins might participate in thrombocyte activation during invasive aspergillosis. Putative consequences could be a platelet-driven antimicrobial response and platelet-mediated stimulation of the innate immune network, but also, on the other hand, negative effects such as thrombosis and thrombocytopenia. Mucormycetes seem to use different mechanisms to influence thrombocytes.

## Treatment strategies and economic aspects in invasive fungal infections

### P844 Efficacy of liposomal amphotericin B alone or in combination with caspofungin vs. caspofungin alone or caspofungin followed by liposomal amphotericin B for treatment of murine *Candida parapsilosis* infection

J.A. Olson\*, D.J. Hahka, N. Nguyen, J.P. Adler-Moore (Pomona, US)

**Objectives:** Given the increased incidence of *Candida glabrata* and *Candida parapsilosis* infections, the limited efficacy of echinocandins against *C. parapsilosis* and the reported synergy of caspofungin (Cs) and liposomal amphotericin B (LAB) for treating murine *C. glabrata* infection, we tested this latter combination in a murine *C. parapsilosis* systemic infection to try to improve treatment outcome.

**Methods:** A broth microdilution assay was used to determine the *C. parapsilosis* (ATCC 22019) MIC of LAB (AmBisome®) and Cs (Cancidas®). Swiss Webster mice were challenged i.v. with  $3.5 \times 10^7$  *C. parapsilosis*, and daily treatment initiated 24 hour later with one of the following regimens: 5 or 7.5 mg/kg LAB, 1 mg/kg Cs, 5 or 7.5 mg/kg LAB + 1 mg/kg Cs, 1 mg/kg Cs (d1–3) followed by 5 or 7.5 mg/kg LAB (d4–6), or 5% dextrose (D5W). Day 4, tissues were collected from seven mice/gp, homogenized and used to determine Log<sub>10</sub> cfu/g (Lcfu) by plating on Sabouraud's agar, and to assess drug concentration in spleen, kidneys and heart using a *Candida albicans* agar bioassay. Another seven mice/gp received 6 days of treatment and were monitored for morbidity for 21 days.

**Results:** *C. parapsilosis* MIC for LAB was 0.5 µg/mL and for Cs, 3 µg/mL. Survival with either dose of LAB alone (100%) or combined with Cs (87% for 5 mg/kg LAB+Cs and 100% for 7.5 mg/kg LAB+Cs) was significantly better ( $p < 0.05$ ) than with Cs alone or Cs prior to LAB (14%). D5W mice all died. Fungal burden (Lcfu) for 7.5 mg/kg LAB, with or without Cs, was 5.1–5.3 (spleen), 5.0–5.4 (kidneys), 4.5–4.7 (liver), 5.6–6.4 (heart), 4.4–4.7 (brain). These values were significantly lower ( $p < 0.05$ ) than those for Cs or D5W (spleen 6.6 and 7.1; kidneys 6.3 and 6.9; liver 5.1 and 5.9; heart 7.3 and 7.8; brain 5.2 and 5.3). Mean tissue concentrations were similar for 5 or 7.5 mg/kg LAB (spleen, 1576–1665 µg/g; kidneys, 17–21 µg/g; heart, 3.2–4.5 µg/g) with Cs levels <2.5 µg/g in these tissues.

**Conclusion:** No synergy was observed with the combination of LAB and Cs for the treatment of *C. parapsilosis* infection since LAB alone was as effective as LAB plus Cs in prolonging survival and decreasing

tissue fungal burden. Animals initially treated with Cs for 3 days could not be rescued by switching treatment to LAB.

### P845 In vivo efficacy of voriconazole therapy in an invertebrate model of *Aspergillus fumigatus* infection

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**Objective:** To investigate whether the greater wax moth *Galleria mellonella* is an alternative model to assess the in vivo efficacy of voriconazole (VOR) against different strains of *Aspergillus fumigatus*.

**Methods:** A total of 180 larvae of *G. mellonella* in the sixth instar larvae were inoculated with  $10^5$  cfu/larva of different *A. fumigatus* strains, including parental strain (wild type for the gene encoding the azoles target cyp51A, MICVOR 0.5 mg/L) and five isogenics mutants with altered cyp51A which confer changes in antifungal drugs susceptibility (MICVOR ranging between 0.25 and 4 mg/L, EUCAST methodology). Within 120 minutes after infection, different doses of VOR were administered. The doses used were calculated taking into account the therapeutic dose in humans and the MIC values of strains studied. Additional control groups of 50 larvae were also included (untouched, pierced, inoculated with PBS; toxicity control of antifungal and DMSO/water). Larvae were incubated at 37°C and the larval survival was monitored daily considering that the larvae die when they do not respond to touch. Each experiment was performed three times. Survival data were plotted using the Mantel-Cox tests (GraphPad Software Inc) and comparisons between untreated and treated groups were assessed with the Long Rank test ( $p < 0.01$ ).

**Results:** Larval survival was dependent both on the dose and the MICVOR. The  $p$  values show no significant larval survival differences between untreated and treated groups with VOR 1.25 and 2.50 mg/kg/day (subtherapeutic doses), with none of the strains included. When a dose of 10 mg/kg/day was administered, the larval survival increased for all, except for those infected with the VOR resistant strain (MICVOR 4.0 mg/L). In all cases, 80% of infected/untreated larvae died between the 3rd and 4th day, while a 100% survival was observed with therapeutic doses of VOR at the last day of the experiment (7 days). The group of larvae infected with the VOR resistant strain all died on day 4.

**Conclusions:** (i) VOR treatment significantly prolonged the survival of *G. mellonella* caterpillars infected with *A. fumigatus* strains that showed low in vitro MICs compared with the ones showing higher VOR MICs. (ii) *G. mellonella* is a relatively simple, nonmammalian model that can be used to facilitate the in vivo-in vitro correlation studies with antifungals. More studies with a larger number of strains and doses would be needed.

### P846 Activity of posaconazole in invasive fungal infections associated with a variety of moulds and yeasts

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**Objective:** Invasive fungal infections (IFIs) cause serious complications in immunosuppressed patients. Although *Candida* and *Aspergillus* species (sp) are the most common pathogens, other rare fungi can occasionally be involved. Extensive use of azoles as first line therapy may result in the emergence of less common fungal infections. Posaconazole is a second generation antifungal drug with wide yeast and mould coverage and could play a role in the treatment of these rare infections.

**Methods:** This is a multicenter single arm phase II study of Posaconazole (400 mg BID) in patients with IFIs who have failed or been intolerant to only one prior line of therapy. The primary objectives are safety and efficacy. Safety was evaluated in all patients; efficacy on those who survived more than 7 days.



**Results:** Forty patients were included in the study of which 29 (73%) had proven or probable IFIs. Conditions leading to IFI were AML (31%), stem cell transplant (26%), lung transplant (11%), renal transplant (9%), pulmonary disease (9%) and others (14%). A specific organism could be identified in 30 patients, with *Aspergillus* sp representing the majority of cases (55%). *A. fumigatus* was the most common sp but *A. niger*, *A. flavus* and *A. cervinus* were also found. *Candida* sp accounted for 18% of fungi: *C. albicans*, *C. glabrata* and *C. krusei* were detected. Less common fungi such as mucormycetes (*Rhizomucor* and *Rizopus*), *Coccidioides*, *Exophiala*, *Fusarium* and *Histoplasma* were also identified. Prior therapy included voriconazole (47%), amphotericin B (28%), fluconazole (16%) and others (9%). Patients were either refractory (70%) or intolerant (30%) to these prior therapies. Efficacy could be evaluated in 32 patients at the time of analysis. Response rate in both proven and probable infected patients based on the EORTC criteria was 53% (9% CR and 43% PR); two patients with early death were classified as non responders. The response rates were similar in all patients including those infected with rare fungi and those with possible infections. Safety was assessed in all 40 patients common toxicities reported were: nausea and vomiting (26%), diarrhea (26%), rash (15%), edema (12%) and elevated liver enzymes (9%). No drug related grade 4 toxicities were reported.

**Conclusion:** Several less common fungi were isolated supporting the emergence of rare sp. Posaconazole was equally effective in all patients and may represent a valuable alternative even after prior exposure to other azoles.

**P847 Treatment of candidaemia in patients with haematologic malignancies**

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Candidemia is a severe complication in children with hematological malignancies leading to a high mortality rate up to 50%. Increasing resistance to azoles of *C. albicans* and prevalence of *C. non-albicans* are two most significant modern trends in candidemia.

**Patients:** Thirty-four patients (24 M/10 F) with median age 6 (0.36–17) years with different malignancies and bone marrow failures developed 43 episodes of candidemia (EC). Systemic antifungal prophylaxis was applied in 35 (82%) before EC, mostly with azoles used in 32 pts. Absolute neutrophil count (ANC) at onset of EC was  $<0.5 \times 10^9/L$  in 26 (60.5%). Clinical manifestations were fever (100%), pneumonia (30%), endocarditis (9.3%), multiorgan failure (7%), septic shock (SS) (4.7%), chronic disseminated candidiasis (4.7%), skin rash (4.7%) and meningitis (2.4%).

**Results:** *C. albicans* was isolated in five pts (eight strains) and *Candida non-albicans* – in 29 pts (36 strains). Twenty (45.5%) strains were resistant to fluconazole: 4/8 (50%) *C. albicans* and 16/36 (44%) *C. non-albicans*. All pts received antifungal therapy (monotherapy 19 EC, combination therapy 24 EC). Central venous catheter (CVC) was removed in 25 (58%) EC. In 21 (49%) EC G-CSF was administered.

**Outcome:** Thirty-seven EC were cured, 6 (14%) patients died. Overall 30 days survival was  $0.7 \pm 0.13$ ; 5-years' OS –  $0.45 \pm 0.09$ . Factors of unfavorable prognosis were: advanced malignancy – 5 death of 10 EC vs 1 death of 33 EC in remission or aplasia after 1-st remission induction ( $p = 0.000$ ), neutropenia at onset of EC: 6 of 26 vs 0 of 17 EC of pts with ANC more and less  $500/mm^3$  respectively ( $p = 0.04$ ), hematopoietic recovery (0 of 35 EC vs 6 of 8 EC with or without recovery, ( $p = 0.000$ ) and CVC removal (1 of 25 EC with CVC removed vs 5 of 18 with CVC remained ( $p = 0.03$ )).

**Conclusion:** Candidemia remains an unresolved issue in pediatric hematology/oncology with high attributable mortality. Most of cases occur in spite of systemic antifungal prophylaxis. Stage of underlying disease, CVC removal and ANC recovery are the main variables influencing survival.

**P848 Correlation between incidence of candidaemia and consumptions of antifungal agents in a medical centre in Taiwan**

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**Background:** To analyze the epidemiology of candidemia and its association with the use of antifungal agents, we review the secular trend and species distribution of candidemia and the consumption of antifungal agents.

**Methods:** Patients with candidemia were identified by central laboratory personnel. The incidences of patients with candidemia were defined as number of patients with candidemia per 1000 inpatient-days, and the incidence of healthcare-associated candidemia (HCAC) was defined as the cases of patients with HCAC per 1000 admissions. Data on annual consumption (defined daily dose [DDD] per 1000 patient-days) of amphotericin B, ambisome, fluconazole, itraconazole, and ketoconazole, from 2000 to 2010 were obtained from the pharmacy department.

**Results:** In this period, a total of 2682 episodes of candidemia were identified. There are 2468 (92%) cases belonged to HCAC and 214 (8.0%) cases were identified as community acquired candidemia. The most prevalent species were *C. albicans* ( $n = 1429, 53.3\%$ ) followed by *C. tropicalis* ( $n = 551, 20.5\%$ ), *C. glabrata* ( $n = 413, 15.4\%$ ), *C. parapsilosis* ( $n = 378, 14.1\%$ ), *C. guilliermondii* ( $n = 46, 1.7\%$ ), *C. krusei* ( $n = 39, 1.5\%$ ). Overall, the incidence of all cases of candidemia remained stable, however, the incidence of *C. parapsilosis* and *C. guilliermondii* fungemia decreased significantly with time. The total incidence of HCAI-candidemia was ranged from 2.66 to 4.27 per 1000 admissions. The consumption of caspofungin, micafungin, and, voriconazole significantly increased, but the consumption of amphotericin remained stable. In contrast, the usage of itraconazole significantly decreased with time. A significant negative correlation was found between the use of echinocandins and voriconazole, and incidence of *C. parapsilosis* fungemia, and the use of caspofungin and the incidence of *C. guilliermondii* fungemia, respectively. In contrast, there are significant positive correlation found between the use of echinocandins and the incidence of *C. tropicalis* fungemia, and the use of azole and the incidence of *C. glabrata*, the use of itraconazole and the incidence of *C. parapsilosis*, and *C. guilliermondii* fungemia.

Correlation between each antifungal agent and the incidence of candidemia caused by each candida species

Candidemia	Total		<i>C. albicans</i>		<i>Non-albicans Candida</i>		<i>C. tropicalis</i>		<i>C. glabrata</i>		<i>C. parapsilosis</i>		<i>C. guilliermondii</i>		<i>C. krusei</i>	
	r	P	r	P	r	P	r	P	r	P	r	P	r	P	r	P
Echinocandins	0.489	0.023	0.384	0.004	0.338	0.021	0.717	0.03	0.461	0.15	0.742	0.022	0.013	0.076	0.369	0.361
Caspofungin	0.368	0.020	0.247	0.022	0.341	0.369	0.323	0.387	0.271	0.326	0.509	0.161	0.802	0.889	0.165	0.663
Micafungin	0.719	0.000	0.471	0.029	0.737	0.243	0.802	0.000	0.383	0.603	0.000	0.112	0.717	0.283	0.499	0.361
Voriconazole	0.364	0.021	0.547	0.024	0.034	0.056	0.238	0.489	0.669	0.024	0.074	0.029	0.023	0.764	0.284	0.328
Fluconazole	0.426	0.016	0.316	0.343	0.332	0.092	0.017	0.019	0.152	0.078	0.132	0.700	0.432	0.163	0.326	0.328
Itraconazole	0.039	0.823	0.110	0.794	0.027	0.949	0.302	0.283	0.199	0.424	0.937	0.081	0.409	0.324	0.133	0.883
Ketoconazole	0.140	0.464	0.213	0.329	0.008	0.902	0.244	0.476	0.094	0.783	0.471	0.024	0.023	0.882	0.287	0.548
Amphotericin	0.141	0.470	0.22	0.355	0.020	0.915	0.118	0.713	0.174	0.612	0.269	0.339	0.663	0.03	0.324	0.697

r, the correlation coefficient; Statistically significant association (marked in bold type,  $P < 0.05$ )

**Conclusions:** This 11-year surveillance study in Taiwan showed the incidence of candidemia, including *C. albicans* and non-albicans *Candida* fungemia remains stable, however, the incidence of HCAI-candidemia was higher than in other regions. The impact of each antifungal agent on the incidence of candidemia caused by different *Candida* species is various.

**P849 How low can you go? Use of low- and standard-dose liposomal amphotericin B for treatment of invasive fungal infections at a USA public hospital**

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**Objectives:** Recommended doses of liposomal amphotericin B (L-AMB) range from 3 to 6 mg/kg/day but 1 mg/kg/day may be equally effective and a lower cost alternative for many indications. Low dose L-AMB (1 mg/kg/day rounded up in 50 mg increments) has been used for most indications in our urban public hospital. We describe and

compare clinical indications and outcomes of patients receiving low-dose (LD: <2 mg/kg/day) and higher dose (HD: ≥2 mg/kg/day) L-AMB for the treatment of invasive fungal infections.

**Methods:** We reviewed consecutive adult L-AMB recipients with probable and proven infections per EORTC/MSG 2008 criteria at a single center from 2006 to 2011.

**Results:** Of 98 adult L-AMB recipients, 38 with proven or probable infections met inclusion criteria. Twenty (53%) received LD and 18 (47%) received HD L-AMB; median doses were 1.5 and 3.0 mg/kg, and median duration of therapy was 8.5 and 8.0 days respectively. Baseline characteristics including ICU admission, need for mechanical ventilation, vasopressors and/or inotropes were similar between the two groups. LD subjects more commonly had HIV/AIDS and a history of invasive fungal disease. Meningitis and pneumonia were the most common sites of infection in both groups. *Cryptococcus* was the most common fungal pathogen (40%) in the LD group whereas *Candida* sp. were more common in the HD group (44%). Other fungal pathogens included *Aspergillus*, histoplasma, blastomyces, coccidioides, and zygomycetes. Fifty percent of subjects in both groups improved clinically. Reasons for failure by frequency of occurrence in both groups were: worsening or absence of improvement, requirement for L-AMB dose escalation, persistent positive cultures, and hospital readmission for the same indication. L-AMB was discontinued to consolidate to azole therapy in 70% of the LD and 50% of the HD groups. Seventy percent and 78% of subjects in the LD and HD groups respectively survived to discharge. Rates of nephrotoxicity and hypokalemia were comparable. Median cost of therapy, based on drug acquisition cost, was \$1140 in the LD and \$2058 in the HD groups.

**Conclusion:** We found comparable rates of clinical improvement and survival to discharge using LD vs. HD L-AMB. LD L-AMB has been an effective and cost-saving treatment option for common invasive fungal infections in our institution.

#### **P850** Impact of an antifungal stewardship programme in a teaching hospital: a prospective study

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**Objectives:** A multifaceted antifungal stewardship program has been in place in our teaching tertiary-care hospital since 2005, with several actions implemented successively, including systematic evaluation of all costly antifungal prescriptions (echinocandins, lipid formulations of amphotericin B, posaconazole and voriconazole). We assessed the impact of this program on antifungal prescriptions and cost (between 2003 and 2010), and on standards of care regarding the management of invasive aspergillosis and candidemia.

**Methods:** We conducted a prospective observational study over an 8-year period.

**Results:** Six hundred and thirty-six antifungal prescriptions were discussed by the antifungal management team from 2005 to 2010, mainly from the haematology department (72%). In 344/636 (54%) cases, a piece of advice was fed back in real time to the physician in charge of the patient, with an 88% compliance rate. Optimal standard of care was achieved for performance of chest CT-scan, galactomannan antigen testing and voriconazole therapeutic drug monitoring for invasive aspergillosis, with no antifungal combination therapies used since 2008. Regarding candidemia, optimal standard of care was achieved for timing of antifungal therapy, recommended 1st-line therapy, duration of therapy and removal of central venous catheters. Antifungal prescriptions and cost were stable between 2003 and 2010.

**Conclusion:** Implementation of an antifungal stewardship program was feasible, sustainable and well-accepted, and we observed an improved quality of care and no increase in antifungal use or cost.

#### **P851** Liposomal amphotericin B in ICU patients with continuous renal replacement therapy

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**Objective:** To assess the effectiveness of liposomal amphotericin B (L-AmB) in ICU patients with continuous renal replacement therapy.

**Methods:** An observational, retrospective and multicenter study was conducted in critically ill patients with continuous renal replacement therapy, treated with L-AmB for at least 3 days. Patients with renal replacement therapy before or within 48 hour after starting L-AmB were included. The primary endpoints were both clinical and microbiological response. Demographics, underlying illness, APACHE II score, fungal infectious disease, use of antifungals, and vital status at ICU and hospital discharge were collected.

**Results:** A total of 36 patients recruited in 16 ICUs were included (range: 1–8). L-AmB was mainly selected because of broad spectrum of the drug and hemodynamic instability of the patients. The mean age of the patients was 47 years, and the majority was men (26 out of 36; 72%). The mean APACHE II score was 21.4 ± 8.8 at the start of L-AmB treatment. Related to infection the reasons for L-AmB use were proven (38.9%), probable (16.7%), possible (16.7%) fungal infection or clinical suspicious (27.8%). Roughly half of the patients had received previous treatment with antifungals (55.6%). The median duration of L-AmB treatment was 13.5 days (range: 4–42), with most patients treated for seven or more days (4 out of 36; 88.9%), and the L-AmB median daily dose was 4.8 mg/kg/day (range: 2.8–7.0). The main reasons for renal replacement therapy were uraemia (47.2%), fluid retention (41.7%) and metabolic acidosis (22.2%). The median time of ICU and hospital stay were 28 and 48 days, respectively. *Candida* spp was present in 73% (19 out of 26) of those patients in whom a fungal species was isolated and *Aspergillus* spp was in 3 out of 26 patients (11.5%). Related to clinical response 66.1% responded to treatment (complete and partial response) and microbiological response was observed in 85.7% of patients with proven infection. Twenty-two out of 36 patients survived (61.1%) and 64.3% of deaths were unrelated to fungal infection.

**Conclusions:** Clinical and microbiological responses of critically ill patients with continuous renal replacement treated with L-AmB were very similar to those already described in ICU patients without it. Although this is a small sample it seems that L-AmB is also effective in ICU patients with continuous renal replacement therapy.

#### **P852** Substudy of the multicentre, retrospective study to evaluate renal function in patients with chronic obstructive pulmonary disease admitted to intensive care units and treated with liposomal amphotericin B

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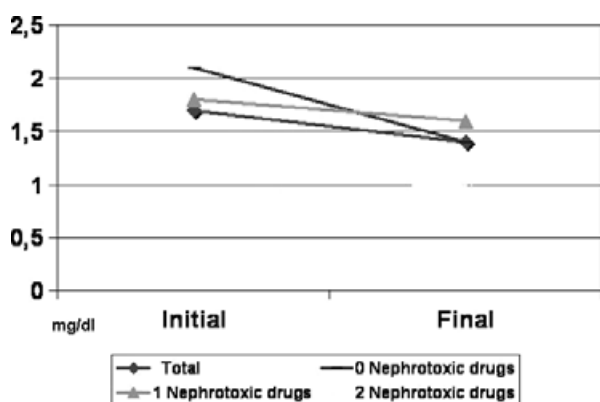
**Objective:** To assess the renal safety of L-AMB in patients with COPD admitted to ICUs in Spanish hospitals.

**Materials and methods:** This is a study of patients admitted to ICU and treated with L-AMB for any reason for at least 3 days during 2008–2009 and 2006. A “study case” was defined as any patient with COPD who had not undergone extrarenal clearance during L-AMB treatment.

**Results:** A total of 37 patients from 21 ICUs were included. On admission, the mean Apache II score was 21.4 (7.5). At the start of L-AMB therapy, 100% of patients had a central venous line and bladder catheter, 89.2% had received antibiotics in the previous 7 days, 84.2%

had an arterial line, 86.5% required mechanical ventilation, and 62.2% needed total parenteral nutrition.

L-AMB was used mainly due to its broad spectrum and the patients' hemodynamic instability and was administered as first-line therapy in 21 patients (56.8%). The mean course of L-AMB was 12.2 (5.9) days and the mean dose was 3.1 (1.0) mg/kg/day. At the start of L-AMB therapy, creatinine was  $\leq 1.5$  mg/dL in 64.9% (24/37) of patients. By the end of treatment, 91.7% of patients with baseline creatinine  $\leq 1.5$  mg/dL still had  $\leq 1.5$  mg/dL. The mean baseline creatinine was 1.71 (1.5) mg/dL, but dropped to 1.40 (1.0) mg/dL by the end of treatment ( $p = 0.089$ ) (Figure 1). In terms of percentages, creatinine had dropped or held steady in 67.57% of patients by the end of treatment. Although 66.7% (24/36) of patients were receiving concomitant nephrotoxic drugs, a two-fold increase in creatinine compared to baseline was observed in only 2 (5.41%) patients. No patients discontinued the treatment for nephrotoxicity or another reason, and there were no serious adverse events.



**Conclusions:** Patients with COPD admitted to ICU experienced no renal impairment during L-AMB therapy and there were no discontinuations due to renal toxicity despite the use of concomitant nephrotoxic drugs. Although the patient sample was small, the data suggest that L-AMB can be safely administered to critical patients with COPD.

**P853** **Costs of posaconazole compared to standard prophylaxis in patients with a high-risk of invasive fungal diseases: an economic analysis from the Cologne cohort of neutropenic patients**

S. Heimann\*, O.A. Cornely, H. Wisplinghoff, M. Vehreschild, B. Franke, J. Glossmann, J.-J. Vehreschild (Cologne, DE)

**Background:** Controversy exists about the cost effectiveness of posaconazole prophylaxis in neutropenic patients with a high risk of invasive fungal diseases. We performed an analysis comparing the direct costs of posaconazole prophylaxis against topical polyene (thrush) prophylaxis in patients with acute myelogenous leukemia (AML) and myelodysplastic syndrome (MDS).

**Methods:** Data of AML/MDS patients receiving remission-induction chemotherapy were extracted from the Cologne Cohort of Neutropenic Patients (CoCoNut) to compare hospital costs of patients before (2003–2005) and after (2006–2008) introduction of posaconazole prophylaxis. All cases were part of an earlier analysis demonstrating effectiveness of posaconazole over topical prophylaxis (Vehreschild et al., J Antimicrob Chemother. 2010 July; 65[7]:1466–71). Duration on general ward, intensive care unit, mechanical ventilation, diagnostic procedures and all anti-infective drugs were included into the cost analysis.

**Results:** Patient groups were well matched according to age, gender, underlying disease, and duration of neutropenia. The mean costs per patient in the posaconazole group ( $n = 76$ ) and the topical polyene group ( $n = 81$ ) were 23 235 (95% CI: 19 722–26 749) and 26 531 (95% CI: 21 887–31 175) per patient, respectively. Average daily

treatment costs were 495 (95% CI: 434–555) and 508 (95% CI: 433–582). Antifungal treatment costs were nominally lower in the posaconazole group (6773 [95% CI: 5187–8360] vs. 7419 [95% CI: 5062–9775]). The costs for other anti-infectives were also numerically decreased in the posaconazole group (4845 [95% CI: 4019–5671] vs. 5402 [95% CI: 4507–6297]). Average duration of ICU stays were 1.79 (95% CI: 0.68–2.90) days per patient for the posaconazole group compared to 3.83 (95% CI: 1.53–6.13) days per patient in the topical polyene group. Costs for diagnostic procedures were 611 (95% CI: 478–744) and 653 (95% CI: 552–754) per patient, respectively.

**Conclusions:** In our hospital, there was a trend towards cost-saving by posaconazole prophylaxis in patients receiving remission-induction chemotherapy. These cost savings were demonstrated in all aspects taken into consideration, including overall treatment costs as well as cost of anti-infective and antifungal medication.

**P854** **Cost analysis of candidaemia in patients on the intensive care unit**

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**Objectives:** Direct costs caused by candidemia in ICU patients are currently unknown. We performed an analysis comparing costs depending on the type of antifungal treatment.

**Methods:** Comprehensive data of patients from the University Hospital of Cologne with at least one blood culture positive for *Candida* spp. while staying on the ICU between 2005 and 2010 were documented into a database provided by the Cologne Cohort of Neutropenic Patients (CoCoNut). Analysis was split for patients treated with new (i.e. echinocandins, liposomal amphotericin B, or voriconazole) or conventional antifungals (i.e. amphotericin B deoxycholate or fluconazole).

**Results:** Out of 120 patients identified, 41 received new and 55 conventional antifungals; 24 patients were excluded (19 died within 96 hours after positive blood culture, five were rated as contamination). Both groups were well matched by age and baseline intubation status. Mean durations of medical care per patient in the new and conventional antifungal groups were as follows: ICU treatment 21.5 days (95% CI: 15.2–27.9 days) vs. 13.7 days (95% CI: 9.4–17.9 days), general ward treatment 10.12 days (95% CI: 5.3–15 days) vs. 9.2 days (95% CI: 5.3–13.2 days), mechanical ventilation 474 hour (95% CI: 323–624 hour) vs. 304 hour (95% CI: 197–410 hour), and dialysis: 31.5 hour (95% CI: 9.6–53.3 hour) vs. 39.7 hour (95% CI: 16.0–63.4 hour). Mean direct costs per patient in the new and the conventional antifungal groups were as follows: ICU treatment 27 291 (95% CI: 19 282–35 300) vs. 17 188 (95% CI: 11 783–22 593,  $p = 0.032$ ), antifungal treatment 4916 (95% CI: 3595–6238) vs. 1812 (95% CI: 866–2758,  $p < 0.001$ ), total direct costs 44 451 (95% CI: 33 157–55 745) vs. 27 844 (95% CI: 19 968–35 720,  $p = 0.014$ ).

**Conclusion:** Our cost-of-illness analysis shows the high treatment costs of patients with candidemia. Actual antifungal drug costs play a minor role compared to the substantial costs of clinical and supportive care. In our analysis, treatment with new antifungals was associated with higher costs and a longer period of hospitalisation. However, as new antifungals are often considered less toxic, less interacting, better tolerated, and/or more effective, there may have been a treatment bias towards sicker patients more likely receiving treatment with these drugs.

**P855** **Pharmacokinetics of intravenous itraconazole followed by itraconazole oral solution in patients with candidaemia**

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**Background:** According to various clinical practice guidelines, antifungals should be administered at least for 14 days after fungal eradication in candidaemia. However, step down therapy from

intravenous to oral antifungal agent might be also recommended from the aspect of pharmacoeconomics.

**Methods:** This randomized, open-label, comparative study assessed the pharmacokinetics and safety of intravenous itraconazole (ITCZ) and oral ITCZ solution in patients with candidaemia caused by *Candida* species with MIC level of under 0.125 mg/L. All patients received ITCZ, 1-hour intravenous drip infusions at the dose of 200 mg twice daily for 2 days, following 200 mg once daily for 8 days. Then, patients were randomized to receive ITCZ oral solution, 200 mg twice daily or 200 mg once daily, for further 14 days. Twenty-one patients were enrolled and analyzed (n = 6 for oral twice daily, n = 5 for once daily). ITCZ and hydroxyitraconazole (OH-ITCZ) plasma concentrations at day 10 and 15 after the first ITCZ administration were assayed with HPLC method.

**Results:** At the final intravenous dosing (day 10), mean trough plasma concentrations of ITCZ and OH-ITCZ were 910 ng/mL and 1720 ng/mL, respectively. During oral dosing, mean trough plasma concentrations of ITCZ and OH-ITCZ were increased in the ITCZ 200 mg twice-daily group, however, almost maintained in the ITCZ 200 mg once-daily oral dose. ITCZ was generally well tolerated and had a favorable safety profile in this study.

**Conclusions:** ITCZ 200 mg given intravenously twice daily for 2 days, following once daily for 8 days, then ITCZ oral solution, once daily for 14 days, would maintain ITCZ trough concentration in the level of over 250 ng/mL, which would be lead to good clinical outcome and low recurrent rate for candidaemia.

#### P856 Pre-emptive/targeted prophylaxis with voriconazole in lung transplant patients: a retrospective cohort study

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**Objective:** Invasive fungal infection is one of the major complications, contributing to high mortality and morbidity among lung transplant (LTx) patients. Data for voriconazole use in pre-emptive/targeted prophylactic setting remain scant. This study aimed to investigate the efficacy and safety of voriconazole pre-emptive/targeted prophylaxis among LTx patients once airway fungal colonisation was confirmed.

**Methods:** A retrospective, single-centre, observational cohort study was conducted. All adult LTx patients who received voriconazole pre-emptive/targeted prophylaxis for the first time between July 2003 and June 2010 were followed up for 1 year. Outcomes were determined at 6 months (primary end-point) and at 12 months after initiation of voriconazole prophylaxis. Chi-square test was used to assess the relationships between variables and outcomes (i.e. fungal infection, *Aspergillus* colonisation, death and drug-related hepatotoxicity). Cumulative survival rate at 12-month after initiation of prophylaxis were estimated using the Kaplan–Meier method.

**Results:** A total of 62 LTx patients were included. *A. fumigatus* (75.8%) was the most common colonising isolate. Median duration of voriconazole prophylaxis was 84 days. At the 6-month end-point, one (1.6%) had developed probable fungal pneumonia, 48 (77.5%) had successful eradication of colonisation, three (4.8%) had persistent colonisation, six (9.7%) had recurrent colonisation, two (3.2%) had aspergilloma and two (3.2%) were clinically unstable but no culture was performed. Sixteen (25.8%) had died by the 12-month end-point, half due to Bronchiolitis Obliterans Syndrome. Ten (16.1%) had drug-related hepatotoxicity. Patients with diabetes mellitus within 30 days before commencing voriconazole prophylaxis (OR: 8.4, 95% CI: 2.1–33.4, p = 0.003) were at higher risk for *Aspergillus* colonisation at 12-month end-point. Preliminary analyses suggest that acute rejection (OR: 4.9, 95% CI: 1.2–19.4, p = 0.026) and chronic rejection (OR: 6.5, 95% CI: 1.3–31.5, p = 0.022) within 30 days prior to voriconazole prophylaxis were significantly associated with 12-month mortality. LTx patients older than 55 years old were 5.9 times more likely to have hepatotoxicity after initiation of prophylaxis (95% CI: 1.1–30.6, p = 0.036).

**Conclusions:** Voriconazole pre-emptive/targeted prophylaxis resulted in low incidence of fungal infection and fungal infection-related mortality in adult LTx recipients, and was generally well-tolerated.

#### P857 Impact of testing posaconazole plasma concentrations on epidemiology of antifungal prophylaxis and therapy in patients with haematologic malignancies: case-control study

M. Hoenigl\*, K. Seeber, R. Raggam, T. Valentin, A. Valentin, A. Strohmaier, I. Zollner-Schwetz, A. Woelfler, A. Grisold, W. Linkesch, R. Krause (Graz, AT)

**Objective:** Therapeutic drug monitoring (TDM) has shown useful for oral antifungal agents in patients with hematologic malignancies. The effect of introduction of TDM on prescribing praxis is unknown. We evaluated the impact of posaconazole TDM on antifungal prophylaxis and therapy.

**Methods:** Applied antifungal prophylaxis and therapy were assessed in patients with underlying hematologic diseases by conducting a prospective observational single-centre study at the Division of Hematology, Medical University of Graz, Austria, for 7 months in 2010. To analyze the impact of PPC testing on antifungal prophylaxis and therapy results obtained were compared to a representative collective of patients assessed by the same investigators at the same institution over 7 months in 2007 before testing of posaconazole plasma concentrations (PPCs) has been introduced.

**Results:** In 2010, 129/729 (18%) of cases with hematologic malignancies received systemic antifungal prophylaxis and therapy. Of those, fifty-seven percent received prophylactic, 44% empiric, 30% preemptive and 6% directed antifungal therapy. Main reasons for prophylaxis were neutropenia in AML patients (40/74; 54%), followed by GVHD (18/74; 24%) and allogeneic HSCT (16/74; 22%). Eleven out of 39 (28%) cases receiving preemptive therapy had clinical/radiological and microbiological evidence of IFI, 26/39 (67%) had clinical/radiological and 2/39 (5%) only microbiological evidence of

	Antifungal therapy 2007 <sup>b</sup>	Antifungal therapy 2010
<b>Number</b>	117/690 (17%)	129/729 (18%)
<b>Sex</b>		
male	70/117 (60%)	85/129 (66%)
female	47/117 (40%)	44/129 (34%)
<b>Age</b>		
median age	51,2	53,5
<b>Chemotherapy</b>		
high dose <sup>a</sup>	55/117 (47%)	81/129 (63%)
palliative <sup>a</sup>	39/117 (33%)	17/129 (13%)
<b>Rational for antifungal therapy</b>		
directed	4/117 (4%)	8/129 (6%)
preemptive <sup>a</sup>	50/117 (43%)	39/129 (30%)
empiric <sup>a</sup>	31/117 (36%)	57/129 (44%)
prophylactic	52/117 (44%)	74/129 (57%)
<b>Antifungal agents</b>		
posaconazole <sup>a</sup>	38/117 (32%)	81/129 (63%)
caspofungin	72/117 (62%)	65/129 (50%)
lip amph B	7/117 (6%)	10/129 (8%)
voriconazole	14/117 (12%)	18/129 (14%)
itraconazole	15/117 (13%)	19/129 (15%)
fluconazole	7/117 (6%)	7/129 (5%)
anisulafungin	0	1/129 (1%)

**Note:**

<sup>a</sup> Significant difference between 2007 and 2010 in patient collective with antifungal therapy  
P < 0.05

<sup>b</sup> Data from 2007 previously published (11)

IFI. In 2010 posaconazole was the most commonly administered antifungal agent followed by caspofungin which had been the leading antifungal agent at the study site in 2007. Posaconazole usage increased significantly after introduction of posaconazole TDM when compared to 2007 ( $p < 0.05$ ). Concerning prescription rates of antifungal agents other than posaconazole no significant difference was found. In both study collectives (2007 and 2010) posaconazole was the primary antifungal agent used for prophylaxis, while itraconazole was used mainly in allogeneic HSCT. Demographic data, chemotherapeutic approach and antifungal modalities for cases receiving antifungal therapy in 2007 and 2010, respectively, are depicted in Table 1.

**Conclusion:** We found a significant increase of posaconazole usage after introduction of posaconazole therapeutic drug monitoring (TDM). As TDM was available for posaconazole only, the feasibility of monitoring plasma concentrations may have influenced the selection of this antifungal agent in clinical routine.

**P858** **Posaconazole plasma concentrations and invasive mould infection in patients with haematologic malignancies: a cohort study**

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**Objectives:** Posaconazole is a new triazole antifungal agent that has broad activity against pathogenic fungi and is increasingly used for prophylaxis and treatment of invasive mould infections (IMI). Posaconazole is available only as oral formulation with varying absorption from the gastro-intestinal tract. Reports correlating posaconazole plasma concentrations (PPCs) with breakthrough IMI, however, are rare.

**Methods:** We analyzed posaconazole plasma concentrations (PPCs) in a prospective observational single-centre study in 2010 and evaluated correlation of PPCs with breakthrough IMI in patients with hematological malignancies. We further evaluated risk factors associated with low PPCs.

**Results:** A total of 109 PPCs were measured in 34 patients receiving posaconazole prophylaxis ( $n = 31$ ) or treatment ( $n = 3$ ). Insufficient levels were detected in 24/34 (70%) of patients; in 15 of these 24 patients concentrations were found under the limit of detection ( $< 0.20 \mu\text{g/mL}$ ). Insufficient PPCs yielded either way in a modification of intake procedures, discontinuation of PPIs or switch of antifungal therapy. In 12 of these 24 cases with insufficient PPC, modification of intake – i.e. with a high fat meal – led to sufficient PPCs. As discontinuation of PPIs led to an improvement of PPC levels in only 1/24 cases, antifungal therapy had to be switched due to insufficient PPCs in another five cases. In three patients with insufficient PPCs, antifungal therapy had to be changed from posaconazole prophylaxis to echinocandin empiric treatment due to development of febrile neutropenia, these patients did not fulfil IFI criteria.

Three patients on posaconazole prophylaxis met the criteria of breakthrough infection. Prior to development of invasive fungal infection (IFI), however, PPCs were insufficient in all three patients.

Fungal species	Posaconazole MIC (mg/L)	Patient's age years/sex	Specimen of fungal detection	Days of posaconazole prophylaxis before breakthrough infection	Last posaconazole plasma level before diagnosis of IMI (days before diagnosis)	Antifungal therapy after IMI diagnosis	Outcome
<i>Aspergillus fumigatus</i>	0.032	58f	BAL	8	0.28 $\mu\text{g/ml}$ (3)	Venconazole	Died
<i>Aspergillus</i> spp.	n.a.	20m	BAL/Serum	23	<0.20 $\mu\text{g/ml}$ (4)	Venconazole	Survived
<i>Geotrichum argillaceum</i>	0.25	52m	BAL/Blood culture	>60	0.31 $\mu\text{g/ml}$ (7)	Venconazole, EpAmph B	Died

Details are depicted in table 1. Associated risk factors for insufficient PPCs varied from previous reports.

**Conclusion:** These data demonstrate that therapeutic drug monitoring (TDM) of posaconazole is mandatory in all patients with hematological malignancies as low PPCs are common and may be associated with development of IFI.

**P859** **Gentian violet can be used as topical oral anti-fungi and anti-stigma agent by people living with HIV/AIDS**

E. Agwu\*, J. Inyang, J. Ihongbe (Ishaka, UG; Ekpoma, NG)

**Background:** The challenges of poor reduction of oral fungal load are complicated by rampant report of refractile (Multidrug resistant) oropharyngeal Candidiasis, permitting the need to search for possible alternative to current treatment regimen.

**Objective:** To demonstrate a concentration of gentian-violet effective against oral yeasts but not stigmatize the patients by painting the mouth with blue/violet.

**Methods:** Department of Microbiology, Kampala International University, supplied for this investigation, the 148 (100 *Candida albicans*, 10 *Candida glabrata*, 10 *Candida norvegensis*, 6 *Candida parapsilosis*, 6 *Zygosaccharomyces* species, 5 *Candida tropicalis*, 5 *Saccharomyces cerevisiae*, 3 *Candida sake* and 3 *Saccharomyces kluyveri*) clinical oral yeast samples previously recovered from the oral lesions of HIV/AIDS patients attending clinics in South-Western Uganda. Standard methods were used to test the serially diluted 0.5% gentian violet solutions against the oral yeasts.

**Results:** It was observed that 1:32 was the least dilution of 0.5% gentian-violet which was able to give the minimum sensitive 14 mm zone diameter according to CLSI with *Candida albicans*. Again 14 mm zone diameter was obtained from 1:10 dilution of *C. glabrata* and *C. sake*; while 1:8 dilutions of *C. tropicalis*, *C. norvegensis*, and *C. sake* respectively. Topical (1:32 dilution of 0.5% GV) antifungal alternative is cheap, available and will not stain the mouth thus removing associated stigma.

**Conclusion:** Therefore, 1:32 dilution of 0.5% gentian-violet can be used as topical anti-*Candida albicans* and 1:8 dilution of GV as topical-anti-non *Candida albicans* respectively. This assures the efficacy, availability and sustainability of oral fungal lesions treatment in resource poor setting.

**P860** **Intrafungal concentration of posaconazole**

F. Farowski\*, A. Hamprecht, C. Müller, A. Steinbach, J.J. Vehreschild, M.J.G.T. Vehreschild, O.A. Cornely (Cologne, DE)

We recently showed that various antifungal drugs, i.e. anidulafungin, micafungin, and posaconazole, accumulate within cells of the peripheral blood. The ratio between the cellular and the extracellular (C/E) concentration depends on the composition, i.e. the protein concentration, of the surrounding medium. However, the composition of the surrounding medium might also influence the antifungal concentration within the fungi themselves and significantly contribute to the efficacy of these drugs. Hence, we currently develop a method to determine intrafungal antifungal concentrations. We present data on the "intrafungal concentration" of posaconazole within *Candida albicans*.

**Method:** Suspensions of *Candida albicans* were prepared in RPMI-1640 medium and incubated with different concentrations of posaconazole (33–990 ng/mL). Following an incubation period of 1 hour the drug was removed by two consecutive washings with sterile water. Afterwards, cell counts were determined using a Neubauer chamber and samples were stored at  $-80^{\circ}\text{C}$  until use. The intrafungal concentrations were determined by a chromatography tandem mass spectrometry (LC-MS/MS) method similar to the previously described method for the quantitation of different antifungals within human peripheral blood cells. An additional step to concentrate the samples was added to increase the sensitivity of the method.

**Preliminary results:** The method is feasible to detect antifungal concentrations as low as 0.5 ng/10<sup>6</sup> cells. The accuracies of all concentrations were within ± 15%. For posaconazole the intra-fungal concentration within *Candida albicans* correlated with the concentration of the medium; however a saturation of the intrafungal concentration was observed (2 ng/10<sup>6</sup> cells).

**Conclusion:** To our knowledge, we established the first method to quantitate intrafungal antifungal drug concentrations by LC-MS/MS. The limit of quantification is sufficient for the expected concentrations.

#### **P861 Invasive aspergillosis in liver transplant recipients in the antifungal targeted prophylaxis era**

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**Background:** Invasive aspergillosis is a devastating complication affecting liver transplantation. Risk factors have been widely described and the use of antifungal prophylaxis seems to be justified in high-risk scenarios. Our aim is to evaluate the characteristics and incidence of IA in the era of antifungal targeted prophylaxis.

**Methods:** All liver transplants recipients from July 2003 to December 2009 were included. Pre, peri and post-transplant variables were reviewed. Proven or probable invasive aspergillosis was defined according to the EORTC/MSG. Categorical variables were compared with the chi-square test or Fischer's exact test. Backward step logistic regression analysis was used for multivariate analysis.

**Results:** A total of 556 patients were evaluated. Overall, 135 patients (24%) fulfilled criteria of high-risk LT, in whom 57 (42%) antifungal prophylaxis was administered. In the study period, 16 patients developed IA (3%) with mortality related to IA in eight patients (50%). According to the presence of risk factors, 8.1% of high-risk LT developed IA vs 1.2% of patients without risk factors ( $p < 0.001$ ). Five out of 16 cases of aspergillosis occurred in patients without criteria for high-risk LT. Most patients (38/59, 64%) received amphotericin B for antifungal prophylaxis. The incidence of IA from 2003 to 2009 was: 2.1%, 1.3%, 2.3%, 5.2%, 4.4%, 1.1%, 3.6% ( $p = 0.348$ ). The species isolated from cases with IA were: *A. fumigatus* 11, *A. terreus* 4, *A. niger* 2, *A. flavus* 1 (two patients had coinfection with two species). Twelve cases of IA occurred in the first 3 months after transplantation of which 11 (fulfilling high risk criteria) could have been prevented with antifungal prophylaxis. Of these 12 patients, two received no prophylaxis, five intravenous amphotericin B, one nebulized amphotericin B, and two fluconazole. Breakthrough aspergillosis occurred in five patients under prophylaxis with amphotericin B. A multivariate analysis adjusted by age and gender show that urgent transplantation (OR 9.2, 95%CI 2.2–38.3) and post-transplant reoperation (OR 5.9, 95%CI 1.9–18.0) were the main risk factors for the development of IA.

**Conclusions:** The incidence of IA in LT has not decreased along time. High-risk definitions for LT were associated with higher risk of IA. We detected 5 out of 12 preventable IA that occurred during or after prophylaxis with amphotericin. Reevaluation of antifungal prophylaxis in LT is warranted.

#### **P862 Recategorisation of reasons for premature discontinuation of antifungal prophylaxis in the IMPROVIT study – focus on IFI-related reasons**

H. Schlamm\*, E.J. Bow, O.A. Cornely, M. Slavin, M. Roden, P. Biswas (New York, US; Manitoba, CA; Cologne, DE; Melbourne, AU; Severna Park, Colledgeville, US)

**Objectives:** In the recently published IMPROVIT study, which compared voriconazole (VOR) to itraconazole (ITR) for antifungal prophylaxis (AFP) after allogenic HSCT, 251/465 (54%) subjects prematurely discontinued within 100 days, primarily for intolerance or toxicity. There were two cases in which AFP was discontinued because of proven or probable IFI, both had received ITR. This analysis further

categorized the reasons for premature discontinuation of prophylaxis in this study, with a focus on IFI-related reasons.

**Methods:** The DRC (OAC, EJB, MS) reviewed data (blinded to study treatment) from all subjects who prematurely discontinued AFP and adjudicated the primary reason for discontinuation using the following categories: proven, probable or possible IFI (using 2002 or 2008 MSG/EORTC criteria), suspected IFI (evidence of IFI not meeting criteria for possible IFI), persistent fever and neutropenia, gastrointestinal intolerance, LFT abnormality, other drug toxicity, progression of underlying disease, other medical condition, administrative reason, or unable to assess.

**Results:** Following are the proportion of subjects who discontinued AFP for the following reasons (VOR vs ITR): Probable or possible IFI: 2 (0.9%) vs 18 (7.5%),  $p < 0.01$ ; Suspected IFI or persistent fever and neutropenia: 8 (3.6%) vs 11 (4.6%), NS; Gastro-intestinal intolerance: 4 (1.8%) vs 45 (18.7%),  $p < 0.001$ ; LFT abnormality: 45 (20.1%) vs 15 (6.2%),  $p < 0.001$ ; Other toxicity: 9 (4.0%) vs 1 (0.4%),  $p < 0.01$ ; Progression of disease: 5 (2.2%) vs 2 (0.8%), NS; Other medical condition: 20 (8.9%) vs 26 (10.8%), NS; Other reason/unable to assess: 11 (4.9%) vs 29 (12.0%),  $p < 0.01$

**Conclusion:** In this retrospective analysis of the IMPROVIT study, more allo HSCT recipients prematurely discontinued AFP with ITR because of IFI-related reasons than previously thought. Broader definitions of IFI-related reasons for discontinuation should be incorporated in future clinical trials of AFP.

#### **P863 Isavuconazole is widely distributed in rat tissue**

A. Schmitt-Hoffmann\*, W. F. Richter (Basel, CH)

**Objectives:** To assess tissue distribution of 14C/3H-BAL8557, a prodrug of BAL4815 (isavuconazole), a novel, broad-spectrum triazole active vs moulds/yeasts, in phase 3 trials for treatment of invasive fungal infections, using quantitative whole body autoradiography (QWBA) in rats. The 14C label was in the active drug moiety and the 3H label was in the pro-moiety.

**Methods:** Male rats received a single IV infusion of 3 mg/kg double-labelled BAL8557 and were sacrificed at 0.5, 8, 24, 72, and 144 hour post dose. QWBA used separate specific detection of 14C and 3H radioactivity; total radioactivity in blood and plasma was analyzed by liquid scintillation counting; urine was collected to assess renal excretion of both radioactive isotopes.

**Results:** 14C radioactivity was widely distributed, with maximum concentrations at 0.5 hour post dose in nearly all tissues. All tissues except eye lens had tissue/plasma concentration ratios above unity, with highest levels found in the adrenal cortex (6.92 mcg equiv/g) and liver (6.14 mcg equiv/g)—tissue/plasma concentration ratios of 15.2 and 13.5, respectively. High levels of 14C radioactivity (tissue/plasma ratios >5) were found in the small intestinal mucosa, brown fat, Harderian gland, pancreas, intra-orbital lacrimal gland, kidney cortex, adrenal medulla, stomach mucosa, and thyroid. Lung/brain plasma ratios at 0.5 hour postadministration were 2.28/1.86, respectively. 14C radioactivity in tissue declined by a factor of 5 at 8 hour post infusion; only adrenal medulla and liver contained quantifiable levels (>0.02 mcg equiv/g) at 144 hour. 3H radioactivity was widely distributed at 0.5 hour and tissue levels declined rapidly, with all tissues containing low/non-detectable 3H radioactivity by 8 hour, except intestinal contents and kidney. Excretion was almost complete within 144 hour, with ~80% 14C radioactivity recovered in bile/faeces and 20% in urine. Tritium levels in blood declined rapidly, with 1.88 and 0.087 mcg equiv/mL at 0.5 and 24 hour post dose, respectively. 3H activity was rapidly eliminated, with no indication of relevant retention in tissues and ~86% recovered in urine; the remainder recovered in faeces/bile within 144 hour. 3H radioactivity was rapidly eliminated from tissues and 14C radioactivity was distributed in most tissues, with no indication of tissue-specific retention of either label.

**Conclusions:** These results show that isavuconazole is widely distributed in tissue, including eye and brain.

**P864** Fibrillar – a novel antifungal delivery method: most unusual case of *Aspergillus fumigatus* empyema and bronchopleural fistulae after right-sided pneumonectomy, treated successfully by innovative salvage therapy

A. Guleri, A. Duncan, M. Hassan, T. Al-Badawi\*, M. Purohit (Blackpool, UK)

**Background:** We present a most unusual and challenging case of *Aspergillus fumigatus*[AF] empyema and bronchopleural fistulae after right sided pneumonectomy. The topography and expanse of fungal growth within the chest cavity made drug delivery and sustained exposure to antifungal agent a challenge. Literature search/consultation with experts was of limited benefit. An innovative and novel antifungal delivery method was planned as a salvage to treat the infection.

**Case Study:** A 52-year old gentleman, heavy smoker for 35 years, was hospitalised with chest symptoms and diagnosed with right upper lobe mass. He underwent an elective pneumonectomy. Histology showed well differentiated squamous cell carcinoma, T3N1M0. His postoperative period was complicated by development of a bronchopleural fistula. An Elossier flap (open stoma) was created to drain the space. This followed long term follow up review in outpatient clinic. In February 2010, review in clinic revealed the presence of thick layer of deposit with a green sheen visible through Elossier flap on the inner wall of chest cavity. Tissue samples for culture isolated pure growth of *Aspergillus fumigatus*. Systemic treatment with voriconazole did not clear the fungus.

Salvage therapy was planned, discussed with patient and consent obtained. Access to inside of chest cavity through Elossier flap, debridement of thick layer of deposit and amphotericin powder insufflation to cover pleural surface. This was packed with Fibrillar [haemostat] to allow sustained exposure to amphotericin. The patient had four such procedures and systemic posaconazole 400 mg q12h po was continued postoperatively. He made slow but good progress and increased some weight by April 2010. He was kept on long term posaconazole and follow up review in clinic. Culture of swabs were negative for fungus. Video of the procedure is available.



**Discussion:** Fibrillar, an absorbable haemostat, conforms, adheres and melts into bleeding tissue. It has antibacterial properties. Body temperature, humidity, nutrients, oxygen and access to environment provided perfect conditions for fungal growth. Systemic antifungals failed to clear the fungus because of limited vascularity of the area. The topography and extensive spread of fungus along pleural surface of chest cavity presented a challenge for choice of delivery method, choice, dose, duration, sustained exposure to a suitable antifungal agent. The patient continues to be infection free and in good health.

**P865** Effectiveness of once-weekly fluconazole on the incidence of invasive candidiasis in a reverse-isolation haematological unit

D. Vuichard, M. Weisser\*, R. Frei, D. Heim, J. Passweg, A. Widmer (Basel, CH)

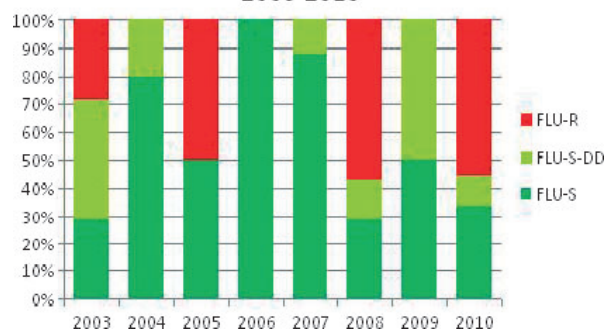
**Objectives:** Fluconazole 400 mg once daily was recommended as first line prophylactic agent for patients with acute leukemia and for those receiving allogeneic HSCT. Since 2001 our institutional policy for hematological high risk patients has been 400 mg fluconazole once weekly despite the fact that doses below 400 mg/day have not shown a significant benefit in preventing invasive fungal infections. Our study aimed to evaluate the impact of this policy on incidence of invasive candidiasis (IC) and the potential of emergence of resistance to fluconazole.

**Methods:** Patients received antifungal prophylaxis with fluconazole 400 mg weekly if they underwent high-dose chemotherapy, allogeneic or autologous stem cell transplantation. Antifungal prophylaxis was routinely stopped when antifungal treatment for invasive fungal infection (IFI) was initiated. Our hematological unit (HU) is located in a separate wing, equipped with laminar air flow, has restricted access and dedicated staff.

Data from patients were prospectively recorded by standardized case report forms. IC was defined as isolation of *Candida* spp. from blood or normally sterile compartments.

**Results:** From January 2003 until December 2010 a total of 1358 patients were treated in the HU, among them, 563 underwent allogeneic or autologous transplantation. Fifty-six *Candida* spp. were isolated from all submitted specimens, among them 28 were regarded as invasive and 28 as colonization. Identification of *Candida* spp. from this unit – including also non-IC isolates – remained stable over the whole period of 8 years with an average of 1.71/1000 patientdays (PD) (all *Candida* isolates) and 0.86/1000 PD for IC, respectively ( $p < 0.05$ ). Fifty-five of 56 strains were tested for fluconazole susceptibility. There was no significant increase of non-albicans *Candida* spp. or an increase of fluconazole resistant isolates of *Candida* spp. ( $p < 0.05$ ). There was no trend for increased resistance over the study period (figure).

**Fluconazole Susceptibility of all *Candida* spp. 2003-2010**



**Conclusion:** This policy of once weekly fluconazole did not result in emergence of *Candida* resistance or a shift towards non-albicans *Candida* spp. and might therefore be considered as alternative to once daily fluconazole.

**P866** Fungi and finance – an innovative project at a district hospital in northwestern England for cost-effective management of candidaemia driving quality and efficiency

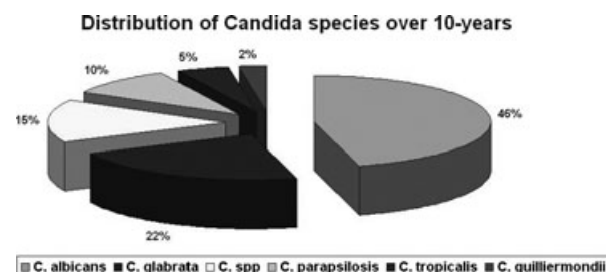
A. Guleri\*, S. Ellershaw, A. Peel, A. Ibrahim, M. Przybylo (Blackpool, UK)

**Background:** *Candida* blood stream infections[CBSI] are 4th commonest cause of BSI, and associated with high mortality and

high potential costs of treatment. The incidence of CBSI in Europe is 0.20–0.38/1000 hospital admissions and 0.31–0.44/10 000 patient days. Literature and recent clinical trials suggest *Candida* BSI due to *Candida* species other than albicans are increasing. The cost of newer antifungals [AF] can potentially erode limited drug budgets. We present an innovative project with potential for adoption at any district hospital. This included in-house *Candida* identification [CID] and fluconazole susceptibility testing [FST], cost comparison of local in-house and outsourcing of CID and susceptibility testing, local epidemiology of CBSI over 10-years and economic modelling to enhance quality and efficiency of patient care and significant cost savings.

**Methods:** Retrospective 10 year review of pathology database for 272 CBSI [2000 – till date]; cost comparison and turn-around-time [TAT] of *Candida* ID and FST [inhouse or outsourced]; cost comparison [BNF 2011] of various systemic antifungals and potential cost savings from in-house ID/FST based antifungal treatment [esp for germ tube negative *Candida*].

**Results:** Key results from 272 CBSI over 10-years included *C. albicans* [CAL] was commonest [48.5%]; followed by 23.5% *C. glabrata* [CGL], 10.3% *C. parapsilosis* [CPA]; 5.1% *C. tropicalis*; and nil *C. krusei*. Incidence of *C. albicans* has remained flat in last 10 years. Fully fluconazole sensitive *Candida* species with the exception of 39% *C. glabrata*. Cost differential between in-house CID/FST incl staff time [£18.32] and outsourcing CID/ST against full panel [£93.73] for 272 CBSI *Candida* is £20 511.52. Cost differential of systemic AF treatment per day/over 2-weeks ranges from £7.78/£108.92 for fluconazole 400 mg IV and £483.45/£6768 for liposomal amphotericin 250 mg IV. A potential of upto £932 272 cost differential in treatments of 140/272 germ tube negative CBSI [inhouse v/s outsourced]. Details to be presented.



**Conclusions:** In-house *Candida* ID and FST is user friendly and easy to setup. MIC using E-test for fluconazole can be available within 24 hour. This has potential for early switch from expensive 2nd or 3rd line antifungals to fluconazole. Knowledge of local epidemiology for different clinical areas offers confidence in fluconazole use as 1st line even for germ tube negative *Candida*. Details of economic modelling and epidemiological profiling to be presented.

#### P867 Mixed treatment comparison of randomised clinical trials of primary antifungal prophylaxis in allogeneic haematopoietic stem cell transplant recipients – focus on invasive aspergillosis

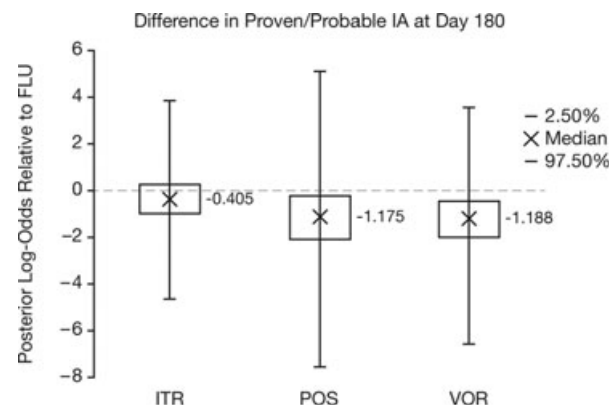
E.J. Bow\*, D.J. Vanness, C. Cordonnier, O.A. Cornely, D.I. Marks, A. Pagliuca, M. Slavin, C. Solano, A. Shaul, S. Sorensen, L. Cragin, R. Chambers, M. Kantecki, D. Weinstein, H. Schlamm (Winnipeg, CA; Madison, US; Creteil, FR; Cologne, DE; Bristol, London, UK; Melbourne, AU; Valencia, ES; Manitowoc, Bethesda, Collegeville, US; Paris, FR; New York, US)

**Objectives:** Invasive aspergillosis (IA) is the most frequent invasive fungal infection (IFI) in allogeneic haematopoietic stem cell transplant (alloHCT) recipients post-engraftment and is associated with high mortality. The oral azoles fluconazole (FLU), itraconazole (ITR), posaconazole (POS), and voriconazole (VOR) can potentially prevent IFI in this setting. The optimum choice for primary prophylaxis of IA in alloHCT is unknown, since these agents have not been directly

compared in a single randomised clinical trial (RCT). Indirect mixed treatment comparison (MTC) is currently the best available approach to evaluate this question.

**Methods:** Based on expert consensus, we conducted a systematic literature review of published RCTs of FLU, ITR, POS, and VOR as primary IFI prophylaxis, in which >50% of patients underwent alloHCT. The respective cumulative proportions of patients with proven/probable IA at 180 days were extracted from each RCT. These data were incorporated into a Bayesian hierarchical random-effects MTC that used an unconstrained baseline (Lu & Ades. Stat Med 2004; 23: 3105–24) to account for potential heterogeneity in study designs and patient populations, as per expert consensus. Using posterior credible intervals estimated from the MTC, we evaluated the following: (i) log-odds of developing proven/probable IA at day 180 for ITR, POS, and VOR relative to FLU and (ii) the probability that each agent was superior to FLU for this endpoint.

**Results:** Six RCTs were identified, of which 1 was excluded from the MTC due to lack of data on IA. The five remaining RCTs (three open-label and two double-blind RCTs) randomized 2147 patients in total. FLU (n = 813) was a comparator in four trials, ITR (n = 485) in 3, VOR (n = 548) in 2, and POS (n = 301) in one trial. Estimates for median log-odds (see figure) suggest that VOR and POS most reduced the probability of proven/probable IA at day 180 relative to FLU (–1.188 and –1.175, respectively), followed by ITR (–0.405). VOR had a higher probability of being superior to FLU (ie, 84.5%) than did POS (79.7%) and ITR (68.9%).



Log-odds below the dotted line indicate reduced probability of proven/probable IA at day 180 relative to FLU.

Boxes indicate interquartile range from 25% (top) to 75% (bottom).

**Conclusion:** The probability of being superior to FLU in preventing IA was high (>75%) for VOR and POS. Mould-active prophylaxis with VOR and POS appeared to result in a greater reduction of IA than ITR. The findings may have clinical relevance, suggesting that in those alloHCT recipients for whom post-transplant IA prophylaxis with an oral azole is deemed valuable, VOR and POS are the most effective options.

#### P868 Evaluation on “real life” prescriptions of antifungal prophylaxis in high-risk patients: preliminary results from a prospective survey

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**Objectives:** To describe the current use of antifungal (AF) prophylaxis in consecutive, unselected adult patient with acute myeloid leukemia patients (AMLs) at first induction of remission and to analyze the



efficacy of prophylaxis with posaconazole (POS) when compared to old azoles in a “real life” setting.

**Methods:** From January 2010 to March 2011, all newly diagnosed AMLs have been consecutively registered and prospectively monitored in 31 Italian participating centers. Only adult cases that received conventional chemotherapy were included in the present study. Principal demographic and clinical data, as well as antifungal treatments were collected. In particular we analyzed data about systemic AF prophylaxis: the drug of choice, the duration of treatment, and its efficacy were thus evaluated. To determine prophylaxis efficacy, incidence of proven/probable IFDs was assessed at 30th day from the end of chemotherapy.

**Results:** Four hundred and ninety-eight AML were evaluated in the present analysis. The most part of them (448, 90%) received systemic antifungal prophylaxis. POS was the most frequently employed drug (224/448, 50%), followed by fluconazole (128, 29%) and itraconazole (86, 19%). When comparing the POS group (224 pts) to those receiving itraconazole or fluconazole (214 pts) (FLU/ITRA) no significant differences emerged in terms of the main risk factors for IFDs (table). In particular the two groups resulted to be comparable in terms of age, sex, frequency and duration of deep neutropenia, days of prophylaxis. On the contrary, there were significant differences in breakthrough IFDs (6.2% in POS vs 11.7% in FLU/ITRA, p-value 0.04). Except for one case of fusariosis, all mold infections were invasive aspergillosis. Yeast infections also were more frequent in the FLU/ITRA group. Caspofungin and amphotericin B compounds were the most frequently employed drugs, as empirical/pre-emptive treatments. There were no significant differences in the response rate, nor in the IFDs attributable mortality rate.

	FLU/ITRA	POS
	214	224
Mean age (years)	57.7	53.3
Sex		
- male	112 (52%)	101 (45%)
- female	102 (48%)	123 (55%)
Deep neutropenia	210 (98%)	213 (95%)
Central venous catheter	119 (56%)	143 (64%)
Mean duration of deep neutropenia (days)	19.5	21.5
Median AF prophylaxis duration (days)	20	20
Empirical/pre-emptive therapies	59 (28%)	71 (32%)
Most used drugs as empirical/pre-emptive therapy	- L-AmB 18 (30%) - Caspo 17 (29%) - Vorico 8 (14%) - CL-AmB 8 (14%) - Others 8 (14%)	- L-AmB 39 (55%) - Caspo 12 (17%) - Vorico 6 (8%) - CL-AmB 2 (3%) - Others 12 (17%)
Proven/probable IFDs	25 (11.7%)	14 (6.2%)
• moulds	- 17 (7.9%)	- 11 (4.9%)
• yeasts	- 8 (3.7%)	- 3 (1.3%)
Molds/yeasts ratio	2.1/1	3.7/1
Favourable responses (RR)	14/25 (56%)	11/14 (79%)
N° deaths (AMR)	7/25 (28%)	2/14 (14%)
• moulds	4	1
• yeasts	3	1

Legend: AF: antifungal; IFD: invasive fungal disease; L-AmB: liposomal Amphotericin B; CL-AmB: lipid complex Amphotericin B; RR: response rate; AMR: attributable mortality rate; pos: posaconazole; flu: fluconazole itra: itraconazole

**Conclusion:** During the last few years the use of POS prophylaxis in high risk pts has significantly increased. Although not randomized, our study demonstrates in a “real life” setting the increased use and the higher efficacy of POS prophylaxis, when compared to FLU/ITRA. Only 14 patients developed a breakthrough IFDs. Surprisingly, POS superiority emerged for both molds and yeasts infections. Previous AF prophylaxis doesn't seem to impact IFDs outcome.

### P869 Antifungal prophylaxis for patients with haematological malignancies in “real life”: a prospective cohort study of compliance, toxicity and outcome

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**Objectives:** This prospective cohort study assesses the compliance with the institution's antifungal prophylaxis (AP) strategy for patients with haematological malignancies and reasons for deviation.

HSCTs patients	89
Full course of itra	50
Itra discontinued, given alternative	22
1. Intolerance to itra	9 ambi=4, posa=4, vori=1
2. Toxicity to itra	6 (ambi)
3. Interaction of itra with other drugs	2 Ambi=1(itra+Efavirenz), caspo=1(itra+cyclosporine+renal artery stenosis)
4. continuing prior antifungal treatment	1(ambi)
5. change from 1 <sup>st</sup> line to 2 <sup>ry</sup> prophylaxis due to clinical suspicion of IFD	4 Vori=3, posa=1
Secondary prophylaxis from the beginning of the course	9 Vori=8, posa=1
Received >2 antifungal drugs as prophylaxis	8
1. toxicity to ambi	2(caspo)
2. intolerance to ambi	4(caspo)
3. change from protocol 2 <sup>ry</sup> to another 2 <sup>ry</sup> prophylaxis	2
Overall RFNE treated with antifungal drugs	28
RFNE treated with 1 drug	20
RFNE treated with > 1 drug	8

AMLs post chemotherapy courses	92 (40 patients)
Full course of itra	68
Itra discontinued, given alternative	18
1. Due to mylotard	13 Ambi=12, caspo=1
2. Due to arsenic	1(ambi)
3. Interaction of itra with other drugs	1 Ambi (itra +everolimus)
4. Intolerance to itra	0
5. Toxicity to itra	3(ambi)
Secondary prophylaxis	6 Vori=5, posa=1
Overall RFNE treated with antifungal drugs	24
RFNE treated with 1 drug	19
RFNE treated with > 1 drug	5

ALLs post chemotherapy courses	51(26 patients)
Full course of itra	23
Itra discontinued, given alternative	27
1. Change of risk for IFD	11 Fluconazole due to maintenance chemotherapy
2. Intolerance to itra	1(ambi)
3. Toxicity to itra	2(ambi)
4. To prevent vincristine toxicity	13 Ambi=11, posa=1, fluco=1
Secondary prophylaxis	1
Overall RFNE treated with antifungal drugs	9
RFNE treated with 1 drug	7
RFNE treated with > 1 drug	2

**Methods:** One hundred and fifty-five consecutive patients admitted for allogeneic haematopoietic stem cell transplantation (HSCT) or chemotherapy for acute myeloblastic (AML) or acute lymphoblastic leukemia (ALL) from 01/08/2008 to 31/07/2010 were reviewed. First line AP on our unit is itraconazole oral solution except during concomitant use with vincristine to avoid potentially fatal interactions. The alternative is IV AmBisome and secondary (2ry) prophylaxis is voriconazole. One hundred and fifty-five HRCTs from 73 patients who developed refractory febrile neutropenic episodes (RFNE) were independently reviewed by two different radiologists. Invasive fungal disease (IFD) was classified according to EORTC/MSG criteria.

**Results:** (i) Eighty-nine HSCTs were studied. Fifty remained on itra for the full course of AP, 22 received itra, stopped and were given an alternative drug for the remainder of AP, 9 were on 2ry AP throughout the course and 8 received >2 drugs as AP. The reasons why alternative AP was given to 22 patients were: intolerance 9, toxicity 6, drug interaction 2, continuing prior antifungal treatment 1, change to 2ry AP due to clinical suspicion of IFD 4. The reasons why eight patients received >2 drugs as AP were: toxicity to AmBi (alternative AP) 2, intolerance to AmBi (alternative AP) 4, change from the protocol 2ry to another 2ry AP 2. (Table 1). (ii) Forty AMLs who had 92 post-chemotherapy courses (CC) were studied. In 68 CC itra was given, 18 CC alternative AP and 6 2ry AP. The reasons for alternative AP were: treatment with mylotarg 13, drug interaction 1, treatment with arsenic 1, toxicity to itra 3 (Table 1). (iii) Twenty-six ALLs had 51 CC. In 23 CC itra was given, 27 alternative AP and 1 2ry AP. The reasons for alternative AP were: change of risk for IFD 11, intolerance 1, toxicity 2, treatment with vincristine 13 (Table 1). (iv) The overall incidence of IFD was 5.8%, probable IFD 1.2% and possible IFD 4.5% patients.

**Conclusion:** AMLs and ALLs tolerate itra well and their course of AP is compliant with our institution's strategy. The prolonged AP duration combined with the interactions between drugs given after transplantation are the major reasons for the low percentage of HSCTs who stayed on itra. However, the overall percentage of IFD is low, which justifies our practice to deviate when necessary from the AP strategy.

#### **P870** Incidence of breakthrough fungal infection during primary antifungal prophylaxis in acute myeloid leukaemia patients in a cancer centre

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**Objectives:** To investigate the patterns of primary antifungal prophylaxis (PAP) use and the incidence of breakthrough fungal infection (BFI) in patients with acute myeloid leukemia (AML) in a cancer center.

**Methods:** We analyzed 75 unselected patients with newly diagnosed AML admitted to MD Anderson Cancer Center who received PAP through August 2009 to March 2011.

PAP drug	# of Patients (%)	Patient-days of PAP	BFI all criteria* / definite or probable EORTC criteria	Incidence of BFI all criteria* / definite or probable EORTC criteria*	Isolated Agents of BFI	1A*
Voriconazole (PO)	41 (55)	5,142	9/6	1.8/1.2	-	0
Posaconazole	28 (37)	3,040	7/4*	2.3/1.3	Mold sterile hyphae; <i>Fusarium</i> sp.; <i>Aspergillus glaucus</i> ;	1
Fluconazole (PO/IV)	23 (31)	2,039	6/4	2.9/2.0	<i>C. glabrata</i> ;	3
Caspofungin	33 (44)	725	4/3*	5.5/4.1	<i>Fusarium</i> sp.; <i>Mucor</i> sp.	1
Anidulafungin	9 (12)	350	2/2	5.7/5.7	<i>Aspergillus fumigatus</i> ;	1
Micafungin	9 (12)	194	3/3	13.8/13.8	<i>Candida glabrata</i> ; <i>Candida krusei</i> and <i>Candida parapsilosis</i> ; <i>Coccidioides</i> sp. (positive IGM serology).	0
Voriconazole (IV)	9 (12)	131	0/0	0/0	-	-
Total AML patients	75 (100)	11,571	30/21	2.6/1.8	-	-

\*definite, probable, possible or suspected BI, <sup>1</sup>/1000 patient-days of PAP, <sup>2</sup>Invasive aspergillosis (IA) detected by *Aspergillus galactomannan* antigen tests, <sup>3</sup>1 case during caspofungin and posaconazole double covered prophylaxis on the 66<sup>th</sup> day of HSCT

**Results:** Patients had a median of two different antifungal drugs as primary prophylaxis; range 1 (24 patients) to five drugs (one patient) for a mean of 154 days (median 103 days, range 3–680 days) during hospitalizations or as outpatients (Table 1).

**Conclusions:** The preliminary findings in this real world study of PAP in a contemporary cohort of AML patients indicate that molds accounted for 85.7% (18/21) of BFIs on PAP. Despite frequent use (82%) of agents having *Aspergillus* activity, no statistical significant variation in the incidence of BFI was observed so far among drugs (p = 0.71).

#### **P871** Breakthrough mycosis in HSCT recipients receiving micafungin prophylaxis

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**Objectives:** To assess incidence and risk factors of breakthrough mycosis under micafungin prophylaxis for hematopoietic stem cell transplantation (HSCT).

**Methods:** A total of 50 HSCT recipients receiving micafungin as prophylaxis in 2010–2011 were analyzed. Patients were followed for 100 days post-transplantation for the development of mycosis defined by EORTC.

**Results:** Of the 50 patients, 38% received HSCT for lymphoma, 18% for AML, 14% for ALL, and 14% for MDS; 40% received allogeneic and 36% autologous HSCT. Breakthrough mycosis developed in 4 (8%) patients, including probable aspergillosis in 3 and definite trichosporosis in 1, after a median of 6 days post-transplant and after micafungin use for a median of 16 days. Of the three patients with probable aspergillosis, persistent elevation of *aspergillus* antigen levels and newly developed pulmonary nodules were the most common presentations. The case with trichosporosis developed fever and disseminated papular rash, and *Trichosporon* species was isolated from the blood. All these four patients were successfully treated with voriconazole. Compared with 46 patients without breakthrough mycosis, positive cases were more like to be older (mean, 53 vs. 43 years old, p < 0.05) and had graft-versus-host-disease (50% vs. 28.3%, p > 0.05). All patients had surveillance nasal and throat fungal cultures post-transplant, and 10 patients had positive results, including *C. albicans* in seven patients, *C. glabrata* in 1, unidentified *Candida* species in 1, *Trichosporon* species in 1, and Mycelium sterile in 1.

**Conclusion:** The incidence of breakthrough mycosis during micafungin prophylaxis for HSCT was 8%. Although micafungin had anti-*Aspergillus* attribute, aspergillosis was the most common breakthrough mycosis.

## Molecular virology

#### **P872** Apoptosis induced by highly pathogenic avian influenza A virus H5N1 NS1 protein and the intrinsic pathway activation

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Recent outbreaks of H5N1 influenza virus infections had important health and economic consequences and raised concerns that a new influenza pandemic would occur in the near future. Non-structural protein 1 (NS1) is an important virulence factor of the highly pathogenic H5N1 avian influenza virus and is found only in infected cells.

The objective of this study is to explore whether intrinsic pathway of apoptosis induced by H5N1 NS1 protein was activated. NS1 protein of influenza A/Jiangsu/1/2007 virus, a highly pathogenic H5N1 strain isolated from an infected human in Nanjing, was cloned to pXJ40-HA vector to construct the plasmid of pXJ40-HA-NS1. pXJ40-HA-NS1 was transfected to human lung epithelial cell line (NCI-H292) with or without apoptosis inducer (staurosporine). The localization of NS1 protein in H1299 cells was detected by immunofluorescence (IF). The apoptosis effects were detected by MTT assay, flow cytometric assay and western blot analyses. The result of IF shows NS1 protein mainly

localizes in cell nucleus (Fig.1). NS1 protein could induce apoptosis in human lung epithelial cell line (NCI-H292) (Figs 2 and 3). Cytochrome C release could be observed dramatically in NS1 transfected cells and the effect was enhanced by apoptosis inducer (Fig. 4). Taken together, these data indicate that influenza A virus NS1 protein serves as a strong inducer of apoptosis in human lung epithelial cells and triggers apoptosis could be via mitochondria-dependant intrinsic pathway.

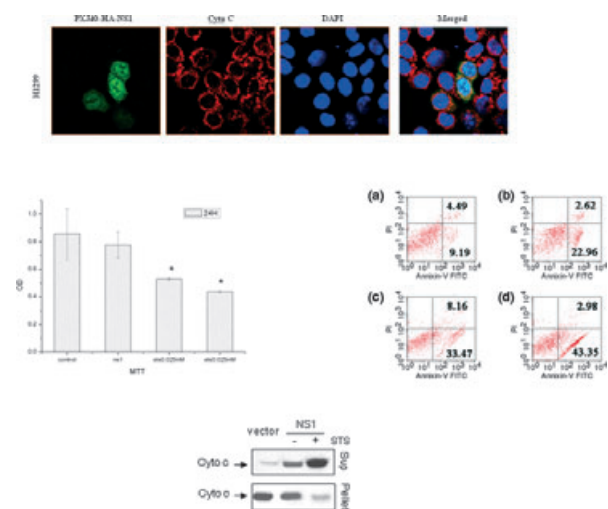


Fig. 1. Analysis the expression and localization of NS1 protein in H1299 cell line by immunofluorescence and confocal assay.

The pXJ40-HA-NS1 transfected H1299 cell staining with anti-HA serum and Alexa488 conjugated secondary antibody (A), anti-Cytochrome C MAb and Alexa555 conjugated secondary antibody (B), DAPI dye (C), respectively; Merged image (D).

Fig. 2. Cytotoxicity detection by MTT assay on NCI-H292 cells transfected by pXJ40-HA-NS1 for 24h. A:vector;B: STS 0.025 nmol;C: NS1;D: NS1 + STS 0.025 nmol.

Fig. 3. Identification of apoptosis by FCM assay of Annexin-V/PI double staining.

A:vector;B: NS1;C: STS0.025 nmol;D: NS1 + STS0.025 nmol.

Fig. 4. Cytochrome C release detection by WB assay in NCI-H292 cells transfected by pXJ40-HA-NS1 and induced by STS (0.025 nmol) for 24 hour.

### P873 Herpes simplex virus in the testis of prepuberal and adult mice: in vivo and in vitro models

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**Objectives:** Although herpes simplex virus (HSV) is associated with male infertility, the mechanisms of its interactions with germ cells and the role of hematotesticular barrier (HTB) are poorly understood. The aim of the present study was to compare germ cells susceptibility to HSV in the testis of prepubertal and adult mice after in vivo and in vitro infection.

**Methods:** Pups 7-day-old (Group I) and 5-month-old (Group II) mice were used to model HSV-infection before and after HTB formation, respectively. Testis organotypic culture was used as in vitro model. It was infected and incubated for 6 days. In vivo infection was performed by intraperitoneal injection of virus. HSV markers in testicular cells were analyzed by PCR, culture method and immunostaining.

**Results:** In culture, HSV-DNA load reached the maximum on day 4 post infection:  $3.5 \times 10^8$  copies/mL in Group I and  $3.4 \times 10^7$  copies/mL in Group II. The content of infectious virus increased till day 6, reaching  $5 \times 10^3$  and 20 plaque forming units (PFU)/mL in groups I and II, respectively. In pup testis 90% of spermatogonia and Sertoli cells contained viral antigen compared with 5% germ cells in adult mice.

In vivo, high levels of HSV DNA ( $1.5 \times 10^5$  copies/mL), infectious virus (140 PFU/mL) and viral antigens (45% of cells) were detected in pups testis. However, no HSV markers were identified in testis of adult mice.

**Conclusion:** Both in vitro and in vivo studies show that testicular cells of mouse pups are more sensitive to viral infection than those of adult mice. These results suggest that HSV-infection of mice in the early neonatal period before HTB formation leads to germ cells infection that can be one of the possible causes of male fertility disorders.

### P874 Post exposure efficacy of AVI-7100 against influenza A in mouse and ferret infection models

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**Objective:** AVI-7100 is a phosphorodiamidate morpholino oligomer containing three modified linkages (PMOplus) that is designed to interfere with expression of the M1 and M2 genes of influenza A virus. The objective was to evaluate the therapeutic utility of AVI-7100 up to one day post viral exposure.

**Methods:** A single 0.1 mg intranasal (i.n.) dose of AVI-7100 was administered to female BALB/c mice (n = 10/group) either 4 hours prior to or 4 hours after viral challenge with with  $5 \times 10^5$  pfu of A/Port Chalmers/1/73 (H3N2). Lung viral load was determined on day 6 post infection. A similar efficacy study in outbred ferrets (*Mustela putorius furo*; n = 7/group) were administered AVI-7100 as a single i.n. dose 4 hours prior to or 1 day post insufflation viral challenge with  $5 \times 10^5$  pfu H1N1 A/Hong Kong/2369/09 per ferret. Negative control groups were treated with saline and positive controls were administered oseltamivir at 10 mg/kg p.o. every other day beginning 7 days prior to infection. A plasma pharmacokinetic study with 16 ferrets (four groups of four ferrets/group) in which a 10 mg/kg or 30 mg/kg i.v. dose was evaluated prior to and three days post viral challenge with H1N1 strain A/Mexico/4108/09 or H5N1 strain A/Vietnam/1203/04.

**Results:** A single intranasal dose of AVI-7100 (0.1 mg/mouse) administered either 4 hours prior to or 4 hours after infection with A/Port Chalmers/1/73 (H3N2) significantly ( $p < 0.05$ ) reduced lung viral titers in each group compared to vehicle controls and oseltamivir treated mice. In the ferret, a single i.n. dose of AVI-7100 administered 4 hours prior to exposure or 1 day after exposure with A/Hong Kong/2369/09 (an oseltamivir resistant pH1N1) significantly ( $p < 0.05$ ) reduced cumulative viral load in nasal wash and in lung bronchiolar lavage compared to saline controls and oseltamivir treated ferrets. A plasma pharmacokinetic study revealed no differences between infected and uninfected ferrets.

**Conclusions:** AVI-7100 is effective against influenza A (H1N1 and H3N2) and in both mouse and ferrets when administered as a single intranasal dose for greater than one day post viral exposure. Post-exposure efficacy data indicate robust antiviral and symptom benefit can be provided by AVI-7100. Infection does not significantly alter plasma pharmacokinetics relative to uninfected ferrets. These data provide a rationale for a therapeutic use of AVI-7100 following influenza exposure.

### P875 Presence of dengue virus genome in kidney tissue of adults without recent dengue infection: another piece of evidence of in vivo persistence of the virus

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**Objectives:** Dengue is the most rapidly spreading mosquito-borne viral disease on a global scale and causes a major public health problem in Thailand. Serosurveillance indicates that almost all Thai adults have been infected, mostly asymptotically. Even though dengue virus is thought to cause only acute infection, some of its peer flaviviruses cause persistent infections in the hosts. Our prior studies revealed that dengue

viral genome could be detected in the bone marrows and the lymph nodes of the patients without evidence of acute or recent infections. Similarly, West Nile virus could be detected in urine for many years after infection in both animal and human hosts. Here, we conduct a study to demonstrate evidence of dengue virus persistence in the kidneys from adults with remote infections.

**Methods:** We enrolled the adults who had an indication for elective nephrectomy and autopsied cases at King Chulalongkorn Memorial Hospital, Bangkok, Thailand from March 2010 to March 2011. Demographic data, especially for the history of recent febrile illness, and laboratory data were recorded. We performed reverse transcription polymerase chain reaction (RT-PCR) using dengue specific primers targeting conserved sequences within the envelope and 3'-untranslated region on the kidney tissues. To confirm that there were no acute or recent infections, paired sera were collected before and 1–4 weeks after surgery for anti-dengue IgM and IgG by standard enzyme-linked immunosorbent assay (ELISA), and were interpreted according to standard criteria.

**Results:** Forty-four surgical cases and five autopsied cases were enrolled. Neoplasm of the urinary system was the most common indication for nephrectomy at almost 50%. Dengue genome was detected in 7 of 49 cases using RT-PCR (table) which was the prevalence of 14.29%. All these seven cases had no prior history of recent febrile illness and there was no evidence of acute or recent infection detected by dengue-ELISA of patients' paired sera.

Patient# & pathological diagnosis	Age (yr) & Gender	ELISA:	
		1st, 2nd IgM & 1st, 2nd IgG	
1. Renal cell carcinoma, unremarkable renal parenchyma	87 M	0, 0 & 0, 0	
2. Infiltrating urothelial carcinoma of urinary bladder, no tumor involving kidney	39 F	11, 14 & 18, 14	
3. Infiltrating urothelial carcinoma, chronic change of renal parenchyma	79 M	4, 0 & 30, 22	
4. High grade infiltrating urothelial carcinoma, chronic pyelonephritis	76 F	8, 0 & 0, 0	
5. Low grade papillary urothelial carcinoma, hydronephrosis of renal parenchyma	58 M	1, 0 & 2, 4	
6. Autopsy: Chronic pyelonephritis with acute tubular necrosis	69 F	0, 13 & NA, NA	
7. Autopsy: Diffuse proliferative glomerulonephritis (LN class IV), bilateral renal infarction, invasive aspergillosis of both kidneys	41 F	0, 6 & NA, NA	

Abbreviations: RT-PCR, reverse transcription polymerase chain reaction; LN, lupus nephritis; M, male; F, female; NA, data not available.

**Conclusions:** This study showed that dengue virus could be detected in kidney tissues more than previous expectation. These findings have implications for dengue pathogenesis and for public health. Persistence of dengue virus genome in the kidney is another step to understand pathogenesis of dengue virus infection. As for public health, a urine-mosquito connection may be another potential route of dengue transmission.

#### P876 Human papillomavirus subtype distribution among women in Singapore: a cross-sectional study

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**Objectives:** Human papillomavirus is the causative agent of the vast majority of cases of cervical cancer, which is the second leading cancer among women worldwide. There are more than 100 human papillomavirus (HPV) subtypes known, with at least 15 subtypes being "high-risk" which will lead to cervical cancer. Epidemiological studies have revealed significant differences in geographical distribution of HPV subtypes across the world. Information on the prevalence of different subtypes of HPV in Singapore is limited to surveys of a small number of HPV subtypes in neoplastic tissues. This study aimed to investigate the HPV prevalence among the different age and ethnic groups of healthy women in Singapore, and study the HPV type-specific distribution to identify the most prevalent HPV subtypes present locally. Such information is important for evaluating the use of anti-HPV vaccines as a public measure in controlling the burden of cervical cancer in Singapore.

**Methods:** A prospective cross-sectional study of 971 healthy women between the ages of 13–82 years was carried out. Informed consent is obtained. Socio-demographic data including age and racial group were obtained. A routine cervical smear (or a vaginal lavage in virginal girls) was obtained from each participant for cytological evaluation. These were collected in liquid-based preservative for HPV DNA analysis. HPV detection and typing was performed using the Roche LINEAR ARRAY HPV (Human Papilloma Virus) Genotyping Test (Roche Diagnostics, Germany) which detects 37 anogenital HPV DNA genotypes.

**Results:** Of the 971 women surveyed, 890 (91.7%) had valid samples. Among the valid cases from 890 women, 83 (9.3%) of the cases had HPV detected. The highest prevalence of HPV and high-risk HPV (HR-HPV) infections were noted in the study population aged 29 years old and below. However, no significant difference was observed in the HPV prevalence of different ethnic groups. Unlike other international studies, HR-HPV subtypes 51, 52 and 58 were significantly more prevalent in the HPV positive cases of normal healthy women in Singapore.

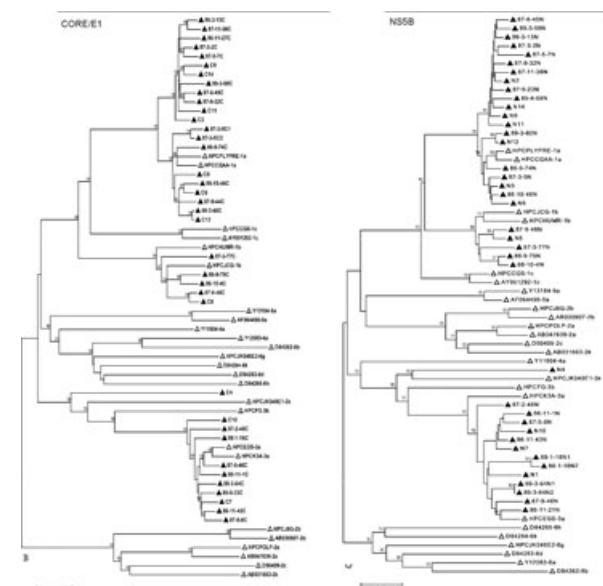
**Conclusions:** The subtype distribution of HPV in Singapore differs from studies conducted in Europe and USA. Findings of this study will be useful in evaluating the cost-effectiveness of HPV vaccine implementation as a national programme in the prevention of cervical cancer.

#### P877 A new and rare subtype of HCV genotype 3 in Iran: phylogenetic analysis of NS5b and Core/E1 gene regions

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**Objectives:** Hepatitis caused by HCV is a serious disease affecting more than 170 million people worldwide. HCV genome is highly variable and based on genetic distances and phylogenetic analyses, all isolates are grouped into six genotypes and many different subtypes. During a research on HCV molecular epidemiology among HIV-HCV dually infected patients admitted to Masih Daneshvari Hospital in Tehran, we found a new variant of HCV genotype 3.

**Methods:** A serum sample from a 41Y/O patient (TB/HIV/HCV infected) was obtained as part of an HCV molecular epidemiology study in the Virology Research Center. The partial genome sequences, including both the core/E1 region and the NS5B region (GenBank accession number JN129985 and JN129986) from this patient's plasma were obtained. Phylogenetic analysis of these partial genome sequences was conducted using MEGA version 4.0 along with representatives of the six HCV genotypes described previously.



**Results:** Neighbour-joining phylogenetic reconstructions revealed that the new sequence placed in HCV genotype 3 sequences, but grouped separately from any of the subtypes described for this genotype in the both gene regions. Genetic distances showed that compared with representatives of the confirmed subtypes of genotype 3, the NS5B and Core/E1 sequences displayed 25.5–35.2% and 41.8–45.4% nucleotide differences, respectively.

**Conclusion:** In this study, we have revealed the existence of a new and distinct variant of HCV genotype 3 from Iran by sequencing and analyzing of partial genome sequences. This genome is as divergent from representative variants of confirmed genotype 3 subtypes. In consequence, we have provided evidence that this isolate could be a truly new subtype. The presence of the new variant in Iran is mostly proven by finding another sequence during BLAST search (isolate 943, GenBank accession number AY654000) with 95.35% similarities in the NS5B region. The variant was also from a dialysis patient in Tehran and reported by Samimi-Rad et al. in 2004. Due to possibly some mistakes, the 943 variant was classified as HCV genotype 5. It is clearly the same genotype as the one we have identified. The rarity and restriction of our new HCV variant to Iran is rather interesting, and might justify some further screening-genotyping of other HCV infected patients.

#### **P878** Vaccine poliovirus associated encephalitis in OPV vaccinated children

A. Kumar\*, D. Shukla, R. Kumar, M.Z. Idris, T.N. Dhole (Lucknow, IN)

**Objectives:** The inheritant problem of Poliovirus genome is subjected to mutation, recombination and may revert back to neurovirulent strain. These neurovirulent strains might cause rare serious disease like vaccine-associated paralytic poliomyelitis in primary vaccine recipients and their contacts. As wild poliovirus is in the last stage of eradication, the characterization of oral polio vaccine (OPV) like strain from central nervous system disease is necessary to completely achieve the goal of poliomyelitis free world and future planning. The aim of the present study was to characterize the OPV like strains associated with encephalitis in children from northern India.

**Methods:** A total of 75 cerebrospinal fluid (CSF) specimen were collected between January and December 2010 from children (age <5 years) with symptoms of acute encephalitis syndrome admitted to the department of Paediatrics, Chhatrapati Shauji Maharaj Medical University Lucknow. Viral RNA was extracted from CSF using a QIAamp Viral RNA mini kit. Enterovirus serotypes were determined by semi-nested RT-PCR and sequencing of partial VP1 region. The categorization of poliovirus into OPV like, vaccine derived poliovirus and wild polio virus was performed according to WHO criteria. The neurovirulence of OPV like strain was done by amplification and sequencing of poliovirus 5' untranslated region (UTR).

**Results:** Out of 75 CSF, 19 (25.33%) were positive for enterovirus, 10 (13.33%) for Japanese encephalitis virus and 8 (10.66%) for chikungunya virus. Out of 19 enteroviruses, type 3 vaccine poliovirus was identified in three encephalitis cases which were vaccinated with OPV. Sequence analysis of 5'UTR region confirms mutation at 472 nucleotide position which is responsible for increased neurovirulence.

**Conclusions:** All three cases were identified as type 3 polio virus probably derived from type 3 OPV strain. The detection of neurovirulent OPV like strain in this highly populated and endemic area of poliomyelitis suggest enhanced molecular surveillance in cases of encephalitis. However, this is the time to debate on the role of emerging oral vaccine poliovirus in central nervous system disease.

#### **P880** The herpes simplex virus type 1 late associated transcript derived miR-H2 drastically reduced SMAD4 expression

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**Objectives:** The Herpes Simplex Virus type 1 (HSV-1) naturally establishes latency in ganglions of sensory neuron cells. The late

associated transcript (LAT) is a spliced noncoding RNA that is transcribed in HSV-1 infected neuron cells. The LAT produces six microRNAs that are able to affect the host cell or viral genes expression. The recent study showed that miR-H2, one of the LAT derived microRNAs, arrests the HSV-1 immediate early ICP0 gene expression. The transforming growth factor-beta (TGF-B) signaling is a critical pathway that regulates cellular process like apoptosis, cell differentiation and growth. The SMAD family member 4 (SMAD4) is crucial protein in TGF-B signaling that in response to TGF-B receptors signals bind to SMAD1 and SMAD2 and as a transcription factor complex regulates gene expression. In this study, we assessed effects of miR-H2 on Smad4 expression.

**Methods:** To prediction of miR-H2 targeting genes the bioinformatical studies were implemented by two software. The targets can and RNA22 with different algorithms were used in forecasting of genes that targeted by miR-H2. The plasmid including miR-H2 was transfected to human neuroblastoma cells [BE-2(c)] by lipofectamine 2000 (Invitrogen) according to manufacture protocol. The expression of miR-H2 was assayed by Real Time PCR. The effects of miR-H2 on Smad4 and its downstream genes were evaluated.

**Results:** The bioinformatical analysis of Targets can predicts that miR-H2 target Smad4 by a site with 8mer seed and RNA22 algorithm forecast two sites with -33 Kcal/mol folding energy. The Real Time PCR assays 48 hours after miR-H2 transfection showed that along with expression of miR-H2 in BE-2(c) cells the Smad4 was drastically reduced.

**Conclusion:** The Real Time PCR results revealed that the SMAD4 expression was reduced in response to miR-H2 overexpression in neuroblastoma cells when bioinformatical studies predicted SMAD4 targeting by miR-H2. Considering TGF-B pathway plays critical roles in many cellular disorders and disease, regulation of SMAD4 as an important protein in this path by miR-H2 could be useful in future investigation on gene therapy.

#### **P881** Genotype variability and clinical features of human metapneumovirus Isolated from Korean children, 2007–2010

M.K. Lee\*, H.R. Kim, T.H. Kim, B.S. Shim (Seoul, KR)

**Objectives:** Human metapneumovirus (hMPV) has been divided into five subgroups based on variations of the hMPV F gene. This study was undertaken to determine the genotype variability of hMPV, its circulation pattern in over a 3.5 year period, and to evaluate its clinical characteristics in Korean children.

**Methods:** We investigated 325 of 4599 pediatric patients with a positive nasopharyngeal aspirate that were referred for a routine respiratory virus test for hMPV by RT-PCR. HMPV genotype analyses were performed using a nested PCR-restriction fragment length polymorphism (PCR-RFLP) assay. Clinical and laboratory data obtained from medical records were reviewed retrospectively.

**Results:** Three hundred and twenty-five (7.1%) of the 4599 samples tested were positive for hMPV, and the co-infection rate among these 325 was 16%. Nested PCR-RFLP analysis clearly identified four of the five previously described hMPV genotypes (A2a, A2b, B1 and B2) in 97.8%. The predominant genotype of hMPV changed over the 3.5-year study period from genotype A2a to B2 and then back to A2a. The most common genotype was A2a (214/325, 65.8%). Evidence of recurrent infection was obtained in one child only. Lymphocytosis was more frequent in children with a co-infection, but sputum was less frequent than in children with a single infection ( $p < 0.05$ ). In genotype A2a hMPV infected-children, sneezing and neutrophilia were more frequent than in genotype B1 or B2 hMPV-infected children.

**Conclusion:** This 3.5-year study broadens knowledge regarding the prevalence, the seasonal incidence, the occurrences of co-infection and re-infection, and the genotype diversity of hMPV in Korea.

**P882** “Moderate” concordance between “modified” Abbott real-time HIV-1 assay and Roche Cobas/Taqman HIV-1 v2.0 procedure for the quantification of human immunodeficiency virus type 1 (HIV-1) RNA at low level of viral load (BELOW 600CP/ML)

A. Amendola\*, P. Marsella, M. Bloisi, C. Angeletti, M.R. Capobianchi (Rome, IT)

**Objectives:** The suppression of HIV RNA below 50 cp/mL is commonly achieved in most patients undergoing current therapeutic approaches. When viremia is reduced below the limit of detection (LOD) of commercial diagnostic tests, residual viremia (RV) can still be detected. The assessment of RV is proving increasingly useful in monitoring viral suppression to determine the relative effectiveness of different regimens, but concordance between commercial assays is weak at low viremia. We compared performances of “modified” procedure of Abbott Real-time HIV-1 assay and standard protocol of Roche COBAS/TaqMan HIV-1 v2.0 for quantification of HIV RNA low levels (<600 cp/mL). The “modified” Abbott assay reaches a LOD of 19 cp/mL (Amendola A. et al. J Clin Virol 2011); the Roche standard procedure shows LOD of 20 cp/mL.

**Methods:** The comparison was performed using: (i) 153 clinical samples chosen with viral load values ranging from “not-detected” to 600 cp/mL with Abbott standard protocol and (ii) the Working Reagent (WR) for NAT assays (NIBSC) as a standard, diluted to 128, 64, 32 and 16 cp/mL (seven replicates/dilution). Quantitative results were compared using correlation, linear regression and Bland and Altman analyses. Concordance on qualitative results (namely “detected” vs “not-detected”) was measured by Cohen’s kappa statistic. Probit analysis of both assays was performed with WR.

**Results:** 1 A good correlation between assays was observed in clinical samples with viral load values ranging from 20 to 600 cp/mL (Pearson  $r = 0.7468$ ); Bland&Altman analysis showed a mean difference of measurement equal to 0.170 log<sub>10</sub> cp/mL. Below 20 cp/mL, the degree of concordance between the two assays for samples given as “detected” vs “not-detected” was “moderate”, with Cohen’s kappa-statistic of 0.450 and an agreement proportion of 0.730.

2 The difference in viral load quantification at low levels was also observed using the WR standard. The Roche assay provided, with high reproducibility, results that were, on average, 3.45 times higher than expected values (equal to +0.52 log<sub>10</sub> cp/mL) at all dilution levels, whilst the Abbott “modified” procedure produced the expected values.

**Conclusions:** Overall, the two diagnostic systems show some differences in the quantification of HIV-1 viral load at low levels and a degree of concordance “moderate”. By probit analysis, performed with WR standard, we observed that Roche procedure is more sensitive, but Abbott “modified” is more precise and accurate.

**P883** Genetic diversity of hepatitis E virus isolates originating from human patients and animals in the Czech Republic

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**Objective:** Hepatitis E virus (HEV) causes an important public health problem in many countries worldwide and its zoonotic potential was recognized recently. Due to high prevalence of HEV in domestic pigs and wild boars, these animals were considered as a reservoir of the virus. According to phylogenetic analysis HEV strains segregate into four major genotypes and their subtypes. Genotypes 1 and 2 correspond to primary circulation within humans, whilst diversity of genotypes 3 and 4 is related to zoonotic origin. Each genotype (even subtype) is dominant in reported geographical areas, which could be used in epidemiological studies. The aim of the study was to perform sequence comparison and phylogenetic analysis of Czech HEV isolates of human and animal origin and thus to find out their possible relation.

**Methods:** Stool and serum from 56 patients with serologically confirmed hepatitis E (HE) and bile, liver and faecal samples from domestic pigs and wild boars were tested for presence of HEV RNA by triplex real time reverse transcriptase PCR. Specific part of HEV genome (242 nt) originating from detected isolates was subjected to sequencing. The sequences were analysed by MEGA 5.05 and compared with the sequences in GenBank. Neighbor-joining method was used for phylogenetic analysis.

**Results:** Comparison of the sequences with GenBank failed to find any 100% identical HEV isolate. The sequence analysis revealed that one human and two swine isolates shared 99.6% (a single nucleotide difference) homology with each other and sequences of two human isolates were 100% identical. Phylogenetic analysis showed that all Czech isolates belonged to genotype 3 subtypes: human isolates 3e, 3f, 3g, 3i and 3g and animal isolates 3f and 3g.

**Conclusions:** Consumption of raw or undercooked meat and meat products or direct contact with infected animals is possible source a HEV infection. Phylogenetic analysis of found HEV isolates revealed presence of subtype 3g and 3f in HE patients and domestic pigs and subtype 3g in HE patients, domestic pigs and wild boars in the Czech Republic. A single nucleotide difference of 242 nt long sequences of human and swine HEV isolates together with HE patients’ anamnestic data support interspecies transmission of HEV in the Czech Republic and zoonotic potential of the virus.

**Acknowledgement:** This work was supported by the Min. Agr. (No. MZE0002716202) and the Min. Edu., Youth and Sports of Czech R. (AdmireVet CZ 1.05/2.1.00/01.0006-ED0006/01/01).

**P884** Multiplex respiratory virus PCR: a comparison of the FilmArray® and xTAG® respiratory panels on bronchoalveolar lavage and other respiratory specimens

J.M. Balada-Llasat\*, C. Kelly, W. Zhang, H. LaRue, P. Pancholi (Columbus, US)

**Objectives:** The clinical presentation of respiratory viral infections may be similar, therefore identification of the causative agent(s) aids in the selection of appropriate treatment and infection control measures. Several multiplex PCR assays have been developed for the detection of respiratory viruses. Among these are the FilmArray® (Idaho Technology Inc.) and the xTAG® (Luminex) assays. We evaluated the performance of the FilmArray compared to the xTAG for the detection of respiratory viruses from various respiratory specimens including Bronchoalveolar lavage (BAL).

**Methods:** Two hundred and sixty-nine respiratory specimens (nares, nasopharynx, throat, sputum, BAL, lung, pleural fluid) were collected from hospitalized patients during the winter of 2010–2011. One aliquot was processed with the xTAG and a second previously frozen aliquot was tested by the FilmArray assay. For the xTAG assay viral nucleic acid was extracted using the EasyMag, reverse transcribed, amplified, and analyzed with Luminex® technology. For the FilmArray, respiratory specimens were processed in a closed RT PCR system that isolates, amplifies, and detects the viral targets. Both assays detect influenza A (Flu A; seasonal H1 and H3) and influenza B (Flu B), adenovirus, parainfluenza 1–3 (Para 1–3), respiratory syncytial virus (RSV), human metapneumovirus (HMPV) and human rhinovirus/enterovirus (HRV/E). The FilmArray additionally detects coronaviruses (NL63 and HKU1), Para 4, and Flu A subtype 2009 H1. The xTAG also differentiates RSV A and RSV B. For discrepant analysis, both assays were repeated on the frozen aliquot. Additionally, sequencing was performed.

**Results:** Of the 269 specimens tested, both assays agreed on 109 negative and 150 positive respiratory specimens. When compared to the xTAG, the FilmArray showed a 95% sensitivity and 98% specificity.

**Conclusions:** The FilmArray was useful for detecting a wide range of viruses in various sample types including BALs. The FilmArray additionally detected coronaviruses. The xTAG requires 6–7 hour with 2.5–3 hour of hands-on time, while the FilmArray takes about an hour with 3–5 minute of hands-on time, making it much easier to perform.

However the FilmArray processes only one sample at a time that can limit its utility in moderate-to high-volume laboratories.

**P885** **Quantification of human cytomegalovirus DNA in transplant recipients by the Abbott real-time HCMV assay**

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**Objectives:** Human Cytomegalovirus (HCMV) infection/disease is the major infectious viral complication in the post-transplant period for hematopoietic stem cell (HSCTR) and solid organ transplant recipients (SOTR). Standardization of protocols for monitoring HCMV infection is a priority in the management of transplanted patients receiving pre-emptive therapy.

**Methods:** The kinetics of HCMV disseminated infections was retrospectively evaluated in surplus whole blood samples of 20 paediatric HSCTR and 17 adult SOTR enrolled in two prospective studies at the IRCCS Policlinico San Matteo, Pavia, Italy aimed at the clinical validation of HCMV DNA cut-off values for preemptive treatment (Gerna et al., *Antivir Ther* 2007; Lilleri et al., *Blood* 2008). All patients had been prospectively tested once a week using an in house developed Real-Time PC (PV assay). HCMV infection was treated preemptively on the basis of two HCMV DNA cut-off values in whole blood: 30 000 copies/mL for HSCTR and 300 000 copies/mL for SOTR.

**Results:** Overall, 266 sequential whole blood samples from 20 HSCTR patients and 247 specimens from 17 SOTR patients were analyzed. HCMV disseminated infection was confirmed by both assays in 18/20 HSCTRs and 15/17 SOTRs, whereas HCMV infection was excluded by both methods in 2/20 HSCTRs and in 2/17 SOTRs. In particular, 352/513 samples resulted concordant positive and 42/513 were concordant negative. 119/513 samples were discordant (Abbott-positive and negative by the PV assay). These samples were restricted to the initial and conclusive phases of infection and were scored as positive by the Abbott assay and as containing <100 copies/mL by the PV assay. A significant correlation was observed between concordant positive samples ( $r = 0.89$ ). Following dilution in whole blood of the WHO international standard for HCMV DNA quantification, a copies/mL to IU/mL conversion factor of 5.04 and 9.83 was calculated for the PV assay and the Abbott assay, respectively. A better concordance between the PV assay and the Abbott assay to score the patients' eligibility to treatment was observed following translation of results in IU/mL, than using the copies/mL readout.

**Conclusions:** (i) the Abbott assay showed greater analytical sensitivity, a characteristic to be taken into account for treatment interruption; (ii) normalization of quantitative results expressed by UI/mL could be useful in order to safely export and use clinical cut-offs for pre-emptive antiviral therapy.

**P886** **Type distribution of human papillomaviruses in cervical samples of women with cytological abnormalities from Primorsko-Goranska county, Croatia**

T. Rukavina\*, B. Ticac (Rijeka, HR)

**Objective:** Cervical cancer is the second most common cancer in women. Most of the cases are caused by infection with HPVs. Certain HPV types have strong oncogenic potential and are considered high-risk (HRT), while certain are considered low-risk (LRT) since their infection results in formation of benign genital warts. Currently, two vaccines for the prevention of these infections are available. Two HRTs (16 and 18) included in vaccines are considered responsible for more than 2/3 of cases of cervical cancer. Several studies from Croatia reported that these two types are less frequent in our female population. Therefore, we decided to determine the HPV types in cervical samples

of women with cytological abnormalities from Primorsko-Goranska County in Croatia.

**Methods:** We have analyzed 108 consecutive HPV-positive samples of women with proven cytological abnormalities. Samples are processed in the Laboratory for Molecular Diagnostics of the Teaching Institute of Public Health of Primorsko-Goranska County, Rijeka, Croatia. They were analyzed by PCR method using commercial kits with electrophoretic detection: HPV6/11; HPV High Risk Screen; and HPV High Risk Typing (Sacace Biotechnologies, Italy). The kits enable detection of LRTs HPV6 and 11 and HRTs HPV16, 18, 31, 33, 35, 39, 45, 52, 53, 56, 58, 59, 66 and 70.

**Results:** Fifty-three of 108 analyzed samples (49.1%) contained 1 type of HPV; while more than 1 (up to 5) HPV types were detected in 51 samples (47.2%). In four samples (3.7%) the HRT detection was positive but the typing was not successful, so we considered them HRT positive, nontypeable. Four samples (3.7%) contained only 1 LRT while all the other samples contained HRTs. The most frequent HRT was HPV16 that was detected in 33 samples (17.2%), followed by HPV31, 52 and 18 that were detected in 25 (13.0%), 21 (10.9%) and 18 (9.4%) samples, respectively. HPV16 and 18 that are considered responsible for more than 2/3 of cases of cervical cancer were found in 48 analyzed samples (44.4%).

**Conclusions:** Most of the women with cytological abnormalities included in study were infected with HRTs (104 out of 108). HPV16 was the most commonly found, followed by HPV31, 52 and 18. The two HRTs covered by currently available HPV vaccines were detected in 44.4% of analyzed samples. This fact should be taken into account during planning of future preventive public health activities.

**P887** **Comparison of Cobas AmpliPrep/Cobas TaqMan Docking Station vs. Cobas AmpliPrep/Cobas TaqMan 48, Nuclisens EasyMag/EasyQ System and System 340 bDNA Analyzer for quantification of HCV, HIV-1 and HBV in plasma**

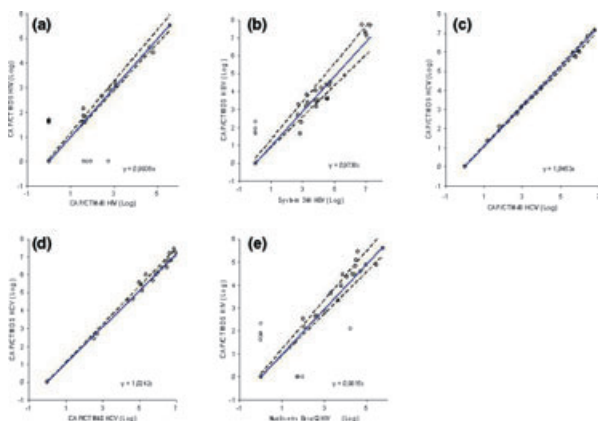
M.J. Munoz-Davila\*, A. Moreno, D. Montero, T. Ramos, M. Segovia (Murcia, Madrid, Barcelona, ES)

**Objective:** Comparative evaluation of correlation rates and management of the Cobas AmpliPrep/Cobas TaqMan Docking Station (CAP/CTM DS) vs. the routine assays (Cobas AmpliPrep/Cobas TaqMan 48 [CAP/CTM 48], Nuclisens EasyMag/EasyQ System [NEQ/NEM] and System 340 bDNA Analyzer [S340bDNA]) used for human plasma or serum measurement of HBV, HCV and HIV-1 in the two laboratories included in the study center (University Hospital Virgen de la Arrixaca [UHVA] and University Hospital La Paz [UHP], Spain).

**Methods:** All samples included in this comparative evaluation were processed on three consecutive days. All tests were performed strictly according to the manufacturer's instructions. Patient samples received at the virology units for viral load quantification of HBV DNA, HCV RNA and HIV-1 RNA were performed in parallel by the routine method and by the CAP/CTM DS at the two hospitals included in the study. Patient samples collected on three consecutive days included positive results in a wide range of quantifications and also samples resulting in target not detected. The patient samples were randomly selected according the order in which they were received at the laboratory, until a statistically significant number was reached. Workflow, throughput, and minimum user time was collected for each assay studied. Statistical analyses: The Passing-Bablok method was used to judge the relationship of the assays to one another. Bland-Altman plots were constructed.

**Results:** The percentages of correlation coefficient of the CAP/CTM DS vs. CAP/CTM 48 were 99.7% and 87.2% for HCV and HIV-1 respectively, at the UHVA. These percentages of correlation coefficient were quite similar to those obtained by the UHP, namely 98.7% for the CAP/CTM DS vs. CAP/CTM 48 for HCV, 86.6% for CAP/CTM DS vs. NEQ/NEM for HIV-1 and 94.2% for CAP/CTM DS vs. S340bDNA for HBV. Linear regression analyses represented by scatter plots are

shown in Figure 1. The specimen throughput for a batch size of 24 was consistently lower for the CAP/CTM DS in all cases except when it was compared with NEM/NEQ for viral load quantification of HIV-1 at the UHP. Importantly, CAP/CTM DS exhibited substantially reduced hands-on-time when compared with the rest of the assays.



**Conclusion:** When compared with these systems, the CAP/CTM DS assay yielded excellent correlation results and improved hands-on time in all cases. Total processing time for 24 samples was also reduced in most cases.

#### **P888** Evaluation of sample collection and handling conditions for the Versant HCV RNA 1.0 assay (kPCR)

W. Lin\*, L. Cheng, P. Lau, S. Ho, J. Canchola, G. Gorrin (Berkeley, US)

**Objectives:** The VERSANT® HCV RNA 1.0 Assay (kPCR)\* (Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA) is an in vitro nucleic acid amplification assay for the quantitation of hepatitis C virus (HCV) RNA in serum or plasma of HCV-infected individuals over the range of 15–1.0E8 IU/mL using the VERSANT® kPCR Molecular System.\*A study was performed to investigate the effect of multiple sample collection tubes and associated processing parameters on viral quantitation in the VERSANT HCV kPCR Assay.

**Methods:** Sixty unique HCV-infected donors were divided among six donor sample sets that were used to evaluate BD VACUTAINER ACD, K2EDTA, PPT, (plasma) and SST (serum) blood collection tubes. Sample hold times before collection tube processing (up to 6 hours at ambient temperature or 24 hours at 2–8°C) and after (24 hours at ambient temperature and up to 72 hours at 2–8°C) were evaluated. In addition, long-term sample storage at –20°C and –80°C plus multiple freeze-thaw cycles at –20°C and –80°C were assessed. Twenty additional patient samples were collected to study the effect of fresh vs. frozen samples on viral quantitation in the four tube types, and primary tubes vs. aliquots in PPT and SST tubes. Results were considered acceptable if the average difference between test and control conditions was within  $\pm 0.2$  log IU/mL.

**Results:** There were no significant differences between the four tube types tested. Serum and plasma were found to be equivalent matrices, as quantitation in serum (SST) was within  $\pm 0.2$  log of quantitation in plasma (K2EDTA, PPT, and ACD). The varying hold times before and after collection tube processing also did not have a significant effect. Long-term sample storage for up to 4 months at –20°C proved to be no different from storage at –80°C for all tube types tested, and samples were stable for up to four freeze-thaw cycles at –20°C and –80°C. Results from the additional twenty patient samples demonstrated that either fresh or frozen samples can be used, (all quantitation differences within the  $\pm 0.2$  log IU/mL). There were also no significant differences in quantitation between primary tubes and aliquots.

**Conclusion:** This study found no significant difference in quantitation between test and reference conditions for all parameters evaluated.

These data demonstrate that the VERSANT HCV RNA 1.0 Assay (kPCR) provides flexibility in sample collection and processing.

\*CE marked. Not available for sale in the US.

#### **P889** Method comparison between the VERSANT HCV RNA 1.0 assay (kPCR), Abbott RealTime HCV, Roche COBAS AmpliPrep/COBAS TaqMan HCV Test, and VERSANT HCV RNA 3.0 assay (bDNA)

H. Huang\*, W. Lin, S. Ho, J. Brooks, P. Lau, J. Surtihadi, J. Canchola, L. Cheng, G. Gorrin (Berkeley, US)

**Objectives:** The VERSANT® HCV RNA 1.0 Assay (kPCR)\* (VERSANT kPCR; Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA) is an in vitro nucleic acid amplification assay for the quantitation of hepatitis C virus (HCV) RNA in serum or plasma of HCV-infected individuals over the range of 15–1.0E8 IU/mL using the VERSANT® kPCR Molecular System.\*Method comparisons were conducted between VERSANT kPCR and Abbott RealTime HCV (ART; Abbott Molecular, Inc., Des Plaines, IL, USA), Roche COBAS® AmpliPrep/COBAS® TaqMan® HCV Test (CAP/CTM; Roche Molecular Systems, Pleasanton, CA, USA) and VERSANT HCV RNA 3.0 Assay (bDNA) (bDNA; Siemens Healthcare Diagnostics Inc.).

**Methods:** In all, 155 samples were tested: 116 HCV-infected serum and plasma samples, collected with IRB approval at BioCollections Worldwide, Inc. (Miami, FL, USA); and 39 samples from HCV genotype panels, purchased from SeraCare (Milford, MA, USA), to ensure all common HCV genotypes were included. Each sample was tested with the VERSANT kPCR Molecular System, which consists of a Sample Prep module and an Amplification/Detection module (Siemens, Berkeley, CA, USA), as well as with bDNA, at Siemens Clinical Laboratory, Berkeley, CA; with Roche CAP/CTM at John Muir Hospital Lab, Walnut Creek, CA; and with Abbott ART at BioCollections Worldwide. Testing was done in singlicate by each of the methods, and results were compared for all samples with paired quantitations within the reporting ranges of each pair of methods. Deming regression was used to determine if methods had a linear relationship (slope within 0.9–1.1). Quantitative equivalence was considered acceptable if the average log difference was within  $\pm 0.5$  log IU/mL.

**Results:** The Deming regression slopes for all paired comparisons were between 0.98 and 1.01, indicating that the VERSANT kPCR has a linear relationship with all of the comparator methods. The average log difference between the VERSANT kPCR and the comparator methods was within 0.38 log IU/mL for Abbott, within 0.13 log IU/mL for Roche, and within 0.31 log IU/mL for bDNA.

**Conclusions:** The results demonstrate a linear relationship and quantitative equivalence between VERSANT HCV RNA 1.0 Assay (kPCR) and the comparator methods:

- 1 Roche COBAS AmpliPrep/COBAS TaqMan HCV Test,
- 2 Abbott RealTime HCV Assay, and
- 3 VERSANT HCV RNA 3.0 Assay (bDNA).

\*CE marked. Not available for sale in the U.S.

#### **P890** Multiplex PCR assays for RSV-A, RSV-B and human metapneumovirus diagnosis in acute bronchiolitis

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**Objectives:** Design and optimization of multiplex real-time RT-PCR for RSV-A, RSV-B and human metapneumovirus diagnosis in acute bronchiolitis in pediatric population.

**Material and Methods:** Three sets of TaqMan primers-probes (Applied Biosystems, USA) were devised for detection of respiratory syncytial virus type A (RSV-A), RSV-B and human metapneumovirus, which are the main causal agents of neonatal and unweaned baby



bronchiolitis. A 7500 Fast real-time thermocycler system (Applied Biosystems, USA) was used to carry out RT-PCR in <1.5 hour. Sequencing of the resulting amplicons using every set of primers and mixtures of the three viruses was made to establish the technique specificity. Sensitivity was determined using serial dilutions of decreasing and known DNA concentrations. Finally, the test yield was proved by means of application of the procedure to 121 nasal aspirate samples from children with clinical findings of bronchiolitis. A rapid detection RSV test (BinaxNow) and a NASBA (Nucleic acid sequence based amplification) technique (EasyQ, Biomerieux, Spain S.A.) for metapneumovirus detection, were previously performed to these samples.

**Results:** Sequencing of the three resulting amplicons compared to sequences from GeneBank database proved an absolute specificity. Also, the technique allows for proper simultaneous differentiation of each virus in a mixture containing the above mentioned viruses. The sensitivity limit showed a PCR able to detect between 1 and 10 DNA copies/ $\mu$ L. The comparison results with the rapid detection RSV test and NASBA technique were as follows: the proportion of positive results for RSV detection by the rapid test was 53/121 (43.8%); 1/121 (0.8%) was positive for metapneumovirus by NASBA technique; 66/121 (54.5%) were positive to RSV (34 type A and 32 type B) and 3/121 (2.5%) to metapneumovirus using multiplex RT-PCR. The operating features of both techniques compared to multiplex RT-PCR are shown in the table.

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
RSV	80,3	100	100	80,9
Metapneumovirus	33,3	100	100	98,3

**Conclusions:** Multiplex real-time RT-PCR has proved both high specificity and sensitivity. Regarding other diagnosis techniques, this PCR procedure has shown a high superior sensitivity. All these advantages, coupled to the high rate and simplicity of the method make this technique very useful in a daily clinical practise for acute bronchiolitis virological diagnosis.

**P891 Multi centre study to evaluate the influence of pre-analytical storage conditions on the RNA concentration of hepatitis C virus**

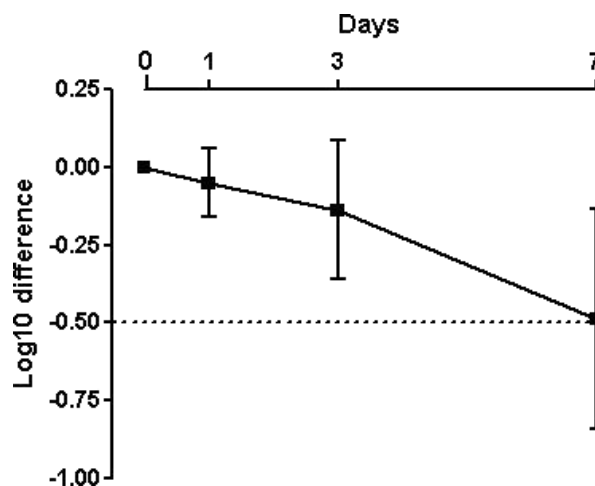
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**Objectives:** Several studies have claimed that hepatitis C virus (HCV) RNA levels are stable in samples when stored at 4°C for 3 up to 7 days. The stability of HCV RNA levels at room temperature (RT) is less clear. Some studies stated that HCV RNA is unstable at RT (18–25°C) whereas others demonstrated that EDTA blood or serum may be stored at 25°C for 4 up to 5 days without significant loss in HCV RNA. In this multi-centre study (10 laboratories) we tested the HCV RNA stability of 15 samples (eight serum samples and seven plasma samples) by storing the samples at RT for 0, 1, 3 and 7 days before analysis.

**Methods:** Laboratories analysing HCV on serum, divided a fresh serum sample in four aliquots, left these aliquots at RT and stored the aliquots below –20°C at day 0, 1, 3 and 7. Laboratories analysing HCV on EDTA plasma, divided an EDTA blood sample in 4 aliquots. The different aliquots were left for 0, 1, 3 and 7 days at RT before centrifugation and immediately stored below –20°C. The RNA extraction and HCV qPCR was done for all aliquots in the same run (avoiding inter-run differences). The concentrations (IU/mL) of the samples at day 0, 1, 3 and 7 were expressed as Log10 values. A Log10 difference >0.5 was considered as a clinical significant difference.

**Results:** One sample was excluded for further analysis because the centre did not determine the RNA level on day 0. One result on day 1 was excluded because of a statistical outlier. The Log10 differences from all samples for the different time points indicate an similar trend:

the HCV concentration decreases over time. For 2 out of 14 samples (14%), a Log10 difference >0.5 was observed at day 3. For 5 out of 12 samples, a Log10 difference >0.5 was observed at day 7. The mean Log10 difference stayed within the 0.5 limit at all days. However at day 7, the mean Log10 difference was  $-0.45 \pm 0.34$ , which demonstrates a significant decrease in HCV concentration for several samples. No statistical difference was observed between serum and plasma samples.



**Conclusion:** Although it is clear that it is the best procedure to store samples as soon as possible at <–20°C is, we can conclude that most samples stored at RT for 3 days do not exhibit a significant loss in measured HCV RNA levels. However, storage for more than 3 days at RT will lead to an underestimation of HCV RNA concentrations or false-negative results in the detection of HCV RNA in serum and plasma.

**P892 Rabies outbreak among wild and domestic animals in Republic of Buryatia, Russia**

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**Objectives:** Rabies is a zoonotic viral disease which infects domestic and wild animals and it is fatal for animals and human. The aim of our study was to determine the molecular characteristics of rabies virus (RABV) outbreak in the Republic of Buryatia (Russia).

**Methods:** We examined three domestic and six wild animals' brain samples on RABV presence. For this goal we have used biological (bioassay), serological (fluoroimmunoassay) and molecular genetic (RT-PCR and sequencing) methods. Several smears on ground slides were prepared from the received material, which were labeled with fluorescent antirabic globulin (Federal Center for Toxic and Radiation Safety of Animals, Russia) after acetone fixation during 2–4 hour. Laboratory not pedigree white mice were infected according to method recommended by WHO. Total RNA was extracted directly from brain tissue (10% suspension in saline) using Riboprep kit (ILS Ltd., Russia). Amplification of nucleocapsid (N) gene was carried out with Amplisens kit (ILS Ltd., Russia). PCR products were sequenced with ABI Prism Big Dye Terminator v.1.1 Cycle Sequencing Kit and Genetic Analyzer 3130 (Applied Biosystems). Sequencing results (1353 bp) were analyzed by BioEdit v.7.0.5.3. software. Phylogenetic analysis was performed by MEGA 5 program using Maximum Likelihood method.

**Results:** The intensity of the granules fluorescence in preparations from the native samples was as much as ++ – +++, the intensity in preparations from the laboratory died mice' samples was as much as +++ – +++. Bioassay samples' nucleotide sequences had 100% of

homology with isolate's sequences. Phylogenetic analysis shows that RABV isolates from Republic of Buryatia (Russia) follows into the clade with RABV strains from Mongolia isolated from domestic and wild animals some years ago. The identity of nucleotide sequences of these strains and Buryat isolates is 98.9–99.2%.

**Conclusion:** Our results show the presence of rabies outbreak among wild animals and cattle on the territory of Republic of Buryatia (Russia). Phylogenetic analysis allows to suggest possible import of rabies virus by wild animals from Mongolia.

#### **P893** Tropism testing using an adaptation of the Trugene assay in plasma, CSF and cell extract (proviral)

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**Objectives:** To adapt an existing rapid HIV drug resistance sequencing test for HIV-1 envelope co-receptor usage detection for plasma, proviral and CSF samples.

**Background:** Knowledge of HIV-1 envelope V3 gene tropism is essential to select most appropriate patients for treatment with the anti-HIV CCR5 drug inhibitors such as Maraviroc. Determination of V3 co-receptor usage is performed primarily by molecular assays which have can be time consuming and involves viral RNA extraction, RT-PCR, sequencing and bioinformatics prediction of HIV coreceptor usage (geno2pheno; Max Planck Institute). There is increasing clinical demand for assessment of V3 tropism in plasma, peripheral blood mononuclear cells and cerebro-spinal fluid (CSF) samples. Here, we describe an adaptation of the Siemens Core Reagent assay which provides a quick and easy method to determine likely co-receptor usage in plasma (Viral RNA), cell extract (proviral DNA) and CSF.

**Method:** RNA was extracted from plasma or CSF using the Qiagen RNA extraction kit and DNA was extracted using the Qiagen DNA miniKit. V3 tropism was assessed using a single approach for all samples with an adaptation of the Siemens Core Reagent method for first round PCR, followed by an in-house nested PCR protocol utilizing a proof-reading enzyme cocktail. PCR products were subsequently directly sequenced using the Siemens Clip Assay and data were analysed online with geno2pheno bioinformatics prediction program. Clade types in V3 samples were compared with Clade types obtained for protease and reverse transcriptase resistance (Pr/RT) typing.

**Results:** The methodology used was found to be equally applicable for the detection of HIV coreceptor usage in viral RNA (plasma/CSF) and proviral (DNA) samples. The Clade types obtained by V3 samples were found to be 95% concordant with the Clade types obtained by Pr/RT resistance typing with discrepancies being confirmed by repeat testing and as such likely to be due to recombination. The majority of samples tested were Clade B (69.8%), followed by Clade A/AG (17.0%), Clade C (9.4%) and Clade G (3.8%). In a small number of samples with both plasma and proviral sequencing data, there was a good concordance between plasma and proviral sequences.

**Conclusion:** Using a single approach, as described here, co-receptor usage can be determined for RNA and proviral DNA samples with equal success.

#### **P894** Design and efficacy of a real-time genome amplification (RT-PCR) for human herpesvirus type 6A/B and 7

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**Objectives:** Human Herpesvirus type 6A/B (HHV6A/B) and Human Herpesvirus type 7 (HHV7) are involved in multiple symptoms, specially in immunosuppressed patients, where a rapid diagnosis of the infection is necessary. Both viruses grow poorly in conventional cell culture, so molecular biology techniques constitute the diagnostic method of choice. Our objective was to design a rapid test based on

real-time amplification (RT-PCR) to detect HHV6A/B and HHV7 genomes, and to check its efficacy in clinical samples.

**Methods:** Two different pairs of primers targeted to the U20 gene from HHV6A/B, and to a non-coding fragment (117250–117350 bp) from HHV7 were designed using Primer Express v.3 software (Applied Biosystems, USA). A RT-PCR was performed with LightCycler FastStart DNA Master<sup>PLUS</sup> SYBR Green I (Roche Diagnostics, Switzerland) according to the manufacturer's protocols. Specificity was determined by melting temperature (T<sub>m</sub>) in positive controls for both viruses (quantitated plasmid dilutions for HHV6A/B, and serial dilutions of positive samples for HHV7). In 60 samples for HHV6A/B, and 38 samples for HHV7, results of RT-PCR were contrasted with the results of the multiplex-nested-PCR performed routinely in the laboratory, using primers directed against the same target genes (see Table 1).

**Results:** RT-PCR detected between 50–500 copies of HHV6A/B DNA, and a 10<sup>-6</sup> dilution of a HHV7-positive clinical sample. The T<sub>m</sub> for HHV6A/B was 80.5°C, and for HHV7 was 84.9°C. In 60 samples assayed for HHV6A/B, RT-PCR was positive in 1 (1.6%), whereas nested-PCR was positive in 8 (13%) (p = 0.03). On the other hand, RT-PCR detected 12 positives of 38 samples assayed (31.5%) for HHV7 DNA, and nested-PCR detected nine positives (23.7%).

	RT-PCR primers	Nested-PCR	
		External primers	Internal primers
HHV6 A/B	5'-GTGTATGCTCAAGCTCTACTCAA-2' 5'-TTCAGATGACATAGACTACAGAT-3'	5'-CRTCTCTCGGATTCCTCA-2' 5'-CTCTARAGACGCTCTCTC-3'	5'-TCTCTGTAAAGAGCTGG-3' 5'-AGAGAGACAGGTTTCTCTC-3'
HHV7	5'-TTCATGTATGATCCGGGCTTT-3' 5'-AACGGTTTACTTCCGCGAATTT-3'	5'-CAGACATCGATGACAGATTTGA-2' 5'-ATTCCGGAAATGATACCAACA-3'	5'-TTCATGTATGATCCGGGCTTT-3' 5'-TAACGGTTTACTTCCGCGAATTT-3'

**Conclusions:** Although the RT-PCR designed for HHV6A/B could detect few DNA copies of a specific plasmid, the poor performance in clinical samples suggest redesigning the conditions of the experiment. RT-PCR protocol for HHV7 is a good alternative to a classic nested-PCR, reducing working time and laboriousness. It also could be possible to simultaneously detect HHV6A/B in a multiplex-PCR with HHV7 because of their different melting temperatures.

#### **P895** Evaluation of Versant<sup>®</sup> HCV RNA 1.0 Assay (kPCR). A new automated kinetic PCR assay for quantification of HCV RNA in plasma

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**Objectives:** To assess performance characteristics of the new VERSANT<sup>®</sup> HCV RNA 1.0 Assay (kPCR) (VERSANT kPCR; Siemens Healthcare Diagnostics) and to compare VERSANT kPCR viral load results with those of the VERSANT HCV RNA 3.0 Assay (bDNA) (VERSANT bDNA; Siemens Healthcare Diagnostics).

**Methods:** Plasma specimens were collected from 451 anti-HCV-positive individuals monitored at two hospitals in Madrid and Barcelona, Spain. Samples having viral loads within the common quantification range of the two assays were aliquoted and stored at -80°C. HCV genotype and subtype were assessed using VERSANT HCV Genotype 2.0 Assay (LiPA). The distribution of HCV genotypes corresponds to the local prevalence. HCV viral loads were tested using VERSANT kPCR and VERSANT bDNA assays, following the manufacturer's instructions. Eight serial dilutions of an HCV concentrated stock provided by Siemens (ranging from 96 969 125 to 48 IU/mL) were used to evaluate precision and linearity. Specificity was evaluated by testing 89 anti-HCV antibody-negative samples from normal blood donors. Statistical analysis was performed using Deming regression and Bland-Altman methods.

**Results:** The slope of the Deming regression for the 256 samples that quantified within the overlapping range of the two assays was 0.95 (R<sup>2</sup> = 0.89) indicating that the two assays have a linear relationship. The Deming regression slopes were 0.98 for Madrid (n = 106), and

0.91 for Barcelona (n = 150). The average log difference between the two assays (kPCR – bDNA) was 0.09 log (95%CI: 0.05–0.14 log). 87.9% of the samples quantified within  $\pm 0.5$  log for the two methods. The mean log difference between assays was also within  $\pm 0.5$  log for all genotypes tested.

A high correlation ( $R^2 = 0.99$ ) was observed between the expected and observed values for quantification of HCV RNA in serial dilutions indicating that the VERSANT kPCR assay has a linear response. Specificity of the VERSANT kPCR assay was 100% (0/89 detected).

**Conclusions:** The VERSANT HCV RNA 1.0 Assay (kPCR) showed excellent specificity, good linearity and genotype inclusivity. This study demonstrated that the VERSANT HCV RNA 1.0 Assay (kPCR) and VERSANT HCV RNA 3.0 Assay (bDNA) have a linear relationship with good concordance. The new VERSANT HCV-1 RNA 1.0 Assay (kPCR) is suitable for monitoring HCV patients following current medical guidelines.

#### **P896** Evaluation of the QIASymphony® RGQ system for the quantification of different BK virus genotypes in biological samples

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**Objectives:** BK virus (BKV) is a ubiquitous human polyomavirus classified into four different genotypes (I to IV) which constitutes an important pathogen among kidney and haematopoietic stem cell transplant recipients. BKV infection monitoring in patients requires the accurate quantification of the BKV genome in biological samples using real-time PCR methods. The aim of this study was to perform a clinical evaluation of the artus® BK Virus QS-RGQ Kit on the QIASymphony® RGQ system.

**Methods:** One hundred and eleven whole blood and 17 urine samples, collected from transplant recipients and stored at  $-80^{\circ}\text{C}$ , were retrospectively selected in order to obtain a broad range of BKV loads. BKV genotype was determined for 71 samples by the sequencing of VP1 gene. BKV load was measured using the QIASymphony® RGQ system according to the manufacturer's instructions (whole blood samples were tested in an off-label capacity). The results were compared to those obtained with the laboratory-developed BKV real-time PCR assay currently used for virological diagnosis.

**Results:** The interassay variation for the four quantitative standards of the kit tested in four consecutive runs was low (CVs ranging from 1.0 to 2.3%). No cross-contamination was observed. The internal control (IC) was detected in all samples apart from seven urines for which BKV load was high (over 7 log). For the 121 remaining samples, the IC mean Ct value (SD) was 26.70 (1.78). For the 128 samples tested, the overall agreement between the laboratory assay and the artus® BKV assay was 96.9%. The four discrepant results corresponded to low BKV loads, below 3 log. The comparison of the 113 paired positive results evidenced a significant correlation (Spearman correlation coefficient: 0.87 [ $p < 0.0001$ ]), and the Bland-Altman analysis showed no significant shift between both assays (bias artus® BKV/laboratory:  $-0.38$  [ $\pm 1.96$  SD:  $-1.35$  to  $0.59$ ]). The comparative analysis of the BKV loads obtained with the QIASymphony® RGQ system evidenced no difference according to BKV genotype, with BKV load differences ranging from  $-0.29$  to  $-0.40$ .

**Conclusion:** This study showed a very good agreement and a high correlation between the artus® BKV and the laboratory-developed assays for whole blood and urine samples. Moreover, the performance of the artus® BKV assay was similar regarding the quantification of all 4 BKV genotypes. In conclusion, the QIASymphony® RGQ system constitutes a suitable and versatile tool in a routine virological laboratory setting.

#### **P897** Comparison of xTAG-Fast respiratory virus panel and CLART PneumoVir for detecting respiratory viruses in nasopharyngeal samples

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**Objective:** Several molecular technologies have been recently developed to detect simultaneously the presence of a broader panel of viruses in infections of lower respiratory tract. We compare xTAG-fast viral respiratory panel (Abbott Molecular diagnostics) based on target-specific primer extension technology and CLART PneumoVir (Zeltia Genomica) based on low density array. This comparison has not been previously analyzed.

**Methods:** Forty nasopharyngeal swabs from hospitalized patients to the Ramon y Cajal Hospital, Madrid, Spain with severe respiratory illness were collected between January to October-2011. The swabs were inoculated into 3 mL of Universal Transport Medium (COPAN Diagnosis, Murrieta, USA) to conserve the sample. Nucleic acids were automatically extracted using NucliSens easyMAG system (Biomerieux, France) from two hundred  $\mu\text{L}$  samples and eluted in 35  $\mu\text{L}$ . These platforms can discriminate between 10 families of viruses. The main differences were CLART was able to discriminate between Enterovirus and Rhinovirus and detect fluA(H1N1)2009 and flu-C. xTAG-fast can detect flu-A (H5N1) and four different strains of coronavirus.

**Results:** Twenty-eight samples lead a positive result at least for one method (71%). Concordance ranged between 76.5% and 42.8% when single and mixed viral infections were compared. The low value of concordance in mixed infection (42.8%) was due to xTAG-fast was not able to detect simultaneously more than two viruses in the same sample. Two samples from ICU patients were negative using xTAG-fast but parainfluenza-4 virus and bocavirus were detected by Clart PneumoVir; moreover parainfluenza-3 virus, respiratory syncytial virus and bocavirus were not detected in mixed infections using xTAG-fast. On the other hand, Clart PneumoVir was not able to detect single infections produced by coronavirus CoV-OC43 and enterovirus/rhinovirus. The xTAG-fast requires around 4 hours whereas Clart PneumoVir takes about 7 hours.

**Conclusions:** Clart PneumoVir seems to be more sensitive to detect multiple viruses in the same sample, although the role of coinfections is not clear. Major differences were observed in the detection of bocavirus and parainfluenza viruses; the first was detected 40% lower with xTAG-fast than Clart PneumoVir and the second was detected 30% lower with xTAG-fast respect to Clart PneumoVir. The observed failures using xTAG-fast are coincident with the target not-approved by US-IVD although they were accepted by CE-IVD Europe.

#### **P898** Evaluation of an automated sample preparation system for detection of human cytomegalovirus in whole blood

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**Objectives:** The VERSANT® Sample Preparation 1.2 (SP1.2) reagents kit\*, which can extract DNA from up to 96 whole blood samples on the automated VERSANT® kPCR Molecular System, was used with the R-gene™ CMV assay to detect human cytomegalovirus (CMV) in whole blood. Analytical performance characteristics of the assay were evaluated.

**Methods:** CMV viral DNA was extracted and captured from panels or patient specimens using VERSANT SP1.2 reagents on the SP module of the VERSANT kPCR Molecular System. The purified DNA target was amplified and detected using the R-gene CMV assay on the Amplification/Detection (AD) Module of the VERSANT kPCR Molecular System. Assay sensitivity, linearity, and precision were determined by testing serial dilution panels prepared with live CMV virus spiked into whole blood. DNA extraction efficiency on the VERSANT SP module using VERSANT SP1.2 reagents was compared

against that of three other automated sample preparation platforms—NucliSens easyMAG (bioMérieux), QIA Symphony (Qiagen), and MagNA Pure LC 2.0 (Roche Diagnostics)—using whole blood samples obtained from patients. A method comparison between the VERSANT kPCR molecular system and the NucliSens easyMAG system was performed using 31 whole blood patient specimens and the R-gene CMV assay.

**Results:** The R-gene CMV assay used with the VERSANT SP1.2 reagents yielded quantitative results for the CMV target in whole blood. Detection limit for CMV was 744 copies/mL with 200 µL whole blood input and 10 µL eluate used in the kPCR reaction. Linearity of the assay was between 2280 and 1E7 copies/mL. Precision results showed a variation of the base-10 logarithm of viral load quantitation of <0.5 at above 1E4 copies/mL. With the R-gene CMV assay as the AD method, the VERSANT SP system in conjunction with the SP1.2 reagents consistently yielded a higher viral load for the same clinical sample than the other three automated sample preparation platforms.

**Conclusion:** Preliminary analytical and clinical data demonstrated that the SP1.2 kit—together with the VERSANT SP module—is an efficient automated method for isolating DNA from whole blood samples. The VERSANT kPCR Molecular System in combination with the VERSANT SP1.2 Reagents kit and the R-gene CMV kit provide an automated method for measuring CMV viral load in whole blood samples.

\*As of November 2011, the VERSANT Sample Preparation 1.2 reagents kit is not yet commercially available. Siemens Reference Number: A91DX-MM-110163-GC1-4A00

#### **P899** Evaluation of the performance of the ProbeTec Qx assay for detection of herpes simplex 1 and 2 using the VIPER platform

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**Objectives:** Molecular assays are routinely used to detect Herpes simplex and are known to be more sensitive than cell culture. Our aim was to determine the performance of the new Becton Dickinson ProbeTec HSV 1 and 2 Qx assay on the VIPER platform in extracted mode. Assay results were compared to cell culture and to an in house HSV 1 and 2 multiplex real time PCR on the BD Max platform. The BD MAX (BD, NJ, USA) is a new flexible platform for performing automated extraction and real time polymerase chain reaction (PCR) on a range of patient specimens.

**Methods:** Six hundred and forty-two virology swabs submitted routinely for herpes simplex 1 and 2 cell culture from patients with genital lesions were included. These were tested by cell culture, anonymised, aliquoted and stored at -80°C until molecular testing was performed. Swabs were expressed in Hanks cell culture media and inoculated onto a monolayer of HEL cells. Cells were examined for a characteristic cytopathic effect (CPE) on a daily basis for a week. Immunofluorescence was performed on all monolayers exhibiting a CPE to differentiate between HSV 1 and HSV 2. Stored aliquots were tested in the BD ProbeTec HSV 1 and 2 Qx assay on the VIPER. Aliquots were then tested using an in house real time multiplex HSV1/HSV 2 PCR assay which has been adapted for use on the BD Max platform

**Results:** 56/642 (8.72%) samples were positive by cell culture for HSV 1 and 103/642 (16.04%) were positive for HSV 2. Using the ProbeTec HSV1 and 2 Qx assay, 73/642 samples tested as positive for HSV 1 and 171/642 tested as positive for HSV 2. The VIPER assay detected 30.4% extra HSV 1 results and 66.0% extra HSV 2 results.

**Conclusions:** The ProbeTec Qx HSV 1 and 2 assay detects more HSV infections than cell culture. Sensitivity and specificity will be determined by comparison to the in house assay using the in house real time multiplex PCR on the BD Max.

#### **P900** Molecular characterisation of clinical enterovirus 71 strains in Greece

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**Objectives:** Six EV-71 strains were recently detected for a first time in Greece in children with hand-foot-and-mouth disease or exanthematous disease with diarrhoea and fever. All these strains were detected within a period of 7 months amongst patients admitted to the same hospital. The present study attempted characterization of almost complete genomic sequences of these strains, in an attempt to elucidate their epidemic association with other EV-71 strains circulating elsewhere in the world and identify possible recombination events which underlie enterovirus evolution and virulence.

**Methods:** RT-PCR assays using primers that specifically amplify the entire genome of all EV-71 strains were employed. Genomic sequences of enteroviruses belonging to Human Enterovirus Species A (HEV-A) were retrieved from GenBank and used for comparison with the partial 5'-UTR, P1, P2 and P3 region sequences of the EV-71 strains obtained during the present study. Multiple alignment of all sequences was made with the aid of ClustalW2 computer software. Genotypic relatedness between the strains identified in the present study and other, previously or currently circulating clinical strains was investigated by construction of the appropriate phylogenetic dendrogram. Finally, all sequences were screened for the presence of recombination events using the SimPlot software.

**Results:** The partial genomic sequences obtained from the six EV-71 strains revealed that they were highly similar (97–99%) to strains isolated from CSF in UK and the Netherlands during 2007 and 2008, belonged to the C2 genetic cluster and were closely related with strains responsible for significant epidemic activity in the Western Pacific Region. No significant genetic recombination events with other HEV-A strains were observed.

**Conclusion:** Molecular characterization of EV-71 strains detected and identified for a first time in Greece revealed the close association of these strains with highly virulent strains that circulated in Europe. Despite the fact that genetic recombination may play a significant role in the evolution of certain genetic groups of EV-71 strains, the lack of such recombination events in the partial sequences of the C2 sub-genogroup strains of the present study comes in accordance with previous studies regarding C1 and C2 sub-genogroups. The complete genomic sequences will have to be obtained first in order to address this issue with certainty.

#### **P901** Performance of the Abbott PLEX-ID viral IC spectrum assay on the PLEX-ID analyser system

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**Objective:** The PLEX-ID Viral IC Spectrum assay is an in vitro test used for the detection and identification of human herpes viruses 1–5 and 8, BK and JC polyomaviruses, parvovirus B19, human enterovirus, and human adenovirus in human plasma. The purpose of these studies was to evaluate the analytical performance of the assay.

**Methods:** The PLEX-ID system couples nucleic acid amplification by PCR and analysis of reaction products by mass spectrometry. Nucleic acids are extracted from plasma samples and amplified by PCR. The reaction products are desalted and then injected into the mass spectrometer for analysis. The base compositions of the products are determined and matched against a reference database to identify any viral nucleic acids in the sample. The Limit of Detection (LOD) of the PLEX-ID Viral IC Spectrum assay was established by analysis of dilute samples (quantified by PCR) of the eleven target organisms. Assay reproducibility was assessed using three assay lots runs over five days, with three different operators using three different instruments. A panel of all 11 target viruses was used. Specificity of the assay was evaluated by testing 100 plasma EDTA specimens from apparently healthy subjects. Sensitivity of the PLEX-ID Viral IC Spectrum assay was

evaluated by testing 133 plasma EDTA specimens with an initial PCR test result positive for 1 of the 11 viruses detected by the assay. All specimens were retested by PCR.

**Results:** LOD: The LOD was defined as the lowest concentration at which  $\geq 95\%$  of replicates tested positive. Reproducibility: At both low (3 X LOD) and moderate (10 X LOD) concentrations, 45/45 (100%) of the sample replicates were detected for each sample virus. Specificity: The specificity rate was 100% (99/99). No viruses were detected in 99 specimens by PLEX-ID. One specimen tested positive by PLEX-ID for Parvovirus B19. This positive result was confirmed by PCR testing and considered a true positive. Sensitivity: The overall sensitivity rate was 94% (123/131 matching results). Thirty-three samples were positive for multiple viruses and further evaluated with additional PCR testing.

**Conclusions:** The PLEX-ID Viral IC Spectrum assay compares favorably to rtPCR detection methods for the viruses studied here. Sensitivity testing results demonstrate the multiplex detection capabilities of the PLEX-ID Viral IC Spectrum assay.

#### **P902** Characterisation of influenza viruses circulating in Latvia in 2010/2011 season

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**Objectives:** To characterise influenza viruses circulating in Latvia in 2010/2011 season comparing to 2009/2010 season and evaluate a likelihood of antiviral resistance and virulence mutations.

**Methods:** Influenza viruses RNA was detected using real-time RT-PCR according to WHO/CDC protocol. Both the neuraminidase (NA) and hemagglutinin (HA) genes were sequenced. Phylogenetic tree was constructed using neighbour-join in MEGA 5. Cell line MDCK was used for isolation of influenza virus strains. Antigenic characterisation based on hemagglutination inhibition test performed using a panel of reference ferret antisera from NIMR, UK. We analyzed 23 sequences of (H1N1)pdm09 viruses from patients hospitalized in 2010/2011 season mostly with pneumonias including severe cases with death (13 cases) and six sequences of B influenza viruses (one lethal case). Sixteen A(H1N1)pdm09 viruses from 2009/2010 season were sequenced in NIMR.

**Results:** All 227 typed A(H1N1)pdm09 viruses from 2010/2011 season showed antigenic similarity to the currently recommended vaccine virus A/California/7/2009. In most (14/16) sequences from 2009/2010 season and in all sequences from 2010/2011 season mutation S203T in HA gen Ca1 antigen region was found. The specific NA gene mutation associated with oseltamivir resistance (H275Y) was not observed in the 2010/2011 nor in the 2009/2010 seasons. Virulence-associated amino acid substitution from aspartic acid to glycine at position 222 (D222G) of the HA1 subunit of HA were detected in two sequences from 2009/2010 season, one of them in fatal case. Other mutation at this amino acid, D222E was found in three sequences, two of them from fatal cases. In 2010/2011 season D222G was observed in one sequence from fatal case and in one sequence other substitution at this position D222N was found. Most of 2010/2011 season circulating A(H1N1)pdm09 viruses belonged to A/StPetersburg/27/2011 group. Influenza B viruses of the B/Victoria/2/87 lineage predominated (164/167) over those of the B/Yamagata/16/88 lineage (3/167) as in other European countries.

**Conclusion:** A(H1N1)pdm09 and B type influenza viruses circulated in Latvia in 2010/2011 season showed the same antigenic profile as viruses circulated in other European countries. The specific NA gene mutation associated with oseltamivir resistance (H275Y) was not found. Virulence-associated amino acid substitution D222G of the HA1 subunit of HA were identified in some sequences from both 2009/2010 and 2010/2011 seasons.

#### **P903** The QIAGEN artus HIV-1 QS-RGQ viral load assay shows high concordance to the Abbott m2000rt HIV-1 viral load and the Roche CAP/CTM HIV-1 test v2.0 assay

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**Objectives:** Quantitative measurement of viral load is one of the major parameters for therapy monitoring in HIV infected patients. The newly available artus HIV-1 QS-RGQ viral load assay provides a complete workflow of sample preparation, PCR setup, amplification and detection as a CE-IVD compliant workflow, whilst remaining highly flexible. As all three platforms employ different target regions for the PCR amplification, with Abbott using Integrase, QIAGEN using LTR and Roche using LTR and gag regions, we analysed clinical routine samples with all three assays in parallel to test for concordance of results. As HIV-1 shows a high genetic diversity we also compared the viral load results from selected non-B-subtype samples between the Abbott and the QIAGEN assay.

**Material and methods:** HIV-1 viral load was determined in 53 routine diagnostic samples using the artus HIV-1 QS-RGQ viral load assay, the Abbott m2000rt HIV-1 viral load assay and the Roche CAP/CTM HIV-1 test v 2.0 assay. All three assays were compared by linear regression analysis and Bland-Altman plotting. In addition to 19 non-B-subtype samples (five subtype A, 3 C, 1 D, 1 F, 1 G, 1 CRF01\_AE, 6 CRF02\_AG, 1 CRF06\_cpx) from clinical routine testing, eight samples from the NIBSC panel were analysed with the QIAGEN and the Abbott assay.

**Results:** Comparison of the QIAGEN assay against the Abbott assay showed a coefficient of determination (R<sup>2</sup>) of 0.9 (QIAGEN vs. Roche also 0.9, Abbott vs. Roche also 0.9). Analysis of Bland-Altman plots showed a slight tendency for higher quantification of 0.2 log<sub>10</sub> c/mL by the QIAGEN and the Roche assays compared to the Abbott assay, while no such difference could be seen between the QIAGEN and the Roche assays. The high coefficient of determination (R<sup>2</sup>) of 0.9 between the QIAGEN assay and Abbott assay could also be confirmed for the non-B subtype samples.

**Conclusions:** All three assays showed a high concordance of results, which could also be confirmed for non-B-subtype viruses for the QIAGEN and the Abbott assay. Although different target regions are used for HIV-1 quantification, standardisation of the assays to the WHO standard material by the manufacturers allows for interchanging these assays in routine diagnostics. The QIAGEN and the Roche assay show a trend for higher quantification compared to the Abbott assay. As the average of the results are only slightly higher there is no risk for falsely classifying these differences as significantly relevant for the patients.

#### **P904** Validation of a real-time multiplex PCR protocol for diagnosis of viral gastroenteritis

*M.J. Hopkins\*, J.A. Booth, N.A. Cunliffe, I.J. Hart (Liverpool, UK)*

**Objectives:** Highly sensitive molecular methods are commonly employed for detection of norovirus in infection control protocols. Molecular techniques are increasingly being used for diagnosis of other causes of viral gastroenteritis, although enzyme immunoassay (EIA) results may correlate better with symptomatic disease. Thus, a cut-off in line with EIA detection limits has been recommended to distinguish sub-clinical rotavirus infection. Here we validate a multiplex PCR for detection of rotavirus, adenovirus, astrovirus and sapovirus which can be used alongside a norovirus PCR to provide a comprehensive viral gastroenteritis screen.

**Methods:** One hundred and thirty-six electron microscopy (EM) screened community stool samples plus 175 paediatric samples previously screened by rotavirus EIA, and a norovirus QCMD EQA panel were included in the evaluation. All samples were blinded and anonymised. Nucleic acid was extracted using the Qiasymphony virus/bacteria kit. Published assays for rotavirus, adenovirus, astrovirus and sapovirus were combined in a single multiplex PCR. An internally controlled norovirus PCR as described previously was run in parallel.

Quantified RNA transcripts were used as positive controls and a PCR targeting VP7 was used to confirm discrepant rotavirus results.

**Results:** The multiplex PCR had detection limits of 4, 45, 5, and 45 RNA copies/ $\mu$ L for rotavirus, adenovirus, astrovirus, and sapovirus, respectively. Low-level RNA could be detected in the presence of higher amounts of the other viral target nucleic acid. Of all 323 samples tested, the multiplex PCR detected rotavirus, adenovirus, astrovirus, and sapovirus nucleic acid in 145, 58, 13, and 13 samples, respectively. Multiple viruses were detected in 60/323 (19%) of samples. No false-negative PCR results were observed in relation to EM and EIA data. One hundred and forty-three of the 145 rotavirus positives were confirmed positive by alternative methods (sensitivity 100%, specificity 98.9%). Receiver operating characteristic analysis identified a cycle threshold cut-off  $\leq 29.07$  for rotavirus EIA positive results in the paediatric samples. A cut-off of 30 was also applied to astrovirus PCR results to avoid false-positives.

**Conclusions:** The multiplex PCR proved a sensitive and specific adjunct to norovirus PCR for detection of additional causes of viral gastroenteritis. A cut-off  $\leq 29$  cycles can be applied to the rotavirus multiplex PCR results if correlation with EIA positive results is required.

**P905** **Cross-sectional study on the performance of quantitative cytomegalovirus (CMV) PCR in stool as a non-invasive diagnostic tool for CMV intestinal disease in comparison to the quantification of CMV-DNA levels in gut biopsies**

*T. Ganzenmueller\*, J. Kluba, J.-U. Becker, C. Schmitt, A. Heim (Hannover, DE)*

**Objectives:** Cytomegalovirus intestinal disease (CMV-ID), a serious complication in immunocompromised patients, is diagnosed by clinical and endoscopic findings and analysing gut biopsies by histopathology and CMV PCR. As endoscopic procedures are rather invasive, CMV PCR from stool samples has been proposed as additional method to identify CMV-ID, but its diagnostic significance has not been studied in detail.

**Methods:** This cross-sectional study included 53 immunocompromised patients with suspected CMV-ID, from whom a lower intestinal tract biopsy and a stool sample was sent to our diagnostic routine laboratory. Biopsy and stool were analysed in parallel by quantitative real time CMV PCR. The aetiology for intestinal disease was classified into "CMV-ID", "Non-CMV" or "unclear" based on thresholds for the CMV-DNA levels in biopsies [quantified as CMV copies/cell by PCR as published previously (J Clin Virol, 2009, 46, pp.254-8)]:  $>0.14$  copies/cell were considered as indicative for CMV-ID,  $>0.01$  as suspicious. Additionally, histopathological, endoscopic and clinical findings were considered to define the diagnosis.

**Results:** 14/53 patients had the diagnosis "CMV-ID" (median 3.96, range 0.05–46.1 CMV copies/cell in biopsies). Thirty-three patients had "Non-CMV" aetiologies of intestinal disease, e.g. graft vs. host disease (3/33 biopsies weakly CMV positive). In 6 "unclear" cases (five CMV positive biopsies; with one exception  $<0.14$  copies/cell) CMV-ID could not be excluded completely.

CMV-DNA was detected in 9/53 stool samples (detection limit of the PCR 500 copies/mL): in 8/14 CMV-ID patients and 1/6 with "unclear" diagnosis. The latter had a negative colon biopsy but subsequently developed CMV duodenitis. None of the 33 patients with "Non-CMV" aetiology had CMV detectable in stool, indicating a very high specificity of the CMV PCR. CMV loads observed in stool samples were relatively low in all cases (range 1000–11 000 copies/mL).

**Conclusions:** CMV-DNA detection from stool samples showed an excellent specificity for diagnosing CMV-ID and might be less prone to sampling errors than biopsies as shown by the CMV duodenitis case. However, compared to analysing biopsies, the sensitivity in stool is low (6/14 CMV-ID cases would have been missed). As the CMV concentrations shed in stool were low, the use of highly sensitive PCR protocols for stool samples requires evaluation. Nevertheless, if

endoscopy is not possible, CMV PCR from stool might be a non-invasive alternative.

**P906** **Aetiology and epidemiology of viral respiratory tract infections in hospitalised and non-hospitalised children using a microarrays platform**

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**Objectives:** To study the etiology of respiratory viral infections (RVI) using a microarrays platform.

**Methods:** Rhinopharyngeal washes were taken from children (1 month–14 years) who were treated as outpatients or hospitalized for upper (URTI) or lower respiratory tract infections (LRTI), from 6/2010 to 6/2011. A microarrays assay (CLART<sup>®</sup> Pneumovir kit – GENOMICA, Spain), that detects 17 different viruses or subtypes simultaneously, was performed to diagnose the etiology of RVI.

**Results:** Samples were taken from 611 children, 299 who were treated as outpatients and 312 who were hospitalized. Two hundred and thirty-five (38%) children were diagnosed with URTI, 320 (53%) with LRTI and 54 (9%) with URTI and LRTI. LRTI was diagnosed more often in hospitalized children and URTI in outpatients ( $p = 0.001$ ). Single infection was found in 228 cases (37.3%), viral coinfections in 169 (25.6%) and no result in 214 (35%). The most prevalent viruses among children with positive samples (397) were Respiratory Syncytial Virus (RSV) in 225 (56.6%) children, Parainfluenza viruses (PIV) in 118 (29.7%), Rhinoviruses (RV) in 73 (18.4%), Influenza viruses (INFL) in 56 (14.1%), Adenoviruses (AD) in 31 (7.8%), Human Bocavirus (HBoV) in 25 (6.3%) and Human Metapneumovirus (HMPV) in 15 (3.7%). No cases of Echovirus infections were detected in our study population. Most common coinfections were RSV-A-RSV-B in 46 (27.2%) children, RSV- INFL in 20 (11.8%), RSV- Rhinovirus in 18 (10.6%), PIV-INFL-RSV in 9 (5.3%), RSV-PIV in 8 (4.7%), RSV-Bocavirus in 5 (3%), PIV-RSV-Adenovirus in 5 (3%). No statistically significant association of viral coinfections with age, LRTI or need of hospitalization was found.

**Conclusions:** Viral coinfections and recently discovered viruses are involved in a significant percentage of acute RVI. Microarray assays could be useful for simultaneous detection of the most common viral respiratory pathogens.

**P908** **Multicentre performance evaluation of the Cobas AmpliPrep/Cobas TaqMan Docking Station**

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**Objective:** Bi-center (University Hospital Virgen de la Arrixaca and University Hospital La Paz, Spain) assessment of reproducibility, limit of detection and workflow of the Cobas AmpliPrep/Cobas TaqMan Docking Station for the viral load quantification of HBV, HCV and HIV-1.

**Methods:** (i) Reproducibility analysis of the system. Analytical standards. OptiQuant HIV-1 and HCV RNA quantification panels and OptiQuant HBV DNA quantification panels (AcroMetrix Corp., Benicia, CA) (Table 1) were used for analytical evaluation of the CAP/CTM DS. (ii) Limit of detection analysis of the system. In this study, we used dilution panels from the international standards recommended by the World Health Organization (WHO). Six serial dilutions were prepared with human plasma/serum for HBV, HCV and HIV-1. (iii) Workflow analysis. Total time required, time required for reporting results, hands-on time and hands-off time were studied.

Statistical analyses: Assay variability was expressed as SD and coefficient of variation (CV), both based on mean log<sub>10</sub>-transformed concentrations. The limit of detection was determined as the 95% probability of obtaining a positive result.

**Results:** (i) Reproducibility analysis: The CAP/CTM DS system was found to be precise within the assay quantitative range of  $2 \times 10^2$  to

2 × 107 UI/mL for HBV (total CV ≤3.36), 5 × 101–5 × 106 UI/mL for HCV (total CV ≤7.02) and 5 × 101–5 × 106 copies/mL for HIV-1 (total CV ≤6.5), which are within the ranges of reproducibility data provided in the respective reagents insert (Table 1). (ii) Sensitivity analysis: The analytical sensitivities of the CAP/CTM DS were also determined to be ≤12.4 UI/mL (95% CI) for HBV, ≤94.02 UI/mL for HCV (95% CI) and ≤26.88 copies/mL (95% CI) for HIV-1 viral load quantification, closely approximating the lower limits of quantification set by the manufacturer of the three assays. (iii) Workflow analysis: The CAP/CTM DS allows emission of 96 results in eight hours.

	N	HBsAg						Anti-HBc						Anti-HBc IgG						
		Sensitivity		Specificity		CV		Sensitivity		Specificity		CV		Sensitivity		Specificity		CV		
		Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	
HBV	200 repeated	4	55.9	104	203	397	645	1120	4533	251	575	407	125	333						
	300 repeated	4	133.75	203	203	655	103	250	1593	4725	558	732	131	44						
	3000 repeated	4	950.1	1563	157	925	136	1875	4659	644	525	107	175							
	30000 repeated	4	3775.8	1563	229	1129	121	136	68003	4812	676	113	107	104						
	300000 repeated	4	46750.8	42525	125	1039	104	105	576003	23623	406	152	106	103						
	3000000 repeated	4	584080.3	385243	413	1593	136	238	8295003	130025	1728	159	118	112						
HCV	50 repeated	5	46.3	378	712	359	108	240	173	338	283	282	122	77						
	500 repeated	6	65.6	1278	1037	644	174	239	37003	5859	1543	685	203	303						
	5000 repeated	6	658.3	20627	3839	875	129	337	49253	4347	961	836	118	115						
	50000 repeated	6	4900.8	38925	1253	1076	112	133	788003	38065	144	109	109	101						
	500000 repeated	4	59395.6	89279	932	1119	116	119	450003	96951	2023	134	122	149						
	5000000 repeated	4	495080.3	50564	846	1736	116	122	5829003	448125	888	154	129	125						
HIV-1	50 repeated	4	41.2	83	192	378	118	449	477	82	133	379	118	460						
	500 repeated	4	362.5	1404	1775	155	162	1162	2303	719	523	54	112	538						
	5000 repeated	4	4962.9	86508	1273	133	133	113	21004	19632	1326	788	125	738						
	50000 repeated	4	28875	110647	477	997	126	505	317003	68825	166	104	118	136						
	500000 repeated	4	380008	242965	1135	1218	112	108	3498003	182374	417	134	141	336						
	5000000 repeated	4	272580.3	1836912	394	1470	104	171	3329003	882925	2543	148	118	136						

**Conclusion:** In this study, we report the results of a two-hospital multicenter evaluation of the CAP/CTM DS assay which yielded very good reproducibility results and an accurate limit of detection for HBV, HCV and HIV-1 viral load quantification. Regarding the new system's workflow, its extraordinary automation and the possibility of 96 results of viral load quantification of the three kinds of virus in an eight-hour workshift are certainly remarkable.

**P909** Frequency of hepatitis D virus infection in HIV-positive and haemodialysis patients in Iran

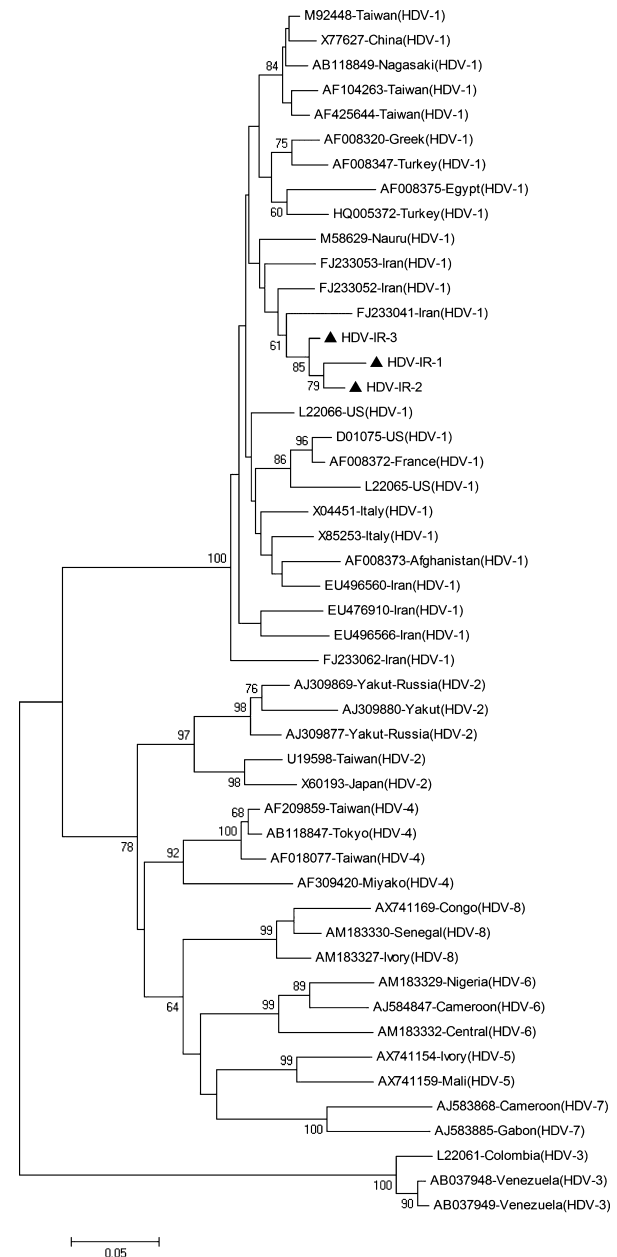
A. Ramezani\*, M. Aghasadeghi, G. Bahramali, A. Aghakhani, M. Banifazl, S. Sadat, M. Foroughi, R. Vahabpour, A. Eslamifaz, S. Siadat, M. Mohraz (Tehran, IR)

**Objectives:** Hepatitis D virus (HDV) is a defective RNA virus dependent on hepatitis B virus (HBV) infection for its replication and expression. It is known that coexistent infection with HDV tends to aggravate the course of HBV-associated liver disease. HDV is a major public health issue in Iran. Studies from different areas of the country show varied prevalence rates. This study was carried out to determine the frequency of HDV infection among HIV positive and hemodialysis patients with HBsAg in Iran.

**Methods:** A total of 720 individuals were enrolled in this study, including 120 hemodialysis (HD) and 600 HIV-infected patients. HBsAg were tested in all subjects. All HBsAg positive cases were evaluated for the presence of anti-HDV antibodies using commercially available enzyme-linked immunosorbent assay kits. Finally anti-HDV and HBsAg positive coinfecting samples were used for HDV active RNA confirmation using nested polymerase chain reaction (PCR). PCR products were sequenced, and the genotype of HDV samples was determined.

**Results:** Out of 120 HD patients, 9 (7.5%) and out of 600 HIV-infected patients 9 (1.5%) were HBsAg positive. Three (33.3%) of HBsAg positive HD samples and 5 (55.5%) of HBsAg positive HIV infected cases, were anti-HDV positive which were then subjected to nested PCR. The amplification results confirmed 3 (37.5%) samples to be HDV-RNA positive. Two of them were HD patients and one was HIV infected case. HDV infection was more common in male than

female patients. All of the HIV-HDV co-infected subjects were intravenous drug users and were co-infected with HCV. Genotypes of HDV samples will be reported in the congress.



**Conclusion:** Our results suggest that the prevalence of HBV/HDV co-infection is relatively high in HIV and HD patients in Iran. Therefore, practitioners and health care managers should be made aware of the risk of dual infection with HBV and HDV especially in high risk patients.

**P910** Molecular epidemiology of non-polio enterovirus circulating in highly endemic areas of central nervous system disease

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**Objectives:** Human enterovirus (HEV) causes asymptomatic infections to a wide range of clinical disease like aseptic meningitis, encephalitis and myocarditis. HEV serotype identification is necessary for their

clinical presentation, emergence of variants and epidemiological surveillance. In northern India, enteroviruses are a significant cause of central nervous system infection presenting either in endemic or epidemic forms. The aim of the present study was identification of enterovirus circulation by molecular method in direct clinical specimen to determine their prevalence in the community.

**Methods:** A total of 320 clinical specimens were collected between January 2009 to December 2010 from children (age <15 years) with suspected enterovirus infection and healthy controls in northern India. Reverse – transcription real time PCR (r-RT-PCR) and semi nested RT-PCR targeting the 5' untranslated region and VP1 region was used for the detection and identification of enterovirus serotypes.

**Results:** HEV genome was detected in 79 (24.7%) of 320 clinical specimens by real time PCR. Central nervous system syndrome (CNS) was the most common clinical manifestations (n = 32, 62.74%) followed by respiratory tract infection (n = 8, 15.69%), acute febrile illness (n = 7, 13.73%) and gastrointestinal disease (n = 4, 7.84%). A total of 32 different serotypes were identified: mainly coxsackievirus (CV) B5 and echovirus (ECV) 6 (11.4% each) followed by CV B3 (6.3%), CV A13, CB6, ECV 3, ECV 20 and ECV 29 (5.1% each), ECV11, ECV24, EV 75 (3.8% each). Phylogenetic analysis of partial VP1 gene sequences from this study showed that many HEV serotypes were showing good similarity with strains from American and European country in comparison to the neighbouring Asian country.

**Conclusions:** High frequency of HEV B species circulation may be an important cause of CNS infection in the children of this region.

#### **P911** A molecular epidemiological survey of viral respiratory tract infections in children

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**Objectives.** Respiratory viruses are known to cause acute respiratory tract infections, including the common cold, bronchiolitis, and pneumonia. The objective of this study was to employ the multiplex Seplex DPO molecular technology (Seegene) to study the epidemiology of respiratory virus infections, the virus-specific positivity rates, and seasonality for respiratory virus infections over a 21 months period in an area of the North East of Italy.

**Methods:** Five hundred and eighty nasopharyngeal specimens were obtained from symptomatic pediatric inpatients between February '10 and October '11. Each specimen was collected with a Copan flocced swab and a liquid universal transport medium. Total nucleic acid was extracted using the easyMAG system (bioMerieux). From February '10 to December '10, 324 samples were assayed by the Seplex RV12, that allows simultaneously detection of Metapneumovirus (MPV), Adenovirus (AD) A/B/C/D/E, Coronavirus (COR) 229E/NL63, Parainfluenza virus (PIV) 1/2/3, Influenza virus A/B (IA/IB), RSV A/B, Rhinovirus (RV) A/B and Coronavirus (COR) OC43. Because Seegene developed a second-generation system, RV15, which allows detection of other additional viruses (Bocavirus 1/2/3/4, Parainfluenza virus 4, RV C, Enterovirus), from January '11 to October '11 respiratory samples were assayed with RV15.

**Results:** Of the 580 specimens tested, 325 (56%) were positive for at least one respiratory virus, including 161 (27.8%) RSV (A or B), 75 (12.9%) RV, 29 (5.0%) PIV, 27 (4.7%) AD, 18 (3.1%) MPV, 17 (2.9%) IA, 14 (2.4%) COR, 9 (1.4%) BOCA, 8 (1.4%) IB and 0/580 Enterovirus. We revealed 22 dual respiratory virus infections (3.8%), and only two triple virus infections. During '11, RV15 allows detection of 8 Bocavirus infections. Most of viruses were distributed across the majority of months, with some peaks: RVs (February/March '10, September/October '10, December 10/March '11, August/September '11), MPV (February/April '10 and December/May '11), PIVs (spring and late summer/autumn). IA/B had an "atypical" distribution during 2010, and a peak from January/March '11, with a prevalence of A/H1N1.

**Conclusion:** RV12 and RV15 increased our understanding of the epidemiology of respiratory viral infections and assist us in the diagnosing the etiology of respiratory tract infections in individual and

in outbreak situation. The high throughput capabilities and potential lower technical requirements afforded by the Seplex system, within easyMAG automated platform, allow a very significant reduction in the turnaround time of viral respiratory tract infections (RTIs) diagnosis.

#### **P912** A novel nucleic acid amplification/signal generation platform with implications as a cost-effective detection system for infectious organisms

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**Objective:** Enzo Biochem set out to develop a low cost, high sensitivity, real-time platform for specific detection of any nucleic acid target.

**Methods:** Primers for the amplification and quantification of HCV RNA were developed as an initial demonstration of utility in infectious disease research. This HCV assay was retrospectively validated against clinical samples.

The Enzo system is designed to amplify a very short target segment which incorporates the primers in close proximity. The primers are manufactured to contain energy transfer dyes such that one primer (eg forward primer) contains an energy donor dye while the other primer (eg reverse primer) contains an energy acceptor dye. When proper amplification occurs the energy transfer dyes are in close proximity and, when exposed to the proper wavelength of light, generate signal that is directly related to the amount of amplicon generated. The Enzo system has very high specificity and low background because the only manner in which signal can be generated is by proper amplicon generation. As such, even after 60 cycles of amplification without target template, there is a lack of any signal generation.

**Results:** The results show a high degree of correlation for the presence or absence of virus as well as the relative quantity of HCV in clinical samples compare to standard clinical methodology. Furthermore, we found a direct correlation between methodologies with smaller sample amount and smaller reaction volumes.

**Conclusion:** We believe this new platform can provide a superior testing methodology through improvement of operational efficiency and reduction of costs. One of the major cost drivers in molecular testing is sample preparation. The Enzo technology requires less sample input and therefore allows paneling or multiplexing of tests per sample preparation. Paneling or multiplexing will reduce the cost of sample preparation per test. Similarly, our technology is highly efficient, requiring less reaction volume and thereby imparting overall cost savings in reagents. We will describe in details the methodology as well as demonstrate its utility in the detection of infectious agents including HCV.

#### **P913** Frequent isolation of Epstein-Barr virus in the intestine of patients with inflammatory bowel disease: a case-control study

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**Background:** Although Epstein-Barr (EBV) infection has been linked to the pathogenesis of inflammatory bowel disease (IBD), the strength of the association is still debatable. The aim of this case-control study was to estimate the frequency of EBV in patients with ulcerative colitis and Crohn's disease and to elucidate the clinical significance of EBV infection in the course of disease.

**Methods:** Ninety four IBD patients-63 with ulcerative colitis (UC) and 31 with Crohn's disease (CD)-and 45 controls with non-inflammatory disease selected from two adult gastrointestinal referral centers in Athens, Greece, were included in the study. The presence of EBV was examined in blood and intestinal tissue samples by molecular assays and comparisons were made among IBD patients and controls. Demographic and clinical characteristics of all participants were also recorded.



**Results:** The mean age of each of the three groups was 47.7 ± 18.3 for patients with UC, 48.1 ± 19.25 for patients with CD and 64.2 ± 14.38 for the control group. UC location was proctitis (n = 6, 9.5%), left-side colitis (n = 36, 57.1%), and pancolitis (n = 21, 33.3%). CD location was small bowel and colon (n = 22, 70.9%), colon only n = 5 (16.1%), and small bowel only n = 4 (12.9%).

The EBV genome in intestine biopsies was detected in 14 (45.2%) CD patients and in 6 (12%) controls (p = 0.001). In blood the EBV genome was detected in 4 (12.9%) CD patients and in 9 (18%) controls (p = 0.3). Regarding UC patients, EBV genome in intestine biopsies was detected in 30 (47.6%) of them and in 6 (12%) controls (p = 0.001). In blood, the EBV genome was detected in 20 (31.7%) UC patients and in 9 (18%) controls (p = 0.07). No differences in demographic and clinical parameters were observed between EBV infected and EBV non-infected patients of both UC and CD. Similarly, no difference in prevalence of EBV genome in blood was indicated among all groups.

**Conclusion:** Higher prevalence of EBV detection in intestinal tissues of IBD patients comparing to healthy controls was observed. In addition, an indication of more frequent EBV detection in blood was established between UC patients and controls. Although a definite causative role of EBV cannot be supported, our findings suggest further investigations on the potential efficacy of antiviral therapy against EBV infection in IBD patients.

**P914** Characterisation of rotavirus circulating in Pakistan

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**Objective:** The objectives are: To identify G and P genotypes based on molecular typing of rotavirus in children with acute gastroenteritis Sequencing and phylogenetic analysis and to the elucidation of rotavirus evolution Comparison of amino acids sequence Genbank sequence submission

**Methods:** Sixty-six samples were characterised for G and P genotype RT-PCR assay. Viral RNA-PAGE was done to determine the electropherotype on a 10% polyacrylamide gel. The stronger amplicon for sequencing is obtained by the full length amplification of VP7, VP8\* and NSP4. Prepared samples were sent to AGRF c/o The Walter and Eliza Hall Institute of Medical Research, Melbourne. Phylogenetic analysis of rotavirus genotypes was performed with MEGA software, version 5. The nucleotide sequence in this study has been deposited in the gene bank database. The accession numbers was assigned for the VP7, VP4 and NSP4 sequences.

**Results:** The 16 samples analysed possessed different electropherotype patterns, each sample contains 11 segments of dsRNA either possessing "short pattern" or "long pattern". Twenty-six samples were genotyped as G1. Prevalence of other genotypes, 2 were genotyped as G2, 8 were

typed as G9, 1 was typed as G3 and. Mixed infection was seen in 16 samples of which 3 were G1/G3, 1 was G1/G8, 7 were G1/G9, 1 was G1/G2/G9, 4 were G1/G3/G9. Twenty-six samples were genotyped as P[8]. Prevalence of other genotypes, 1 was typed as P[4] and 3 were genotyped as P[6]. Mixed infection of P[4] P [8] was seen in 26 samples. The most common genotype combination was G1P[8] at 25.75% followed by mixed infection of G1P[4]P[8] at 9.09%, G9P[4]P[8] at 7.57%, G1/G9P[8] at 6.0%, G1/G3/G9P[8]P[4] at 6.0%, G1/G3P[8]P[4] at 6.0%, G9 P[8] at 4.54%, G1/G9P[8]P [4] at 4.54%, G2P[6] at 3.0%, G12P[8] at 3.0%, G3/G9P[8]P[4] at 1.5%, G3/G9/P[8]P [4] at 1.5%. Sequencing and deduced amino acids analysis of VP4, VP7 and NSP4 were found to be closely related to globally circulated rotavirus strains. Comparison between Faisalabad samples and globally circulating strains identified different amino acids substitutions in variable regions at different positions.

**Conclusion:** The rotavirus strains originating in Faisalabad, Pakistan are globally circulating strains. Some noticeable mutations were detected in regions known to increase pathogenicity, the amino acids substitutions present may have contributed to the emergence of the new strains.

**P915** Development and evaluation of a multiplex real-time assay to detect herpes simplex virus types 1 and 2, and varicella-zoster virus

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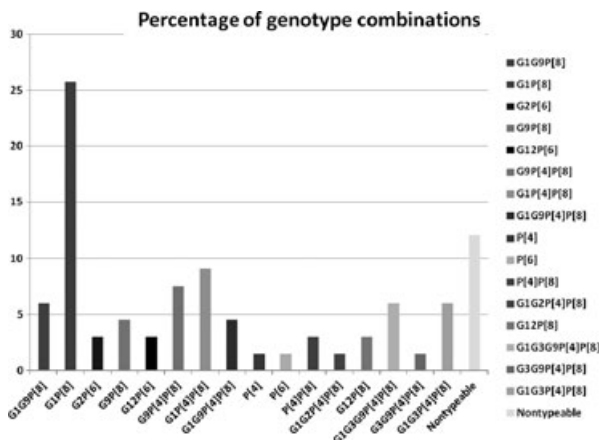
**Introduction:** Herpes simplex virus types 1 and 2 (HSV-1, HSV-2), and Varicella-Zoster virus (VZV) are implicated in multiples types of infections, some of them as important as encephalitis. Real-time PCR is a significant improvement over viral isolation and immunofluorescence for routinely detecting viruses in order to management the infection.

**Objective:** To check the efficacy of a multiplex Real-Time PCR (RT-PCR) designed to detect simultaneously HSV-1, HSV-2, and VZV infections in different types of samples.

**Methods and samples:** primers and labeled-probes against different regions of HSV-1, HSV-2, and VZV were designed using Prime3 software (ABI, USA) (table 1). The assay was developed with Fast- 1-Step Mix (ABI, USA), according manufacturer protocol.

From february, 15th and july 15th, 463 different samples (97 respiratory swabs, 95 genital swabs, 72 skin samples, 18 conjuntival swabs, 135 CNS, and 41 biopsies) belonging to 438 patients were processed by new multiplex RT- PCR, and conventional multiplex nested PCR with primers located in same regions (table 1). In 268 samples, immunofluorescence and viral culture were also performed.

**Results:** Viruses were presented in 109 (24.88%) individuals. In 102 (93.5%) were detected by classical multiplex PCR, and in 98 (89.9%) by new multiplex RT-PCR. HSV-1 was found in 62 samples: 58 (93.5%) by nested-PCR and 52 (83.8%) by RT-PCR. HSV-2 was found in 30 samples: 29 (97%) by nested-PCR, and 30 (100%) by RT-PCR. VZV was detected in 17 samples: 15 (88.2%) by nested-PCR, and 17 (100%) by RT-PCR. In 268 samples which other methods were assayed, genomic amplification (RT-PCR or nested PCR) was positive in 91 (34%), immunofluorescence in 22/250 (8.8%), and culture in 67/265 (25.3%). RT-PCR was positive in seven CNS, and nested-PCR in 3. In the opposite, RT-PCR found viruses in three biopsies and nested-PCR in 8. RT-PCR was able to detect 50 copies in HSV-1, and VZV. Nested-PCR detected 50 copies in HSV-1, and 500 copies in VZV. No quantitative control for HSV-2 was available.



Region	Primer	RT-PCR		nested PCR	
		probe	External primers	Internal primers	
HSV1	GPO	g1g9p[8]	g1g9p[8]f	g1g9p[8]i	g1g9p[8]i
		g1p[8]	g1p[8]f	g1p[8]i	g1p[8]i
		g2p[6]	g2p[6]f	g2p[6]i	g2p[6]i
HSV2	GPO	g1g9p[8]	g1g9p[8]f	g1g9p[8]i	g1g9p[8]i
		g1p[8]	g1p[8]f	g1p[8]i	g1p[8]i
		g2p[6]	g2p[6]f	g2p[6]i	g2p[6]i
VZV	HEE	g1g9p[8]	g1g9p[8]f	g1g9p[8]i	g1g9p[8]i
		g1p[8]	g1p[8]f	g1p[8]i	g1p[8]i

**Conclusions:** Multiplex RT-PCR provides a rapid, sensitive, specific and simultaneous detection of HSV-1, HSV-2, VZV in clinical samples even with a low viral load, making it as a useful tool for diagnosis of these viruses, reducing laborious and time-work consuming.

#### **P916** The effect of interpretation method for quantitation of cytomegalovirus by real-time PCR

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**Objectives:** The real-time PCR using Ct value and standard curve is well established quantitation method assuming the equal efficiency between unknown specimen and standard materials. However, in the presence of inhibitory agents, amplification efficiency difference could cause significant inaccuracy and numerous methods have been proposed to correct this problem. In order to know the actual effect in clinical practice, we compared Ct method with Cy0 and maxRatio method.

**Methods:** A total of 93 batch run data including 610 clinical specimen from Jan 2010 to Sep 2011 are analysed; Each batch includes four quantitation standard material equivalent 10000, 1000, 100, 10 copies/ $\mu$ L and one negative control (ntc). QIAcube with QIAmp blood DNA mini kit (Qiagen) was used for sample preparation and Rotor-Gene Q with Artus CMV RG PCR Kit (Qiagen) was used for real-time PCR experiments. Threshold for Ct method were determined by Rotor-Gene Q – Pure Detection Software version 2.0.2 below 0.05 (AutoCt). Cy0, maxRatio method were analyzed by qpcR package in R version 2.13.1 (R Foundation for Statistical Computing).

**Results:** In analysis of standard material, autoCt method shows best coincidence in the point of mean CMV copies and also showed least CV. In NTC sample results, autoCt method showed 1.72 copies/ $\mu$ L in one sample and negative for other samples; Cy0 method couldn't calculate 12 samples and showed above 1000 copies/ $\mu$ L in 81 samples; MaxRatio method couldn't calculate 11 samples and showed three samples below 100 copies/ $\mu$ L and 79 samples above 20 000 copies/ $\mu$ L, however, in maxRatio method, MR were <0.1 in 80 sample and only two sample were above 0.1 which suggest false elevation. In clinical samples, 25 samples between 10 and 100 copies/ $\mu$ L, seven samples between 100 and 1000 copies/ $\mu$ L, and four samples between 1000 and 10 000 copies/ $\mu$ L in autoCt method coincided with Cy0 and maxRatio method. In 203 samples showing <10 copies/ $\mu$ L in autoCt method, Cy0 method showed one sample above 1000 copies/ $\mu$ L and maxRatio method showed two samples above 10 000 copies/ $\mu$ L with low MR. Efficiency calculated by Cy0 method were between 1.016 and 1.149 in standard samples and seven clinical samples were out of that range.

**Conclusion:** Although Cy0 and maxRatio method could be helpful in samples with reduced efficiency, care must be taken and individual amplification curve or supplementary index should be examined for proper evaluation especially when interpret low concentration samples.

#### **P917** Outbreak of acute enterovirus intestinal infection in Sakhalin region in August 2010

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**Introduction:** Enterovirus diarrhea (gastroenteritis) is an acute febrile illness with lesions of the gastrointestinal tract. Outbreaks of enterovirus gastroenteritis usually are local. Large epidemics occur extremely rare.

**Objectives:** To investigate cases of acute intestinal infections in the Sakhalin region in August 2010, the identification of the causative agent.

**Methods:** We analyzed 102 samples of faeces received from the Sakhalin region from patients with acute intestinal infection. Isolation of RNA/DNA from the test material was carried out using a reagent kit "Ribo-Sorb" (AmpliSens, Russia), reverse transcription reactions –

using the reagent kit "Reverte-L" (AmpliSens, Russia). All samples were analyzed for the presence of adeno-, noro-, astro-, rota-, enteroviruses, *Yersinia enterocolitica* and *Listeria monocytogenes* sets of (AmpliSens, Russia). All samples were additionally tested for enterovirus by PCR with primers for genotyping on the 5'UTR (Demina A.V. et al., 2011) Determination of nucleotide sequences was performed using an automatic sequencer ABI Prism 3130xl. The nucleotide sequences were analyzed using Lasergene 7 and compared with nucleotide sequences database GenBank.

**Results:** The study showed that in these clinical samples, genetic material (viral RNA or DNA), adeno-, noro-, astro-, rotavirus, as well as DNA *Yersinia enterocolitica* and *Listeria monocytogenes* is absent. In 93 fecal samples there were detected RNA enteroviruses. After determining the nucleotide sequences of DNA fragments in the positive samples there were identified enteroviruses: Coxsackie A2–42 samples (45%), Coxsackie A4–31 sample (34%), Enterovirus 71 – six samples (6.5%), coxsackievirus B5 – six samples (6.5%), coxsackievirus B3 – four samples (4%) and Coxsackie B1 – four samples (4%). Phylogenetic analysis showed that the closest prototype of nucleotide sequences of these genotypes were previously identified in Japan (AB126199, AB126200, AB550333), China (GU109481) and Korea (AY875692) in 2000–2010.

**Conclusion:** A molecular epidemiological investigation of outbreak of acute intestinal infection in the Sakhalin region in August 2010, the etiologic agents that caused diseases, are enteroviruses of different serotypes. Specific nucleotide sequences (fragments of the 5'UTR) were deposited by us in GenBank (23.08.2011: JN603367 – JN603368).

#### **P918** Usefulness of herpes consensus PCR methodology in the diagnosis of herpesvirus ocular infections

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**Objectives:** Herpetic eye diseases exhibit various clinical manifestations making a diagnosis difficult in some patients. Molecular techniques are most useful in such instances for an accurate and rapid diagnosis since conventional methods are less sensitive. The purpose of this study was to assess the usefulness of simultaneously amplifying herpeviruses DNA in various ocular samples by using a multiplex polymerase chain reaction (PCR) assay.

**Methods:** A total of 278 samples (aqueous fluid-160, eye swabs and tears-102, corneal scrapings-9, vitreous fluid-7) from consecutive patients with keratoconjunctivitis, hypertensive iridocyclitis, retinitis and uveitis were tested using the Herpes Consensus PCR methodology which is based on gene amplification to search for the six main human viruses: herpes simplex virus 1 and 2 (HSV-1 and HSV-2), varicella-zoster virus (VZV), cytomegalovirus (CMV), Epstein–Barr (EBV) and human herpesvirus 6 (HHV-6).

**Results:** Thirty-five out of 278 ocular specimens (13%) tested positive and most of them were obtained from adults. HSV-1 DNA was detected in majority of the clinical samples (80%), most frequent being eye swabs (60.7%) and aqueous fluid (32.2%). Comparatively, HSV-2, VZV and CMV infections were detected in small number of specimens (2.86%, 14.28% and 2.86% respectively). No sample was positive for EBV and HHV-6 DNA or more than one herpesvirus. The patients with detectable HSV-1 and VZV DNA in aqueous fluid experienced in their majority (eight out of nine and three out of five respectively) hypertensive iridocyclitis. In four patients with acute retinal necrosis syndrome (ARNS), VZV was detected in aqueous tap in two cases, HSV-1 in one case and HSV-2 in one case.

**Conclusion:** According to our experience, Herpes Consensus PCR assay can be useful to facilitate the routine diagnosis of herpetic ocular infections- especially in difficult clinical cases as hypertensive iridocyclitis or ARNS- within a single assay, thereby allowing earlier and prompt administration of a specific antiviral treatment and better clinical management.

**P919** Evaluation of suitability of various novel swab devices for the molecular detection of influenza A from surveillance samples in France

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In France, the influenza surveillance in the general population is carried out with the GROG network that includes general practitioners and paediatricians. These practitioners collect clinical samples (nasal swabs) with Sigma-virocult®. The samples are sent to the laboratory by post with an average delay of 4 days.

**Objective:** In this study, we compared the performance of four different swab collection devices over 4 days for the detection of the pandemic A/California/7/2009 virus using an in-house RT-PCR technique. The devices used were Sigma-Virocult® with polyurethane foam bud swab (SVP), Sigma-Virocult® with Hydraflock Swab (SVH), Sigma-VCM™ with Hydraflock Swab (VCH) and Copan UTM® with Flocked Swab (CU).

**Methods:** Briefly, a titrated suspension of influenza A(H1N1pdm)09 prepared on MDCK cells was used as infectious material to be seeded on different swabs. In this protocol, 10-fold dilutions of the virus suspension (ranging from 10.3 to 10.5) were seeded and subsequently tested at Day 0 and Day 4 post-seeding by RT-PCR. For the latter point, seeded swabs were stored at room temperature, to mimic postal transportation of a GROG specimen.

**Results:** The results showed a high stability of the detection of the virus by RT-PCR in the 4 transport devices up to 4 days. The performance of the foam bud device (SVP) is similar to the 3 hydraflock swabs whatever the concentration of the virus.

**Conclusion:** From this study, we confirm that both foam and flocked swabs are suitable for molecular diagnostic of pandemic influenza from surveillance samples.

**P920** Association of mutation in HCV core region and response to interferon therapy in patients from Rawalpind, Pakistan

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**Objectives:** The global prevalence of Hepatitis C Virus (HCV) infection corresponds to about 130 million HCV positive patients worldwide. The only drug that effectively reduces viral load is interferon-alpha (IFN-alpha) and currently combination of IFN and ribavirin is the choice for treatment. HCV variants with mutations within the core protein appeared to be more sensitive to therapy, suggesting that core protein played a role in conferring IFN-resistance. The present study is aimed to resolve the genotypes that affect the response to interferon therapy.

**Methodology:** To achieve the objectives seven HCV ELISA positive subjects who have completed IFN treatment were randomly selected. All patients received injections of pegylated-IFN-alpha once each week plus oral ribavirin daily for 48 weeks. Nested RT-PCR was performed after reverse transcribing the viral RNA. Sequencing of the purified PCR samples was carried out to assign the genotypes; core region was the target site for direct sequencing. Neighbor Joining (NJ) method was used to study the evolutionary relationship among the studied samples and HCV strains reported from different geographical regions of the world.

**Results:** Four different genotypes were revealed out of which two samples could not be typed. Two new genotypes have been reported for the first time: 4a from sub urban region of Rawalpindi and 6e from all over the Pakistan in addition to HCV genotype 3 (3a and 3b).

**Conclusion:** The emergence of new genotypes, untypable genotypes and new quasispecies is due to mutations that emerged during therapy due to drugs resistance. Rapid emergence of new resistant strains/quasi-species occurs in a population of viruses already present, allowing the new/resistant virus to become the leading strain, therefore, further understanding of the complex mechanism involved in disrupting the

viral response to therapy would facilitate the development of more effective therapeutic regimens.

**P921** Molecular detection and identification of enteroviruses and parechoviruses in hospitalised children

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**Objectives:** The present study attempted the prospective molecular detection and identification of human enteroviruses and parechoviruses in children admitted to a university hospital with signs and symptoms suggestive of enterovirus or parechovirus infection, as well as the investigation of their possible epidemic association with virulent strains.

**Methods:** During a period of 3 years, 220 consecutive samples [189 stool, 20 cerebrospinal fluid (CSF), 7 whole blood, 2 throat swabs and 2 vesicular fluid swabs] were collected from 188 patients. Clinical presentation included febrile illness variably accompanied by gastrointestinal disorders, possible viral meningitis, exanthematous disease and hand-foot-and-mouth disease. An one-step Real-Time RT-PCR protocol targeting the 5-UTR was used as a screening method for rapid, simultaneous detection of enteroviruses and parechoviruses. Genotypic identification of the different serotypes was performed by sequencing of the VP1-coding region.

**Results:** Twenty-seven enterovirus strains were detected and identified in 25 stool samples, one CSF, one whole blood sample, one throat swab and two vesicular fluid swabs from 27 out of 188 examined patients (14.4%). In contrast, only two parechoviruses were detected in stool samples from two different patients, which were identified as human parechovirus type 1 (HPeV-1) and HPeV-3 respectively. Fourteen enterovirus strains (eight serotypes) belonged to Human Enterovirus Species B (HEV-B) and 13 (five serotypes) to HEV-A. HPeV-1, coxsackieviruses A2, A4, A6, A10 and particularly, the emerging enterovirus 71 and the potentially neurovirulent for neonates HPeV-3 were detected for the first time in Greece. All enterovirus 71 strains were detected within a period of 7 months, indicating a possible epidemic outbreak in the community. Phylogenetic analysis confirmed that most of the strains were closely associated with virulent strains circulating in Europe and elsewhere, but not with strains identified in Greece during the previous years.

**Conclusion:** The present study showed the simultaneous circulation of different enterovirus serotypes and elucidated the epidemic association of these strains with other virulent strains that circulate globally. Most notably, detection of enterovirus 71 for the first time in Greece is particularly alarming and further emphasizes the significance of applying informative molecular assays to improve surveillance for emerging strains with increased virulence.

**P922** Molecular epidemiology of human rhinovirus in general population in Catalonia during 2010

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**Objective:** To determine the prevalence of infections by the three human rhinovirus (HRV) species in general population with upper respiratory disease in Catalonia (Spain) during 2010.

**Methods:** Nasopharyngeal specimens were collected from patients with mild upper respiratory tract infection through sentinel surveillance network in Catalonia during last year. HRV and the most common human pathogenic respiratory viruses were detected using two nested-PCR. In laboratory-confirmed samples of single HRV infection, nucleotide VP4/2 region was used for phylogenetic analysis to establish the HRV specie (HRV-A, HRV-B or HRV-C).

**Results:** HRV infection was laboratory-confirmed in 241 (24%) samples out of 1009 respiratory specimens collected during 2010, of which 188 (78%) positive samples were in single infection. The prevalence of rhinovirus in general population in Catalonia was high

during late winter (weeks 3–10) and early autumn (weeks 41–48). The incidence was especially high in children between 2 and 6 years old. Phylogenetic analysis of nucleotide sequences VP4/2 region obtained from 154 (82%) HRV positive samples identified: 94 (61%) HRV-A, 19 (12%) HRV-B and 41 (27%) HRV-C.

**Conclusion:** High prevalence of HRV infection and co-circulation of the three HRV (A, B and C) species in general population with mild upper respiratory illness in Catalonia was shown. Interestingly highest incidence of HRV was observed in youngest children. HRV-A was the most predominant rhinovirus specie. Better acknowledgment of molecular epidemiology of circulating HRV among general population in Catalonia is good for further studies in hospitalized population.

#### **P923** Use of real-time PCR for the detection of EBV in rare clinical manifestations

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**Objectives:** Herpesviruses may cause wide range of infections which manifest with various and often non-specific symptoms and signs, because specific therapy is available in certain cases, rapid and accurate diagnosis is essential. In our laboratory, real-time quantitative PCRs are generally used for the detection of CMV, HSV-1/2, and EBV in immunocompromised patients and in patients with central nervous system infections. Our aims were to retrospectively analyze the results of real-time EBV PCR and to show three, rare clinical presentations of EBV infection.

**Methods:** We analyzed the results of real-time EBV PCR (artus<sup>®</sup> EBV LC PCR; Qiagen) given during the last 36 months. Clinical specimens (plasma, cerebrospinal fluid, biopsy, urine and other specimens) were sent to the laboratory from mainly inpatients treated in the local university hospitals.

**Results:** During 36 months, 349 specimens were set up for EBV real-time PCR from 273 patients. Thirty-four samples collected from 20 patients proved to be positive for EBV. Positive PCR results were given in the case of 16 plasma, six biopsy, nine CSF and three other specimens. Three unusual manifestations of EBV infection were selected from positive cases. In one patient after 4 years of renal transplantation, on the basis of brain MRI, and histological examination the possibility of PTLD has been arisen, this was confirmed by EBV PCR positivity from brain biopsy, CSF and plasma specimens. The patient was successfully treated with ganciclovir, rituximab, mycophenolate acid, and everolimus and lesions in the brain on MRI were completely resolved. The second case was an EBV-associated oesophagitis in an immunocompetent patient, in this case after acyclovir treatment, the patient were symptom-free for 1 year until 1 month ago that he developed relapse. In the case of a 15-year-old girl real-time EBV PCR and serology revealed cerebellitis with concomitant hydrocephalus. Treatment consisted of acyclovir, mannitol, furosemide and ceftriaxone and ventriculostomy. Nine days after ventriculostomy catheter insertion control MRI showed regression and the catheter was removed.

**Discussion:** Because of the importance of early recognition of PTLD in transplant patients, and rare manifestations of EBV infection in some patients, besides serology the use of molecular methods may give rapid and reliable results to set up the clinical diagnosis.

#### **P924** Evaluation of GeneXpert enterovirus assay for detection of enteroviral RNA in paediatric cerebral spinal fluid

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**Background:** Traditional viral culture for detection of enteroviruses in cerebral spinal fluid (CSF) is time-consuming and less sensitive when compared with clinical impression. The molecular assay directly detects enteroviral RNA in CSF may help physicians to act in a timely manner especially when encountering a catastrophic condition.

**Objectives:** The Cepheid GeneXpert EV assay is a commercial and in-vitro diagnostic reagent performed on an automated GeneXpert system. This study was to evaluate the performance of GeneXpert enterovirus (GEV) assay for detection of enteroviral RNA in pediatric CSF.

**Methods:**

CSF specimens submitted to viral culture that obtained from unrelated pediatric patients under clinical impression of enteroviral infection with CNS involvement were investigated. After inoculation, CSF was appropriately frozen until analysis for enteroviral RNA using GEV assay. The results were compared and sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated.

**Results:** A total of 42 CSF specimens were enrolled. Ten and 32 were positive and negative for CSF culture, respectively. Among 32 patients having negative CSF culture, 20 had a positive throat/rectal culture for enteroviruses in the same course. When GEV assay was compared with CSF culture, the sensitivity and specificity for GEV assay were 90.0% (9/10) and 87.5% (28/32), respectively. When adding other culture results in addition to CSF culture as laboratory evidence of enteroviral infection, PPV and NPV of GEV assay for diagnosis of CNS enteroviral infection were 92.3% (12/13) and 96.6% (28/29), respectively.

**Conclusions:** The GEV assay was demonstrated as a useful tool for its good performance for rapid detection (2.5 hours) of enteroviral RNA in CSF of pediatric patients under impression of enteroviral infection with CNS involvement.

#### **P925** A clinical laboratory evaluation of the Luminex xTAG respiratory viral panel FAST version 2.0

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The Luminex xTAG respiratory viral panel FAST version 2.0 (RVP FASTv2.0) is an updated assay which detects 19 human respiratory virus strains per single well test, including identification of the 2009 Pandemic H1N1 Influenza strain. This assay was run on new MAGPIX platform. We studied RVP FAST 2.0 efficacy in detecting respiratory viruses from 121 Nasopharyngeal Aspirates (NPA) samples collected from patients with acute respiratory infection during the winter 2010–2011 season and compared RVP FASTv2.0 results to those obtained with the RVP FASTv1.0. Considering the 2009 pandemic Influenza strain RVP FAST v1.0 detected 41 of 48 isolates as matrix positive, whilst RVP FASTv2.0 detected all 42 as matrix positive and Hemagglutinin (HA) positive with three other isolates as HA positive but with no matrix signal. A secondary real-time PCR was used to confirm all pandemic positive results. Findings for all other respiratory viruses indicate equal sensitivity and specificity between the two assay formulations using our clinical specimens.

In addition, a significant difference between the assays occurred when the QCMD RSV 2011 panel for was tested. The RVP FASTv2.0 assay comprehensively detects all the clinically significant viruses in a single measurement making it a useful application for detecting respiratory tract viral infections. Use of RVP FAST v2.0 would be a significant advantage over RVP FAST v1.0 in simplifying and organising laboratory workflow and increasing speed to result over current reflex testing of untypable influenza positives.

#### **P926** Evaluation of three molecular assays for detection of respiratory virus

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**Objective:** To evaluate three molecular methods for detection of respiratory viruses. After comparison of different kits, to implement a molecular method suitable for routine laboratory analysis, evaluating cost, speed and ease of technical implementation.

**Methods:** For comparison purposes, we studied 58 samples (nasopharyngeal lavage) collected between July 2009 and June 2010,

mostly from children under the age of five, with respiratory disease. Results were compared with IF (Biotrin<sup>®</sup>, Diagnostics Hibrids<sup>®</sup>). The molecular analysis included nucleic acid extraction, amplification and detection of respiratory viruses by three different kits: RV15 ACE Detection, Seegene; Pneumovir CLART<sup>®</sup>, Genomica, Magicplex<sup>™</sup>RVPanelReal-TimeTest, Seegene.

After implementation of Magicplex<sup>™</sup> RV Panel in laboratory routine, 263 samples were studied between February and August 2011, mostly from Pediatric ward and emergency.

**Results:** Considering only the virus detected by IF and PCR, positivity rate was 26% and 64% respectively. In the comparative molecular study, the number of viruses detected by the three techniques was different (RV15-68; Pneumovir-76; Magicplex-87), the most commonly detected virus was RhV (RV15-34%, n = 23; Pneumovir-28%, n = 21; Magicplex-33%, n = 29) followed by VSR (RV15-28%, n = 19; Pneumovir-25%, n = 19; Magicplex-26%, n = 23). The three techniques allowed identification of co-detections (RV15-17; Pneumovir-19; Magicplex-21) the most common being the association AdV/RhV (Pneumovir 38%, n = 5; Magicplex 33%, n = 4) and VSR/RhV (RV15-43%, n = 6).

After implementation of Magicplex<sup>™</sup> RV Panel, of 263 samples studied, 210 were positive and 53 negative for the detection of respiratory viruses. The most commonly detected virus was AdV (57%, n = 120) followed by RhV (35%, n = 74). In 90 double detections, the most frequent association was AdV/RSV (22%, n = 20).

**Conclusion:** The comparative study demonstrated the superior sensitivity of molecular methods, which lead us to choose the kit Magicplex<sup>™</sup> RV Panel Real-Time Test, since it showed a better cost/benefit ratio, as well as better suitability for laboratory implementation. The results after 6 months of implementation in the routine laboratory, revealed a high number of co-detections, as well as the difficulty in clinical interpretation of these results. Thus, the use of complementary methodologies, as well as viral load quantitation, may be the next steps in completing this work.

#### **P927** Clinical performance of the xTAG<sup>®</sup> respiratory viral panel FAST v2 with subtyping capability of influenza A 2009 H1N1 non-seasonal variant

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**Objectives:** Since the 2009 pandemic, FluA 2009H1N1 strain has become a predominant strain of influenza A H1 virus worldwide. The xTAG<sup>®</sup> Respiratory Viral Panel FAST v2 (RVP FAST v2) is a qualitative nucleic acid multiplex test intended for the simultaneous detection and identification of multiple respiratory viruses including the 2009H1N1 strain in NP swabs, nasal aspirates and BALs from individuals suspected of respiratory tract infections. The viruses and subtypes detected by the assay are: FluA, FluA subtype H1, FluA 2009H1N1 (pandemic variant), FluA subtype H3, FluB, RSV, hMPV, HRV, Adenovirus, HPIV-1, HPIV-2, HPIV-3, HPIV-4, HCoV-229E, HCoV-OC43, HCoV-HKU1, HCoV-NL63, and HBoV. Here we describe the clinical performance of RVP FAST v2 for all viral targets probed by the assay.

**Methods:** Diagnostic accuracy of RVP FAST v2 for FluA 2009H1N1 was evaluated using clinical specimens that tested positive or negative for FluA 2009H1N1 by real-time RT-PCR assay or bidirectional sequencing. Clinical performance of RVP FAST v2 for all other targets was evaluated using clinical specimens that tested positive or negative by xTAG<sup>®</sup> RVP FAST. Clinical specimens were collected from multiple sites in the US, Canada and China. All specimens tested were run on both the Luminex<sup>®</sup> 100/200 and MAGPIX<sup>®</sup> systems.

**Results:** A total of 179 clinical specimens were tested to establish the diagnostic sensitivity and specificity of RVP FAST v2 for FluA 2009H1N1. Diagnostic sensitivity and specificity of RVP FAST v2 for FluA 2009H1N1 were 98.10% (95% CI: 93.3–99.8%) and 100% (95% CI: 95.1–100%) respectively. A total of 329 clinical specimens were tested by both RVP FAST and RVP FAST v2 in order to assess

equivalence for analytes probed by both assays, all of which were represented in this sample set. Overall agreement between the two assays for common analytes was 100% for positive calls (95% CI: 99.0–100%) and 99.1% (95% CI: 98.8–99.3%) for negative calls. Results were comparable between the Luminex<sup>®</sup> 100/200 and MAGPIX<sup>®</sup> systems.

**Conclusion:** This evaluation shows that RVP FAST and RVP FAST v2 are substantially equivalent in terms of clinical performance and that RVP FAST v2 is well suited for diagnosis of respiratory viruses including subtyping of FluA 2009H1N1 non-seasonal variant. Additionally the study demonstrated clinical utility of the MAGPIX<sup>®</sup> system for molecular diagnostic tests used as an aid in the diagnosis of respiratory tract infections.

#### **P928** Evaluation of a new real-time PCR for the detection of HSV1, -2 and VZV in CSF and lesion scrapings

*A.K Petrich\*, F. Gharabaghi, S.E. Richardson (Toronto, CA)*

**Objectives:** Nucleic acid amplification testing has greatly facilitated the detection of herpes simplex viruses (HSV-1, -2) and varicella-zoster virus (VZV) in CSF. The goal of this study was to evaluate a new multiplex real-time PCR assay that detects and identifies HSV1 and -2 and VZV (Altona Diagnostics, Hamburg, Germany) in CSF and lesion scrapings.

**Methods:** Ten-fold dilutions of culture lysates of HSV-1, -2, and VZV were used to spike pooled CSF and CSF shunt fluid. Five hundred microlitre of mock sample was extracted into 110 µL using the easyMag (biomérieux, France). Reactions were run in triplicate near the detection limit to compare the sensitivity of two conventional in-house PCR assays (HSV-1/2 and VZV, modified from Johnson et al., 2000) and the RealStar<sup>®</sup> alpha Herpesvirus real-time PCR assay (Altona Diagnostics). Ten microlitre of extracted nucleic acid was added to each reaction mix. Detection of the conventional PCR products was performed by gel electrophoresis with visualization using ethidium bromide. Fifty nine left-over CSF specimens (100 µL) from pediatric and adult patients were extracted and tested using the in-house conventional PCR assays and the real-time assay (19 pos. and 40 neg). Discordant results were repeated with the conventional and real-time assays. The real-time PCR assay was also evaluated with material from 22 known positive lesion scrapings extracted using a manual guanidine thiocyanate method and compared to the conventional PCR (modification of Johnson et al., 2000) for detection and characterization of the three viruses.

**Results:** The RealStar<sup>®</sup> alpha Herpesvirus assay was significantly more sensitive than the conventional PCR for all three targets; HSV1 (2–3 logs difference), HSV2 (1–2 logs difference), and VZV (2–3 logs difference) using end-point analysis on mock CSF and CSF shunt fluid samples. The RealStar<sup>®</sup> alpha Herpesvirus assay detected 19/19 (sensitivity 100%) known positive clinical CSF specimens and was negative for 40/40 known negatives (specificity 100%) For the lesion scrapings, both assays detected and identified 6 VZV, 12 HSV-1 and 4 HSV2 in 22 known positive lesion scrapings.

**Conclusion:** The new RealStar<sup>®</sup> alpha Herpesvirus PCR kit is a multiplex assay capable of detecting and characterizing HSV-1, HSV-2 and VZV in CSF and lesion scrapings with high sensitivity and specificity. The assay was 1–3 logs more sensitive for all targets in mock CSF and shunt specimens than conventional PCR.

#### **P929** Multicentre performance evaluation of the VERSANT<sup>®</sup> HCV RNA 1.0 Assay (kPCR) in quantitative detection of hepatitis C viral RNA

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**Objectives:** The VERSANT HCV RNA 1.0 Assay (kPCR)\*\* (VERSANT assay) is a reverse transcription, kinetic polymerase chain reaction (kPCR) method for quantifying hepatitis C virus (HCV) RNA in human plasma and serum, using the VERSANT kPCR Molecular

System (System). This system combines a fully automated sample preparation module and a fully automated amplification and detection module. This study examined the performance characteristics of the assay, including a comparison between the VERSANT Assay and the Abbott RealTime HCV assay (Abbott assay).

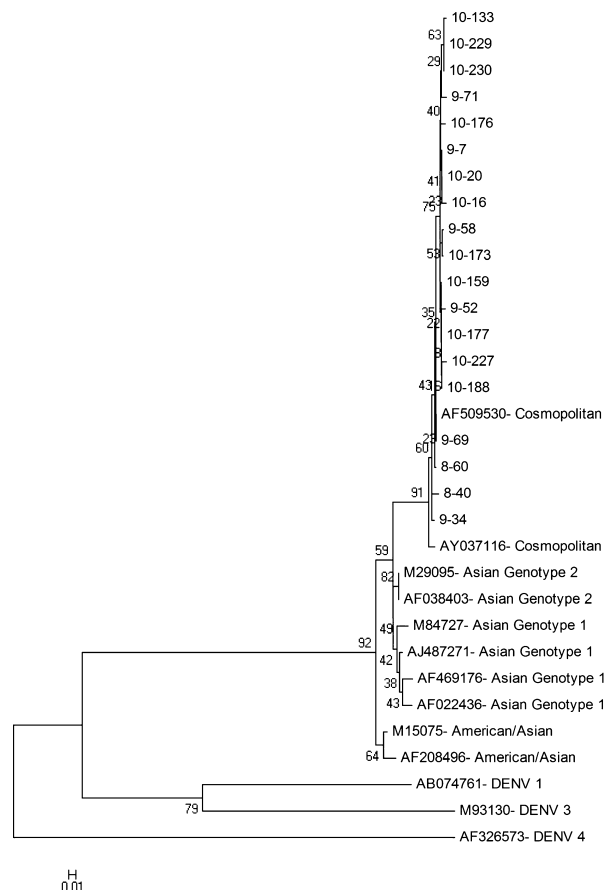
**Methods:** Assay performance characteristics were evaluated at three laboratory sites, using two assay reagent lots and six Systems. Assay specificity was assessed using plasma and serum samples from 1054 HCV-seronegative individuals. A 14-member panel prepared from high-titer HCV RNA samples or HCV Armored-RNA, diluted in defibrinated human plasma basepool, was used to determine reproducibility (precision), linearity, accuracy, quantification range and analytical sensitivity (LoD). Panel concentrations range from 5 to 139 711 676 IU/mL. The VERSANT and Abbott assays were compared using 243 paired HCV RNA-positive clinical samples across the quantification range, with HCV genotypes 1 to 6 represented.

**Results:** The overall assay specificity was 100%. The estimated limit of detection (LoD) was 10.5 IU/mL, and the claimed LoD was 15 IU/mL. The claimed lower and upper limits of quantification were 15 IU/mL (LLOQ) and  $10^8$  IU/mL (ULOQ). Within this range, the assay showed good reproducibility (total log SD range: 0.10–0.37), linearity (log observed = 0.97 log expected + 0.20), and accuracy (log recovery range: -0.1 to 0.2). Deming regression analysis of log quantitative results obtained from VERSANT vs. Abbott assay showed good correlation ( $y = 1.1 \times -0.28$ ;  $R = 0.93$ ). On average VERSANT assay quantified 0.24 log IU/mL higher than Abbott assay, with 95% of the differences falling within the range of -0.51 to 0.99 log IU/mL.

**Conclusions:** The VERSANT HCV RNA 1.0 Assay using the VERSANT kPCR Molecular System is a reliable and accurate assay for the quantification of HCV RNA in plasma and serum from HCV infected individuals.

VERSANT is a registered trademark of Siemens and all other trademarks are the property of their respective owners.

\*\*CE marked in Europe; not available in the US.



### P930 Molecular genotyping of dengue serotype 2 in the Philippines

R. Destura\*, J.A. Petronio, R. Vinarao, K.M. Flores (Manila, PH)

**Objectives:** Dengue fever and dengue haemorrhagic fever (DF/DHF) have emerged as the most important arboviral diseases of mankind. The characterization of circulating dengue virus serotypes is important in surveillance, since the introduction of a new variant to areas affected by pre-existing serotypes constitutes a risk factor for DHF and Dengue Shock Syndrome (DSS). This study aims to determine the genotype of Dengue Virus (DENV) 2 using the capsid-premembrane (C-prM) gene junction sequence of isolates during the 2008–2010 outbreaks.

**Methodology:** De-identified serum samples positive for dengue infections were obtained from a tertiary hospital during outbreaks from 2008 to early 2010. Dengue virus serotyping was carried out by heminested RT-PCR targeting the C-prM gene junction. DNA sequencing was performed using an ABI PRISM BigDye<sup>®</sup> Terminator v1.1 Cycle Sequencing Kit on an automated sequencer. The C-prM gene was sequenced using the primers, mD1 and D2. Sequence alignments were performed using the ClustalW function of MEGA 4 (Molecular Evolutionary Genetics Analysis) software. Comparisons were made with DENV-2 reference strains. Phylogenetic trees were constructed by the neighbour-joining (NJ) method and reliability of neighbour-joined trees was estimated by bootstrap analysis.

**Results:** Nineteen DENV 2 were identified from nucleotide sequences generated from the C-prM gene junction and compared with ten other DENV 2 isolates with known genotypes. Alignments revealed eight point mutations distributed in different isolates, five of which were missense. Analysis using Maximum Composite Likelihood method showed that the number of substitutions per site of the nineteen isolates was 0.011. Seventy three out of the 386 base pairs in the final dataset were variable, 47 of which were parsimony informative and 26 are singletons. Phylogenetic tree was drawn including DENV 1, 3, and 4 as

outgroups. Isolates investigated claded in the cosmopolitan genotype and was supported by a bootstrap value of 91%.

**Conclusion:** Sequence analysis and phylogenetic data suggested that the Philippine DENV 2 isolates are primarily Cosmopolitan genotype. Constant monitoring of the DENV 2 genotypes is essential to detect possible shifts within the serotype in the future and in understanding phylodynamic patterns of disease outbreaks in the era of global warming and climate change.

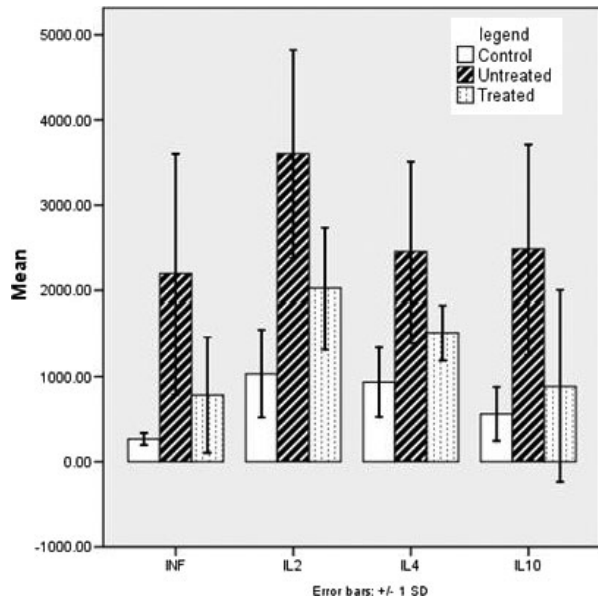
### P931 Serum profile of T helper 1 and T helper 2 cytokines in HCV-infected patients

A. Ramezani\*, M. Sofian, A. Aghakhani, A. Farazi, M. Banifazl, A. Eslamifard, A. Velayati (Tehran, Arak, IR)

**Objectives:** T-helper (Th) lymphocyte cytokine production may be important in the immunopathogenesis of hepatitis C virus (HCV) infection. Th1 cytokines (interleukin [IL]-2, interferon gamma [IFN-gamma]) are necessary for host antiviral immune responses while Th2 cytokines (IL-4, IL-10) can inhibit the development of these effector mechanisms. The aim of the present study was to assess the serum profile of Th1 and Th2 cytokines in treated and non-treated HCV infected individuals.

**Methods:** This study was carried out in 63 HCV infected patients (31 under treatment and 32 untreated) and 32 matched HCV-seronegative healthy subjects. The serum samples were checked with enzyme-linked immunosorbent assay (ELISA) for IL-2, IL-4, IL-10 and IFN-gamma.

**Results:** Levels of circulating IL-2, IL-4, IL-10 and IFN-gamma were significantly elevated in HCV patients vs. normal controls ( $2822.6 \pm 1259.92$  vs.  $950.8 \pm 286.9$  pg/mL;  $1987 \pm 900.69$  vs.  $895.91 \pm 332.33$  pg/mL;  $1688.5 \pm 1405.1$  vs.  $519.03 \pm 177.64$  pg/mL and  $1501.9 \pm 1298$  vs.  $264.66 \pm 71.59$  pg/mL respectively;



$p < 0.001$ ). The serum levels of all cytokines were significantly lower in under treatment patients than those of untreated patients ( $p < 0.001$ ). **Conclusion:** On the basis of our data, the simultaneous increase of Th1 and Th2 related cytokines may indicate that both Th1 and Th2 cytokines have been involved in the pathogenesis of HCV infection. Besides, this activated T-cell response in HCV infected patients could be regulated by treatment.

#### P932 Molecular detection and sub-typing of human papillomavirus on Albanian women

A. Mitre\*, F. Bakiri, T. Bare, A. Xhani, R. Bajro (Tirana, AL)

Human Papillomavirus (HPV) is estimated to be the common sexually transmitted infection in the Albanian population. Genital HPV types are categorized according to their epidemiological association with cervical cancer. High-risk HPV types can cause cervical and other genital cancers. Here, we introduce a new broad HPV assay based on high-density DNA microarrays, with which single and multiple HPV infections are easily recognized, and the corresponding HPV types can be identified. With this format it is possible to detect and identify 35 HPV types and the assay has the ability to recognize multiple infections.

**Aim:** The use of DNA microarrays for detection and sub typing of the multitude of HPV types in endocervical samples.

**Materials and methods:** Cervicovaginal swab specimens were collected from the 111 females aged 18–50 years, during period of time 2009–2010. The patients were recommended from gynecologist of University Hospital “Mbretresha Geraldine”. The identification and subtyping of HPV was performed with high density AND microarray in “Intermedica” laboratory.

**Results:** In the studied population we observed 27.9% incidence of HPV infection, from which 70.9% with high risk. The following HPV types were encountered in our clinical sample collection: 51(h), 84(p), 6(l), 16(h), 39(h), 51(h), 6(l), 31(h), 59(h), 56(h), 18(h), 52(h), 53(h), 83(p), 81(l), 73(h), 35(h), 66(h). The percentage of serotypes are: High risk HPV: HPV-16 (10%), HPV-18 (4%), HPV-31 (14%), HPV-35 (2%), HPV-39 (2%), HPV-51 (4%), HPV-52 (4%), HPV-53 (6%), HPV-59 (4%), HPV-62 (2%), HPV 66 (2%), HPV 73 (2%). Low-risk HPV: HPV-6 (28%), HPV-81 (6%). Indeterminate-risk HPV: HPV-83 (2%), HPV-84 (2%), which were found only as co infections with HPV-high risk types. We have found 71.7% of the positive cases as co infections with other HPV types, from which 46.7% with two type, 14% with three and the 9.5% with four types of HPV.

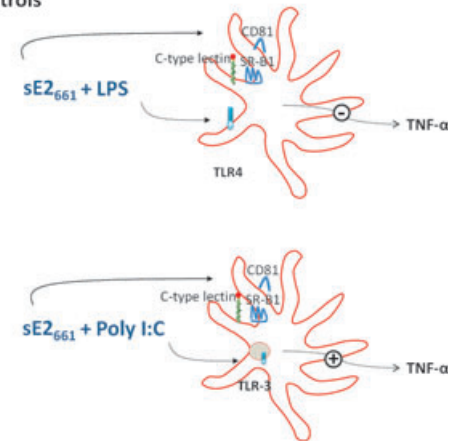
**Conclusion:** The prevalence of high-risk HPV in female genital tract shows high level. DNA microarrays were a good method for detection and sub typing of the multitude of HPV types in endocervical samples.

#### P933 Activation of monocyte derived dendritic cells (MoDCs) by hepatitis C virus (HCV) glycoproteins

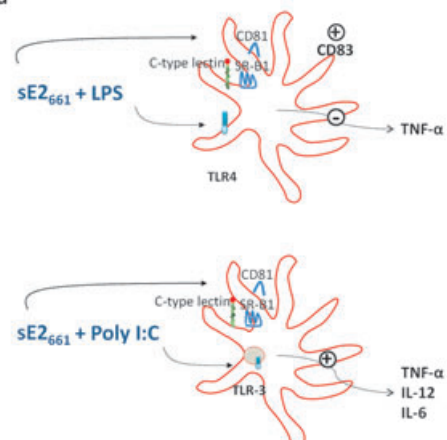
A. Abdulhaq\*, A.W. Tarr, L. Martinez-Pomares, W.L. Irving (Nottingham, UK)

Hepatitis C Virus causes a persistent, chronic infection in more than 80% of the estimated 170 million infections worldwide. Persistent infection can lead to complications such as cirrhosis and hepatocellular carcinoma. Around 20% of HCV infected patients are able to spontaneously clear the infection. Clearance is associated with the presence of broadly neutralizing antibodies. HCV glycoprotein E2 mediates HCV entry, and as such is the primary target for neutralizing antibodies. Dendritic cells have a critical role in priming the adaptive immune response to pathogens. This study aimed to investigate the recognition of HCV E2 envelope glycoproteins by dendritic cells. sE2 interacted with multiple receptors on the surface of MoDCs, including CD81 and DC-SIGN. These interactions resulted in moderate activation of MoDCs, but was associated with different profiles of cytokine release compared to cells stimulated with either LPS or Poly I:C. In both healthy donors and HCV positive patients similar expression of CD86 was observed following stimulation, either with sE2, LPS or Poly I:C. In contrast, expression of CD83 was significantly reduced in HCV infections, compared to healthy donors. MoDCs isolated from HCV infected individuals displayed a normal cytokine

#### (a) Healthy controls



#### (b) HCV infected



production compared to healthy donors. When MoDCs were activated with combinations of sE2661 with LPS or poly I:C, differences were observed in the phenotype and production of cytokines between MoDCs isolated from healthy controls and HCV infected patients. These results demonstrate that the HCV E2661 protein is recognised by some MoDCs, resulting in up-regulated expression of the DC maturation markers CD83 and CD86 and altered patterns of cytokine secretion, compared to un-stimulated cells. This activation is not similar to that achieved with LPS or poly I:C. HCV sE2661 does not possess the capacity to induce either Th1- or Th2-type immune responses in MoDCs from both healthy and HCV infected patients. However, this protein induced production of TNF-alpha from MoDCs isolated from some healthy donors, but not from MoDCs isolated from HCV-infected patients. In addition, sE2661 was found to influence MoDCs function when combined with TLR ligands. It is concluded that MoDCs from some HCV infected patients possess different in phenotypes to healthy controls. This may play a role in the inability to control HCV infection.

## Virology – diagnosis, epidemiology, prophylaxis and therapy

### P934 Prophylactic efficacy of AVI-7100 against influenza A in mouse and ferret infection models

*P. Iversen\*, F. Schnell, S. Crumley, D. Mourich, T. Voss (Bothell, New Orleans, US)*

**Objective:** AVI-7100 is a phosphorodiamidate morpholino oligomer containing three modified linkages (PMOplus) that is designed to interfere with expression of the M1 and M2 genes of influenza A virus. The objective was to evaluate the prophylactic therapeutic utility of AVI-7100.

**Methods:** A single 0.1 mg intranasal (i.n.) dose of AVI-7100 was administered to female BALB/c mice (n = 10/group) at 7 days, 3 days or 4 hours prior to viral challenge with  $5 \times 10^5$  pfu of A/Port Chalmers/1/73 (H3N2). Lung viral load was determined on day 6 post infection. In a separate study, a single i.n. dose was administered to mice infected with A/PR/8 (H1N1) and plasma and lung oligomer concentrations were determined. Outbred ferrets (*Mustela putorius furo*; n = 7/group) were administered AVI-7100 as a single i.n. dose at 7 days, 5 days, 3 days or 4 hours prior to insufflation viral challenge with  $5 \times 10^5$  pfu H1N1 A/Hong Kong/2369/09 per ferret. Negative control groups were treated with saline and positive controls were administered oseltamivir at 10 mg/kg p.o. every other day beginning 7 days prior to infection.

**Results:** A single intranasal dose of AVI-7100 (0.1 mg/mouse) administered 7, 3 days or 4 hours prior to infection with A/Port Chalmers/1/73 (H3N2) significantly ( $p < 0.05$ ) reduced lung viral titers in each group compared to vehicle controls and oseltamivir treated mice. PMOplus concentrations in the lungs of mice following a single insufflation dose follow zero order elimination and tissue concentrations above the AVI-7100 EC50 are maintained for >3 days. In the ferret, a single i.n. dose of AVI-7100 administered 7 Days, 5 days, 3 days or 4 hours prior to exposure with A/Hong Kong/2369/09 (an oseltamivir resistant pH1N1) significantly ( $p < 0.05$ ) reduced cumulative viral load in nasal wash and in lung bronchiolar lavage compared to saline controls and oseltamivir treated ferrets. The decrease in viral load in nasal wash samples was directly proportional to the interval of time between prophylactic treatment and viral exposure (a zero order reduction in activity).

**Conclusions:** AVI-7100 is effective against influenza A (H1N1 and H3N2) and in both mouse and ferrets after a single intranasal dose up to seven days prior to viral exposure. Zero order elimination of AVI-7100 from the lung was observed. These observations support the prophylactic use of AVI-7100 in preventing influenza A infection.

### P935 The usefulness of mice monoclonal antibody in dengue virus diagnosis and research

*M. Pupo-Antunez\*, Y. Vazquez, N. Amin, S. Vazquez, Y. Caballero, L. Morier, L. Morier, V. Capó, G. Guzmán (Havana, CU)*

**Objective:** The aim of this work was to generate, characterize and mainly use mice monoclonal antibodies in serodiagnosis and dengue researches.

**Methods:** Mabs were generated by conventional technology of Kohler and Milstein. Mice immunizations were used dengue-2 virus (D-2V) New Guinea C and Cuban (A15) strains. Mabs were characterized to determine: immunoglobulin isotype, protein recognition, and biological characteristics. In some cases, they were used in different assays: serological diagnosis, passive protection, viral protein expression and used in the identification of dengue mimotopes by a phage display peptide library (pVIII-9aa).

**Results:** H3-6 Mab specific to D-2V New Guinea C strain showed IgG 2a isotype and was able to recognize E protein from four dengue serotypes by Immunofluorescence assays. This Mab was capable of detecting anti dengue IgA antibody by ELISA as a possible marker in early and recent dengue infection. From the biopanning process, three mimotope peptides were identified. These showed similarity in their amino acid sequences with E dengue protein. One peptide was synthesized containing E dengue mimotope which was recognized by anti dengue antibodies in sera from convalescent infected patients. 8H8 Mab was raised against core (C) dengue protein specific to D-2V A15 Cuban strain. It was the IgG1 isotype and it had no hemagglutination inhibition, neither complement fixation nor neutralization properties. 8H8 Mab followed kinetically C dengue protein expression in mosquito cells (from 6 to 96 hour post-inoculation) and immunolocalized in brain tissues from D-2V infected mice (from 24 to 78 hour post-inoculation). In preliminary studies, 8H8 Mab was competent to recognize the C dengue protein in viremic serum from infected patients which made possible the quantification of a C recombinant dengue protein.

**Conclusions:** Our results suggest that mice monoclonal antibodies continue being useful in Dengue diagnosis and research. Mabs can be used directly in serodiagnosis or indirectly through phage-displayed peptide library for the development of diagnostic systems and a potential vaccine against this pathogen. Murine Mabs with defined virus specificities were efficient analytic tools for detection and expression of dengue C protein in vivo and in vitro.

### P936 Development of a new method for BK polyomavirus genotyping: application to transplant recipients

*S. Burrel, M. Gits-Muselli, Z. Ait-Arkoub, S. Nguyen, N. Arzouk, H. Agut, D. Boutolleau\* (Paris, FR)*

**Objectives:** In immunocompromised patients, BK virus (BKV) reactivation may result in nephropathy in kidney transplant (KT) recipients or haemorrhagic cystitis in haematopoietic stem cell transplant (HSCT) recipients. BKV strains are classified into four different genotypes (I to IV). BKV genotyping has been historically based on the analysis a 327-bp variable region of the gene coding for the major capsid protein VP1. The aim of this work was to develop a new method for BKV genotyping based on the sequencing of the full-length gene coding for VP1 and to determine BKV genotypes among KT and HSCT recipients.

**Methods:** Primers were designed to amplify, by nested PCR, and to sequence the full-length VP1 coding region (1089 bp). Amplified products were analyzed with the automated sequencer ABI 3100 Genetic Analyzer (Applied Biosystems). This new method was applied to 52 EDTA whole blood specimens obtained from 40 KT and 12 HSCT recipients (34 men, 18 women, median age: 48 years) experiencing BKV active infection (median BKV load in blood: 5.5 log). All nucleotide and amino acid VP1 sequences were aligned with SeqScape v2.5 software using BKV Dunlop strain as a reference (GenBank accession number V01108). A phylogenetic tree was



constructed by the neighbor-joining method using ClustalW program, including the VP1 sequences from the different reference BKV strains representing the main genotypes obtained from GenBank.

**Results:** The sensitivity of the VP1 nested PCR was 500 copies/mL. At the nucleotide level, the interstrain identity of VP1 gene ranged from 91.9% to 99.2%. At the amino acid level, a total of 45 amino acid changes were identified, that is 12.4% of the total codons of the protein. Each strain harboured a mean number of 8.8 amino acid changes. Seven amino acid changes were evidenced among at least 50% of the BKV strains. As a whole, the distribution of BKV genotypes among transplant recipients was as follows: I (81%), II (8%), III (0%), IV (12%). Among genotype I, BKV Ia, Ib1, Ib2, and Ic subgroups represented 5%, 31%, 64%, and 0%, respectively. Only BKV subgroups Ib1 and Ib2 were identified among HSCT recipients.

**Conclusion:** We report here the development of a new method for the sequencing of the full-length VP1 coding region allowing BKV genotyping. Our results evidenced the high variability of VP1. This method constitutes a useful tool for further studies on BKV pathogenicity according to the genotypes.

### **P937** Comparison of the INNO-LiPA genotyping extra and the Hybrid Capture 2 assays for detection of carcinogenic human papillomavirus genotypes

*L. Barzon, V. Militello\*, S. Pagni, G. Palù (Padua, IT)*

**Objectives:** The objective of this analysis was to compare the performance characteristics of two human papillomavirus (HPV) DNA detection assays, the Hybrid Capture 2 assay (HC2) and the INNO-LiPA Genotyping Extra assay (LiPA), for the detection of carcinogenic HPVs.

**Methods:** Cervical specimens collected from a total of 1184 consecutive women, attending our Unit for HPV testing and genotyping for opportunistic screening and investigation of HPV-related lesions, were analyzed by both HC2 and LiPA tests.

**Results:** Among the 1184 paired analyses, 559 (47.2%) specimens were negative by both tests, 418 (35.3%) were positive by HC2, while 638 (53.9%) were positive by LiPA, including 371 (31.3%) which were positive for carcinogenic HPV types (i.e., IARC groups 1 and 2A). Seventy nine specimens (6.7%) were HC2-positive but carcinogenic HPV type-negative by use of the SPF10-LiPA system. Similarly, 45 (3.8%) were carcinogenic HPV type-positive by use of the SPF10-LiPA system but HC2-negative. Agreement between the two assays for carcinogenic HPV type detection was 89.7%. HC2 identified as positive between 83% (HPV51) and 100% (HPV45, HPV56, and HPV59) of specimens with carcinogenic HPV types detected by LiPA. Ninety-two and ninety percent of the samples identified as HPV16- and HPV18-positive, respectively, by SPF10-LiPA were called positive by HC2. Among patients infected with a single HPV type detected by LiPA, HC2 positivity for carcinogenic HPV types ranged from 61.9% (HPV51) to 100% (HPV33, 35, 39, 45, 59), from 0% for HPV73 to 44% and 50% for the possibly carcinogenic IARC 2B HPV66 and HPV53, respectively, while non-carcinogenic HPVs were negative at HC2 assay, with the exception of same single HPV6 and HPV74 infections testing HC2 positive in 14.3% and 5.6% of cases, respectively. When carcinogenic HPV type detection by HC2 and the SPF10 system was stratified by cytology diagnosis, both HPV assays performed similarly for all cytology interpretations, except for women with low-grade squamous intraepithelial lesions. Discordant results were mainly due to infection with HC2 cross-reactive types HPV53 and HPV66.

**Conclusion:** A very good agreement was observed between HC2 and INNO-LiPA Genotyping Extra assays for carcinogenic HPV type detection. In addition, HC2 probes showed cross-reactivity with the possible carcinogenic HPV53 and HPV66.

### **P938** Prospective evaluation of a norovirus bedside test in unselected patients with gastroenteritis

*E. Weise\*, M. Vergoulidou, B. Friedrichs, S. Siedler, S. Pfaffenbach, J. Hofmann, R. Somasundaram, S. Schwartz (Berlin, DE)*

**Objectives:** Infections with Norovirus (NV) are highly contagious, requiring rapid identification of infected pts. However, NV-specific PCR testing, currently regarded as the diagnostic standard, is not always readily available and time consuming. We prospectively evaluated a Norovirus (NV) bedside test, which gives a result within 15 minutes, in unselected pts with gastroenteritis (GE).

**Methods:** Pts who presented with acute gastroenteritis (GE) were enrolled after written consent. Clinical details were recorded by a standardised questionnaire. The study was approved by the ethical committee. Stool specimens were investigated using a commercially available, immunochromatographic bedside test (RidaQuick NorovirusTM, R-Biopharm, Darmstadt, Germany) and in parallel by NV-specific, quantitative PCR.

**Results:** A total of 63 pts (33 male; age 20–96 year, median 70 year) were enrolled during the past winter season and eligible for a final analysis. Another 16 pts were excluded: no stool specimen 14, no consent 2. Eligible pts (63) were tested 0–19 (median 1) days after onset of symptoms, which were emesis (1), diarrhoea (26) or both (36). In all, 18 pts had a positive bedside test result and in 28 pts Norovirus (NV) GE was confirmed by NV-specific PCR. The sensitivity, specificity, positive predictive value and negative predictive value of the bedside test were 61%, 97%, 94% and 76%, respectively. The median ct-value of 17 pts with NV GE and correctly assigned by the bedside test was significantly lower compared to 11 pts with a false-negative bedside test (22.5 vs. 27.2,  $p = 0.03$ ). There was only a slight, not significant trend towards a higher median clinical severity score (Rockx, Clin Infect Dis 2001, 35:246) among 17 pts with Norovirus (NV) gastroenteritis (GE) and correctly assigned by the bedside test compared to 11 pts with a false-negative bedside test (10 vs. 8,  $p = 0.31$ ).

**Conclusion:** In this clinical study, we recorded a limited sensitivity but good specificity, which makes this test a useful adjunct diagnostic tool, particularly in NV outbreak settings. We found a trend towards false-negative bedside test results in pts with likely low replicative NV GE.

### **P939** Evaluation of interleukin-10 gene promoter polymorphisms and hepatitis B virus infection outcome

*M. Sofian, M. Banifazl, A. Aghakhani, N. Zarin far, A. Farazi, A. Ramezani\* (Arak, Tehran, IR)*

**Objectives:** Single nucleotide polymorphisms (SNP) in the promoter region of the interleukin (IL)-10 gene has been reported to have a role in determining of hepatitis B virus (HBV) infection outcome. The present study was undertaken to evaluate the association between HBV infection and SNP in the promoter region of the IL-10 gene in a central Iranian city.

**Methods:** A total of 127 cases including 32 patients with chronic hepatitis B infection and 34 healthy carriers collapsed as persistent group, 30 subjects who had spontaneously recovered from HBV infection and 31 healthy controls were enrolled in this study. The three biallelic (–819, –592, –1082) polymorphisms in the IL-10 gene promoter were analyzed by polymerase chain reaction and direct sequencing.

**Results:** No significant difference was found in frequencies of genotypes and haplotypes of IL-10 gene promoter region at position –1082, –819 and –592 among controls, individuals spontaneously recovering from HBV infection, carriers and patients with chronic hepatitis B infection. However, frequencies of A/A genotype at position –592 and T/T genotype at position –819 were higher in the HBV clearance group, while frequency of G/G genotype at position –1082 was higher in persistence group. GCC/GCC and GCC/ACC haplotypes were significantly more frequent in anti-HBe positive patients.

**Conclusion:** It seems that genetic polymorphisms of IL-10 promoter region are not associated with HBV infection outcome. However, patients with high and intermediate producer haplotypes of IL-10 had more ability to produce anti-HBe than those with low producer haplotypes.

**P940 Investigation of interleukin 28b gene polymorphism in patients with chronic hepatitis C**

Ü. Savasçi, Ö. Coskun\*, S. Kozan, A. Karakas, H. Gül, C. Eyiğün (Ankara, TR)

**Introduction:** The rate of response to treatment can be predicted by IL28B genotyping. Thus, decisions related to treatment can be tailored and the cost can be reduced. The frequency of IL28B polymorphism and the effect of this on response to treatment were investigated in patients with chronic hepatitis-C virus.

**Methods:** The study was planned as a study for the determination of attitude and DNA was isolated from the blood sample obtained from the patients with chronic hepatitis-C, using “spin column” method. In the analysis of rs12979860 polymorphism, Tetra primer amplification refractory mutation system method was used. In the analysis of rs12979860 polymorphism, a fast and cheap polymerase chain reaction method was developed.

**Results:** As a result of the analysis for the correlation of rs8099917 polymorphism with genotypes performed using chi-square test, TT genotype was found in 21 of 37 patients among responsive patients and in 4 of 16 patients among unresponsive patients. In the evaluation of the difference between rs8099917 polymorphism and other genotypes using logistic regression, we found that the presence of GG genotype increased the therapeutic response by three times compared to TG, but this difference was not statistically significant. The presence of TT genotype in the patient increased the therapeutic response by 5.25 times compared to TG and the difference was statistically significant.

**Conclusion:** Future studies should address to the explanation of the genetic mechanisms involved in IL28B. Correlation between IL28B polymorphism and the therapy will be of a great value for the drugs used in the treatment of hepatitis-C infection.

**P941 Detection of hepatitis C virus proteins in peripheral blood mononuclear cells of patients with chronic hepatitis C: correlation with activity and stage of hepatitis**

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**Objectives:** To study hepatitis C virus (HCV) RNA and HCV protein expression in peripheral blood mononuclear cells (PBMC) of patients with chronic hepatitis C (CHC), and to determine the relationship between HCV replication in PBMC and the activity and stage of hepatitis C.

**Methods:** PBMC were isolated from peripheral blood of 83 patients with CHC in a Ficoll gradient. PBMC from 20 HCV-negative donors served as a control. HCV proteins (core, NS3, NS4A, NS4B and NS5A) in PBMC were identified by immunocytochemical staining (ICS) with 17 monoclonal antibodies (mAb). Intracellular expression of viral proteins was also studied by flow cytometry with FITC-conjugated mAb. Plus- and minus-strands HCV RNA in PBMC were detected by RT-PCR. Histological activity and hepatitis stage were determined by analysis of liver biopsy specimens.

**Results:** By ICS, HCV proteins in PBMC were detected by at least one Mab or mixtures of Mabs in 71 out of 83 (86%) CHC patients. NS5A had the highest detection rate (61%), those for other proteins varied from 35% to 44%. The proportion of antigen-positive (AG+) cells and the intensity of staining varied considerably from patient to patient, being different for individual proteins. The HCV proteins were located

in the cytoplasm of predominantly monocytes and rarely – of lymphocytes. Flow cytometry detected HCV proteins in 11 out of 21 (52.4%) patients, in four patients the proteins were located in lymphocytes and monocytes and in seven patients only in monocytes. The proportion of AG+ cells reached 32% of total population for monocytes and 5% for lymphocytes. Imbalance in the lymphocyte/monocyte ratio was typical of the majority of patients. HCV detection rate correlated with a decrease in the total count of mononuclear cells and lymphocytes:  $r = -0.66$ ,  $p = 0.001$  and  $r = -0.64$ ,  $p = 0.002$ , respectively. Plus-strand HCV RNA in PBMC was detected in 95% patients and minus-strand RNA in 51% patients. The presence of minus-strand RNA in PBMC correlated with expression of core and NS5A ( $p < 0.05$ ). The accumulation of HCV Core protein in PBMC positively correlated with histological activity and stage of hepatitis ( $p < 0.05$ ). Detection rate for HCV proteins was the highest for PBMC of patients with elevated alanine aminotransferase level ( $p < 0.05$ ).

**Conclusion:** PBMC are a site of active HCV replication and viral proteins accumulation, which may lead to immune disorders, aggravated biochemical and necro-inflammatory activity of liver disease and hepatitis stage.

**P942 Cloning and expression of HCV polytope-HBV surface antigen fusion protein under optimised condition in *E. coli* for vaccine study**

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**Objective:** Hepatitis C virus (HCV) is a major cause of liver disease worldwide. HCV infection is associated with high morbidity and has become a major problem in public health. Until now, there has been no effective prophylactic or therapeutic vaccine for HCV. Cellular immune responses, specially cytotoxic T-lymphocytes (CTLs) play a critical role in immune response against HCV. Considering that polytope vaccine has the ability to stimulate the cellular immunity, the aim of this study is construction of a recombinant fusion protein as an HCV vaccine. This fusion protein contains HBs-antigen as an immunocarrier and five immunogenic epitops (polytope) of HCV to induce specific CTL responses.

**Materials and methods:** Five immunogenic epitops from core, E2 and NS3 antigens of HCV were selected based on evaluation with SYFPEITHI software. Coding sequence of this polytope was synthesized and fused to the HBsAg sequence by overlapping PCR. The product was digested by NcoI and XhoI enzyme and cloned in to pET-28a plasmid, which was sequence-confirmed and transformed to different *E. coli* strains of BL21 (DE3), Rozetta (DE3) and BL21 (DE3) pLysS and BL21-CodonPlus (DE3)-RIL. Expression of recombinant fusion protein was induced by addition of IPTG and evaluated using SDS-PAGE analysis and western blotting.

**Result:** The results of immunoinformatic survey led to the selection of five immunogenic epitops with high HLA-binding scores. Sequencing reactions confirmed the authenticity of final plasmid. Optimization of inducer concentration, host strains, induction time, temperature and media resulted in the expression of the highest level of recombinant protein, nearly up to 50% of total protein, and the corresponding protein band with the molecular weight of 32 kDa was detected on SDS-PAGE and confirmed using western blotting.

**Conclusion:** Recombinant protein containing the fusion of HCV polytope and HBsAg was efficiently produced in *E. coli* cells. Polytope vaccines are new approach to immunize host against pathogens. A polyepitope-based strategy combining of HCV epitops including conserved T-cell epitops of core, E2 and NS3, could impulse cellular immunity. The recombinant polytope fusion protein properties represented a good implication as a potential vaccine candidate and this guarantees the further investigations towards the assessment of its immunogenicity, which are currently under process.

**P943 A new tool to study ribavirin-induced haemolysis**

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**Background and aims:** Today's treatment of chronic hepatitis C is based on a synergistic combination of pegylated interferon and ribavirin with antiprotease inhibitors. Anemia, which is the major side effect of ribavirin disrupts ribavirin treatment compliance and vary significantly from one patient to another. There is an individual susceptibility to ribavirin hemolysis.

**Methods:** With a view to studying hemolysis and thus optimizing the treatment response, we developed an in vitro tool for analyzing the ribavirin-induced lysis of red blood cells.

**Results:** In this model, the degree of hemolysis depended on the ribavirin concentration used and could be inhibited by the addition of dipyridamole with an IC50 of 30  $\mu$ M. Intra- and inter-individual variability of the hemolysis test were evaluated and were about 5%. We observed a strong decrease in red blood cells hemolysis in the presence of the ribavirin prodrug viramidine (Taribavirin®). When testing the performance of this assay with blood from 24 patients, we obtained a strong correlation between in vitro hemolysis before treatment and in vivo hemoglobin levels decrease during subsequent treatment ( $p < 0.001$ ).

**Conclusions:** Thus, with this new tool, it seems now possible to better evaluate individual susceptibility to ribavirin-induced hemolysis before the start of treatment. In addition, the mechanism of anemia with ribavirin can be further explored, which allow then to screen and test in vitro molecules that could reduce ribavirin hemolysis. This approach could help optimize current and future therapeutic strategies involving ribavirin in the treatment of chronic hepatitis C.

**P944 A report on a large measles outbreak in Lyon area, France, 2010–2011**

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**Objectives:** A large outbreak of measles has been occurring in France since 2008. The city of Lyon, located in Rhône-Alpes region (South-East of France) experienced one of the highest burden in Europe with a regional incidence of 94 cases/100 000 inhabitants between January and May 2011. The objective was to describe the cases of measles diagnosed in Lyon University Hospitals between 2010 and mid 2011.

**Methods:** A prospective surveillance of measles cases based on mandatory report and virological data was done in Lyon University Hospitals from 1/1/2010 to 8/7/2011. Healthcare practitioners had to report each suspected or confirmed case of measles. A standardized form with clinical signs, biologic confirmation and vaccinal status was completed for each case. A clinical case was defined as: fever  $\geq 38.5^{\circ}\text{C}$  and maculopapular rash and at least one of the following signs: conjunctivitis, coryza, cough, Koplik sign.

**Results:** Overall, 407 measles cases were diagnosed (Figure 1, Epidemic curve), the higher number of incident cases was observed in the populations under 1 year old ( $N = 129$ , 32%) and those aged

between 17 and 29 years old ( $N = 126$ , 31%). Totally, 72 (18%) had complications of measles: 51 (13%) had pneumonia, and 2 (1%) had encephalitis, no patient died. Among the measles cases, 224 (55%) were not vaccinated, 52 (13%) had received a single dose of measles vaccine whereas 11 (3%) had received two doses. For 120 (29%), information about vaccination status was not available. Overall, 2789 individuals were tested for serology and/or PCR because of clinical suspicion or exposure to a measles case: 1487 patients and 1302 healthcare workers (HCWs). Among patient, 235 (27%) had positive IgM, 648 (70%) had positive IgG, and 345 (61%) had positive PCR. Among HCWs, 8 (1%) had positive IgM, 1238 (95%) had positive IgG, and none had positive PCR. The proportion of patients aged  $\leq 30$  years without immunization was 36% vs. 12% in individuals aged  $>30$  years ( $p < 0.001$ ). The proportion of HCWs aged  $\leq 30$  years without immunization was 11% vs. 3% in individuals aged  $>30$  years ( $p < 0.001$ ).

**Conclusion:** A catch-up vaccination campaign must be implemented, focused on population under 30 years old who did not receive two doses of measles vaccine and on HCWs. Hospital-based surveillance of measles is relevant to estimate the rate of hospitalization related to measles during this outbreak and to help for early detection and control of nosocomial cases.

**P945 Surveillance of measles outbreaks in Veneto region, Italy, November 2010–October 2011**

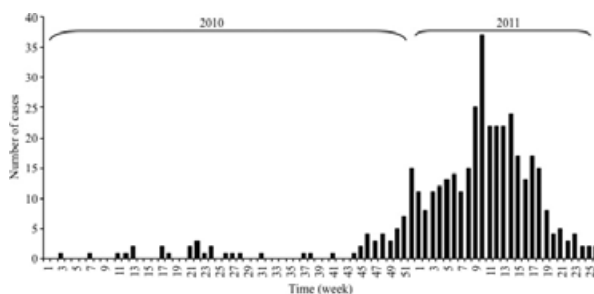
M. Pacenti\*, L. Barzon, E. Franchin, R. Cusinato, G. Marcati, E. Lavezzo, L. Gottardello, G. Palù (Padua, IT)

**Objectives:** An enhanced surveillance programme was activated in Veneto Region since October 2010 to improve case investigation and laboratory confirmation of diagnosis.

**Methods:** For each suspected measles case, the local health authorities carried out epidemiological investigation and obtained specimens for laboratory confirmation and genotyping. Confirmed cases were notified to regional and national health authorities. In our regional reference lab, real-time RT-PCR was used to detect measles RNA from throat swabs and/or urine specimens. As recommended by the WHO, strains were genotyped by viral nucleoprotein sequencing.

**Results:** Out of a total of 185 possible cases reported in Veneto Region during the surveillance period, 105 (56.7%) were classified as laboratory-confirmed. Two incidence peaks of confirmed cases occurred in December 2010 and in April 2011, followed by a progressive decrease and no case reported in August–October 2011. All provinces of Veneto Region were involved, but incidence varied among municipalities. Children younger than 1 year and young adults aged 20–35 years were the most affected age groups. Overall, 70% of cases were unvaccinated, 8% had received only one dose of vaccine, 2% had received two doses, while information on vaccination was unavailable for 20% of cases. Measles virus was genotyped in all confirmed cases and D4G4 (53%), D8 (45%), and D4G3 (1%) genotypes were identified. D4G4 circulated in all Veneto Region until February 2011, when it was replaced by D8 genotype, which circulated in central and southern provinces. A new genetic cluster of D4G4 genotype appeared in April 2011, with a peak of cases in May 2011. Measles subtyping by sequencing the whole haemoagglutinating gene identified temporarily and geographically-related measles clusters. One case of D4G3 genotype was imported from France and one case of Edmonston A vaccine genotype was isolated in a recently vaccinated subject.

**Conclusions:** Like other Italian Regions and countries in the WHO European Region, Veneto Region did not meet the 2010 elimination target. An enhanced surveillance programme led to the identification of several measles cases in 2010 and 2011 and the determination of their circulation in the regional territory. Unvaccinated children and young adults were the most affected age groups, indicating the need to strengthen interventions to increase vaccination coverage.



**P946** Parallel epidemics with a range of globally appearing rhinovirus types – a 4-year molecular epidemiology study

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**Objectives:** Human rhinovirus (HRV) is a major cause of acute respiratory tract infections. HRV appears to have less seasonality than most other respiratory viruses. Our objective was to study the biodiversity of HRV strains circulating in Western Sweden over a period of four consecutive years and to evaluate their relationship with previously described strains.

**Methods:** One-hundred-seventy diagnostic respiratory samples positive for rhinovirus by real-time PCR collected during November 2006 through September 2010 were chosen for further analysis. Selection criteria were a Ct-value  $\leq 33$  indicating a relatively high viral load, and a dispersed distribution across seasons. These samples represent approximately 10% of the total amount of HRV-positive samples from the region during this time period. Sequencing of the VP4/VP2 region was used for typing and phylogenetic trees were constructed using neighbour-joining maximum distance matrix analysis of the amplified regions by MEGA 4 software.

**Results:** Typing was successful in 112 samples, of which 54 came from children (age  $\leq 18$ ). HRV-A was found in 62, HRV-B in 11 and HRV-C in 37 cases and 33 different HRV-A types, 9 HRV-B types and 21 HRV-C types were identified. The mean genetic distance between the HRV-A strains was 0.23 as compared with 0.38 between HRV-C strains, ( $p < 0.0001$ ). Enterovirus (CAV9 and EV68) were found in two cases. Twelve of the subtypes appeared during several seasons, in some cases with a span of 4 years. Six samples showed  $< 85\%$  similarity with reference sequences suggesting putative new subtypes. Comparison with published sequences in Genbank was made and revealed similarity with sequences distant in time and geographical location.

**Conclusion:** HRV-infections are highly globalised. Subtypes may prevail during several seasons and seem to circulate as parallel global epidemics. HRV-A and HRV-C represent dominant subtypes with a greater genetic diversity within subtype C. Our results raise new questions regarding HRV epidemiology, viral evolution and persistence. Further study of subtype or strain-specific circulation patterns is needed to reveal the dynamics of HRV diversification or association with clinical outcome.

**P947** Influenza admitted in intensive care units: a comparative study of seasonal and pandemic influenza A (H1N1) 2009

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**Objective:** Pandemic A (H1N1) 2009 influenza was associated with an unprecedented rate of admission in intensive care units (ICU), mostly due to acute respiratory distress syndrome (ARDS). Characteristics of these patients were described, but they have not been compared to patients with severe seasonal influenza.

**Methods:** We performed a retrospective study of all patients with documented influenza admitted in one medical ICU for influenza A between 1993 and March 2011. Patients were included if influenza was documented on respiratory samples by RT-PCR and/or immunofluorescence and/or cell culture and/or serology (complement fixation). A standardized questionnaire was used to extract data from medical charts, including demographics, comorbidities, influenza vaccination, date of 1st symptoms, hospital, and ICU admission, patients characteristics including body mass index and pregnancy, severity score (IGS-II), ARDS, treatment (including antivirals, corticosteroids, and mechanical ventilation), bacterial infection, and final outcome. Patients with influenza A (H1N1) 2009 were compared to patients with seasonal influenza, using nonparametric Mann Whitney test for quantitative variables, and Fisher exact test for categorical variables.

**Results:** Between 1993 and 2011, 35 patients with documented influenza were admitted in our ICU, including 18 patients with seasonal influenza (1993–2006), and 17 patients with pandemic influenza A (H1N1) 2009. Patients with pandemic influenza were younger than patients with seasonal influenza, with a median age of 41 years (IQR, 29–55) vs 56 (41–68), ( $p = 0.04$ ), more likely to be obese (41% vs 6%,  $p = 0.03$ ), and current smoker (65% vs 28%,  $p = 0.01$ ). Influenza-related symptoms, and complications, were not different between the two groups, including ARDS, bacterial infections, and in-ICU mortality (respectively, 18% for pandemic, and 33% for seasonal influenza). The diagnosis delay (time between admission and influenza documentation) was shorter for pandemic influenza patients (median 1 day vs 4,  $p = 0.01$ ). More patients with pandemic influenza were treated with oseltamivir (88% vs 11%,  $p < 0.01$ ).

**Conclusion:** As compared to severe seasonal influenza, severe pandemic influenza A (H1N1) 2009 affected younger patients, more frequently obese and smokers. Although pandemic influenza A (H1N1) 2009 was diagnosed faster, and more likely to be treated with oseltamivir, outcomes were no significantly different.

**P948** Sequencing of influenza A(H1N1) pdm viruses in the Philippines

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**Objective:** Few studies have been done on the genetic characteristics of influenza strains in the Philippines. Mutations for drug resistance in local influenza isolates have also not been widely studied. This study is part of a larger project in collaboration with the Department of Health and Department of Science and Technology to develop a web-based genomic library for the surveillance of influenza A in the Philippines. Our aim is to generate a genomic library and resistance profile of local influenza strains by sequencing the matrix (M), neuraminidase (NA) and haemagglutinin (HA) genes.

**Methods:** RNA extracts of confirmed influenza A (H1N1) and seasonal influenza A (H3N2) from 2009 to 2011 were obtained from a tertiary hospital, The Medical City, Pasig City, Philippines. Matrix 1 and 2 (M), neuraminidase (NA), and haemagglutinin (HA) genes of influenza A were amplified using gene-specific primers. The amplification products were electrophoresed to determine the presence of amplified product. The amplified products were then sequenced.

**Results:** Of the samples sequenced, 33 have been sent to Genbank. None of the neuraminidase genes tested showed mutations for resistance to oseltamivir. However, all of the matrix genes had resistance mutations for adamantanes. Phylogenetic analysis did not yield any distinct clades of influenza A. However, this may be due to the fact that only one site was involved in the pilot project.

**Conclusion:** The results indicate that resistance mutations for adamantane are likely to be widespread in the Philippines, and the drug should not be used to treat influenza. Resistance mutations for oseltamivir, on the other hand, are likely to be rare. The pilot project will now be scaled up and integrated into all influenza A surveillance specimens in the Philippines.

**P949** A 5-year retrospective review of patients with norovirus infection: factors associated with prolonged viral shedding

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**Objectives:** Norovirus gastroenteritis is a leading infection control problem. Existing UK guidelines recommend isolation/cohorting of individuals for 48 hours after symptom resolution and ward closure for 72 hours after the last new case. Individuals with norovirus gastroenteritis continue to shed virus in their stool after acute infection, but the factors which determine the duration of shedding

are not established. We aim to determine which factors may be associated with prolonged viral shedding.

**Methods:** We retrospectively reviewed all patients with norovirus diagnosed by RT-PCR in stool or vomit at the Royal Liverpool University Hospital between 2006 and 2011. Inclusion criteria was the detection of norovirus in stool or vomit on >1 occasion >1 day apart to be able to assess duration of shedding. Discharge summaries and laboratory records were reviewed and key demographic details, past medical and medication histories were collated. To compare the duration of viral shedding depending on exposure to various factors (age, sex, past medical and medication history) we calculated incident rate ratios (IRR) using negative binomial regression. Significant factors on univariable analysis were included in a stepwise multivariable negative binomial regression model to determine which factors were independently associated with duration of viral shedding.

**Results:** Eight hundred and forty-nine cases were identified of which 53 met the inclusion criteria, 49% male, with a median age of 77 years (range 33–98). The median duration of viral shedding was 8 days (range 3–90), 50% of cases shed norovirus for <10 days, while 25% shed for >3 weeks. 29.8% were on immunosuppressant therapy. On univariable analysis younger age, immunosuppressant therapy and renal transplant were associated with prolonged viral shedding. However, on a multivariable regression model only immunosuppressant therapy and younger age remained significant factors.

**Conclusion:** Current guidelines may not be adequate to prevent secondary nosocomial norovirus infection due to extended viral shedding. Younger patients and those receiving immunosuppressive therapy may have delayed clearance of norovirus infection. The role of prolonged shedding in secondary transmission of norovirus requires further investigation. Limitations of this study include retrospective analysis and a strong bias towards cases with persistent symptoms.

#### **P950** No resistance detected to PMOplus antisense oligomers that protect nonhuman primates against marburgvirus

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**Objectives:** Marburgvirus (MARV) is highly virulent RNA virus of the family Filoviridae and a causative agent of viral hemorrhagic fever (VHF). The postexposure therapeutic efficacy of AVI-6003 [a PMOplus combination targeting MARV nucleoprotein (NP, AVI-7287) and VP24 (AVI-7288)] has reproducibly provided for 100% survival in nonhuman primate (NHP) MARV lethal challenge infections. The objective was to evaluate the viral sequence fidelity in viral genome regions targeted by AVI-6003 in nonhuman primates infected with MARV Musoke in treated vs. untreated groups.

**Methods:** Three independent studies were conducted in which cynomolgus macaques were infected with 1000 pfu of MARV Musoke. Five groups of infected NHPs included daily doses of AVI-6003, AVI-7287 alone, AVI-7288 alone, a negative-control PMOplus agent, and saline. Viral genomic RNA was obtained from infected animals immediately upon sample collection by mixing one volume of serum with three volumes of Triazol LS. Samples with >300 pfu/mL were amplified using random hexamer DNA primers and reverse transcriptase PCR amplification. Viral genome sequence was determined using dye-terminator (studies 1 and 2) and pyrosequencing (study 3) methods.

**Results:** The amplicon DNA sequence was determined to an average depth of up to 3300 for the NP site and 6700 for the VP24 site including an approximate 200 base flanking region. Determination of the entire viral genome sequence is currently in progress. The sequence observations encompass three independent studies, evaluating blood or serum samples from 35 different NHPs (29 treated at doses from 7.5 to 40 mg/kg/day and six saline controls), and samples were evaluated from days 8 to 30 post infection. The PMO targeted viral genome sites

were found to demonstrate high fidelity and show no sequence changes indicating no development of resistance to AVI-6003.

**Conclusions:** These results indicate viral resistance to AVI-6003 and its components is unlikely in the genome of this single-stranded RNA human pathogen and support the further development of PMOplus therapies for use in humans.

#### **P951** Respiratory viruses other than influenza as cause of severely respiratory infections in adult patients admitted to an intensive care unit during 2009/2010 influenza season

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**Objectives:** To evaluate the frequency of viral pathogens in adult patients with severe respiratory infections (SRI) requiring admission to an Intensive Care Unit (ICU).

**Design, setting and Patients:** A prospective single-center observational study including all consecutive patients admitted to ICU due to SRI between July 17th, 2009 and January 31st, 2010 was conducted. Clinical, microbiological and radiological variables were assessed. In addition to RT-PCR for 2009 A(H1N1) pandemic influenza which was performed prospectively in all patients admitted to ICU with SRI, respiratory samples were retrospectively studied for multiple respiratory virus (Respiratory Virus Panel [RVP]) assay on the CLART<sup>®</sup> PneumoVir. RVP targeted the following virus types and subtypes: Influenza A, Influenza B, Respiratory Syncytial Virus subtype A, Respiratory Syncytial Virus subtype B, Parainfluenza 1, 2, 3 and 4 virus, Human Metapneumovirus, Rhinovirus, Coronavirus and Adenovirus. Other microbiological work-up was performed upon clinician request.

**Results:** Forty-nine patients were included. The median age was 48 years-old and associated comorbid conditions were present in 26 patients (53.1%), being chronic lung disease the most commonly observed comorbidity (13 patients-26.5%). No solid-organ transplant nor hematological patients were admitted during the study period. Median APACHE II score was 14. Inotropic drugs were needed in 17 patients (34.7%). Pulmonary infiltrates consistent with pneumonia were found in 23 patients (45.1%). Nine patients died during ICU admission. Etiological diagnosis was reached in 30 patients (61.2%), being Influenza the most frequently documented agent: Influenza C was identified in one patient (2.04%) and nH1N1 in 16 (32.7%). Respiratory Syncytial Virus in five patients (10.24%), Rhinovirus in 4 (8.16%) and Parainfluenza virus in 1 (2.04%). Six patients had documented bacterial infections (4 *S. pneumoniae* and 2 Legionella).

**Conclusions:** Respiratory viruses other than Influenza, especially Respiratory Syncytial Virus and Rhinovirus are involved in adult patients with SRI requiring intensive care support.

#### **P952** A novel study on rapid molecular characterisation of group A rotavirus in Turkey using PCR-reverse line blot hybridisation

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**Objectives:** Group A rotavirus related acute gastroenteritis in infants and young children has evolved a significant impact on morbidity and mortality not only in developing but also in developed countries. The study was done to determine the diversity of co-circulating rotavirus strains in Turkey, and to provide a baseline for future assessment of possible effects of vaccine implementation in selecting novel vs. common rotavirus strains. Detection and genotyping of rotavirus strains

from stool samples in children with gastroenteritis in Turkey were examined using polymerase chain reaction-reverse line blot hybridization (PCR-RLB). Compared to conventional RT-PCR, PCR-RLB has known to be more robust, cheap, time saving and suitable to be implemented in developing country.

**Methods:** Stool samples were collected from 495 paediatric patients with acute gastroenteritis and screened for rotavirus infection from several hospitals in Turkey. The samples were provided by different paediatric clinics in Anatolian regions of Turkey such as Afyon, Bursa, Istanbul, and Ankara from May 2010 through May 2011.

**Results:** Of 495 stool samples, 238 samples were rotavirus ELISA positive and subsequently characterized by PCR RLB. Most of strains (97%) belonged to genotypes G1–G4, and G9, associated with either P[8] or P[4], commonly found in humans worldwide. We found that the percentage of G1P[8] is a predominant strain of Rotavirus (43.3) and followed by G4P[8] (26.5), G9P[8] (19.3), G2P[4] (13), G2P[8] (1.7), G1P[4] (1.3), G12P[8] (1.3), G12P[4] (1.3), G9P[4] (0.8) and G4P[6] (0.4). In 22.1% of cases, viruses exhibited either a G or P type typical of animal viral strains (G9 and G12), suggesting gene reassortment events between rotaviruses of different origin. Novelty, this study has characterized G12 as a newly emerging genotype in Turkey. In addition, we detected 17 patients with mixed infection in RLB hybridization membrane. However, there were untypable G genotype in 30 patients and P genotype in six patients.

**Conclusion:** The numbers and genotypes of likely natural reassortants of common genotype rotaviruses were found to be correlated with the observed numbers and genotypes of mixed infections. Large variation in the relative frequency of different rotavirus genotypes was observed between different seasons and/or areas of Turkey, suggesting independent evolution or differential introduction of viral strains with respect to both time and space.

#### **P953** Presence of multiple dengue serotypes in various body compartments in different time points of single clinical episodes

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**Objectives:** Dengue is the most wide-spread arthropod-borne virus. The virus consists of four serotypes, DEN1-DEN4. Frequent concurrent multi-serotype infections in a single clinical specimen in hyperendemic areas is well documented. Here we demonstrate for the first time that multiple serotypes could also be present in various compartments during different time points of each clinical episode.

**Methods:** Plasma, peripheral blood mononuclear cells (PBMCs), saliva and urine collected during febrile and convalescent periods of 22 serologically-confirmed dengue patients were subjected to nested reverse transcription PCR (RT-PCR) using two envelope (E) gene specific PCRs (E1 and E2 protocols). E2 differentiates each serotype by product size. E1 PCR products were used for both direct sequencing and cloning. The 10–15 colonies of each sample were sequenced. Direct sequencing results were blasted with GenBank for serotyping. Sequence of each clone was also aligned with that of direct sequencing.

**Results:** All patients were of secondary infection (DHF II). One of three patients (N40) clearly showed mixed DEN1 and DEN2 infections. Plasma, PBMCs and urine during febrile period contained DEN1 whereas convalescent PBMCs showed DEN2 by serotype-specific RT-PCR. Interestingly, clones from convalescent PBMCs PCR product showed mixed populations of DEN1 and DEN2 (%DEN2 > %DEN1). While PCR product direct sequencing of the other two patients clearly presented single serotypes of DEN4 and DEN1, some clone sequences showed populations of mixed serotypes. In patient N33, febrile plasma, PBMCs, saliva and convalescent urine were of DEN4 whereas febrile urine was of mixed DEN4 and DEN2 (% DEN4 > % DEN2). In patient N34, febrile PBMCs, saliva and urine were of DEN1 while convalescent urine was of mixed DEN1 and DEN3 (% DEN1 >

Code	Clinical diagnosis	Days of fever	Febrile period				Convalescent period			
			Plasma	PBMCs	Saliva	Urine	Plasma	PBMCs	Saliva	Urine
N33	DHF II	6	DEN4 (100%) [7]	DEN4 (100%) [7]	DEN4 (100%) [7]	DEN4 (72.73%) DEN2 (27.27%) [7]	-	-	-	DEN4 (100%) [18]
N34	DHF II	6	-	DEN1 (100%) [8]	DEN1 (100%) [8]	DEN1 (100%) [8]	-	-	-	DEN1 (83.33%) DEN3 (16.67%) [14]
N40	DHF II	4	DEN1 (100%) [4]	DEN1 (100%) [4]	-	DEN1 (100%) [4]	-	DEN1 (83.33%) DEN2 (16.67%) [21]	-	-

- = negative result  
( ) = % of each serotype of DENV (total clones of each serotype of all selected clones)  
[ ] = the day of illness that each specimen was collected

DEN3). E2 PCR also confirmed multi-serotype infections in both patients.

**Conclusions:** The phenomena of multi-serotype dengue infections occur in a more sophisticated manner than previously described. All the studied patients, along with almost all adult dengue patients in hyperendemic regions, are re-infected cases from a different serotype from that of the first, earlier infectious episode. We are unable to tell if these three cases were concurrently multi-serotype-infected, or whether the prior-infected and hidden, persistent serotype was reactivated to join the other new one. Further investigations could add up to the incomplete jigsaw of dengue pathogenesis.

## Virology – non-HIV/non-hepatitis

### **P954** Respiratory viral infections during the 2009–2010 winter season in the West Midlands Region, UK: incidence and patterns of multiple virus co-infections

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**Objectives:** Acute viral respiratory infections are the most common infections in humans. Co-infection with different respiratory viruses is well documented but not necessarily well understood. The aim of this study was to utilise laboratory data from the winter season following the 2009 Influenza A(H1N1) outbreak to investigate rates of respiratory virus co-infections.

**Methods:** The Health Protection Agency Public Health Laboratory Birmingham, UK routinely uses PCR to detect a range of common respiratory viruses: influenza A and B, respiratory syncytial virus (RSV), human metapneumovirus (HMPV), parainfluenza, rhinovirus and adenovirus. Results from all specimens received for respiratory virus investigations from late September 2009 to April 2010 were downloaded and multiple virus detections in single specimens were identified. For each virus, the differences between the expected prevalence (i.e. the prevalence in all specimens) and the observed prevalences in specimens positive for other viruses, were calculated. Poisson regression was employed in order to determine the statistical significance of any associations found between different pairs of co-detected viruses.

**Results:** A total of 4821 specimen results were analysed. Of these, 2447 (50.8%) had at least one respiratory virus detected. Three hundred and twenty three (13.2%) had co-detections of two viruses, 22 (0.9%) had three viruses and four (0.2%) had four viruses. Reciprocal patterns of positive or negative associations between different virus pairs were found. Statistical analysis confirmed the significance of negative associations between influenza A and HMPV (p value 0.013) and influenza A and rhinovirus (p value 0.026). Positive associations between parainfluenza and rhinovirus (p value 0.003) and rhinovirus and RSV (p value 0.003) were also significant.

**Conclusion:** This study found that that co-detection of different respiratory viruses is not random and most associations are reciprocal, either positively or negatively. The most striking association observed was the lower prevalence of influenza A(H1N1) in patients positive for adenovirus, RSV, parainfluenza, HMPV and rhinovirus and vice versa, which reached statistical significance with HMPV and RSV.

**P955** **Viral load relative quantification of influenza virus in paired lower respiratory tract and nose-throat swabs samples from patients admitted in intensive care units**

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**Background:** Influenza is a well known cause of admission to intensive care units (ICU's). Nasopharyngeal aspirates or Nose-Throat Swabs (NTS) specimens are considered the specimens of choice for the detection of influenza although most of the data come from paediatric population and are based on viral culture as the reference. In many ICU patients Lower Respiratory Tract (LRT) samples are readily and available. The aim of the study was to compare the yield of NTS with LRT for detection of Influenza virus by real time RT-PCR in patients admitted to ICU's.

**Methods:** From December 2010 to February 2011, we performed a prospective comparative study to detect influenza virus by RT-PCR in diagnostic NTS and LRT specimens from adults admitted in ICU's with suspected influenza. Relative quantification of viral load was performed in paired samples from patients in whom both samples were positive for influenza.

**Results:** During the study period, we included 52 patients admitted to ICU's, with suspicion of influenza, with paired NPA and LRT specimens. In 21 patients influenza virus was detected in at least one sample. All but one (influenza B) viruses detected were influenza A (H1N1) 2009. In 13/21 (62%) patients the virus was detected in the NTS and in 19/21 (90.5%) patients in the LRT. In 11 (52.4%) patients the virus was detected in both specimens. The validity values of NTS and LRT were respectively as follows: Sensitivity (62%, 90.5%), and Negative Predictive Value (79.5%, 93.9%). We detected higher amount of virus in LRT than in NTS samples but the difference did not reach statistical significance (median LRT/NTS for paired samples: 2.025; IQR: 0.039–6.374;  $p = 0.638$ )

**Conclusion:** Influenza detection by RT-PCR was more efficient in LRT than in NTE. In ICU patients with clinical suspicion of influenza, a negative RT-PCR in NTS is insufficient to rule out the entity. We recommended to search for influenza virus both in NTS and LRT.

**P956** **Identification and complete genome analysis of a novel feline picornavirus from the domestic cat in Hong Kong**

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**Objectives:** Picornaviruses are known to infect human and various animals in which they can cause many diseases of varying severity. Since the role and existence of picornaviruses in cats have been largely unknown, we attempted to study the presence of previously undescribed picornaviruses in domestic cats in Hong Kong.

**Methods:** A cat surveillance study was conducted during a 39-month period, in which samples from 662 stray cats captured from 32 different locations in Hong Kong were collected. Viral RNA extracted from respiratory, fecal, urine and serum samples was used as the template for RT-PCR. Initial picornavirus screening was performed by RT-PCR of 3Dpol gene of picornaviruses using conserved primers. And subsequent screening of novel "feline picornavirus" ("FePV") was performed by RT-PCR of 2C gene using specific primers, which were designed from the sequence of the first positive sample detected in the initial screening. Five genomes of "FePV" were amplified and sequenced, and the sequences were compared to those of other picornaviruses.

**Results:** "FePV" was detected in fecal samples of 14 cats and urine samples of two cats by RT-PCR among 662 cats. Analysis of five "FePV" genomes revealed distinct phylogenetic position and genomic features. The five "FePV" strains formed a distinct group among known picornaviruses in all three phylogenetic trees constructed using the P1, P2 (excluding 2A) and P3 (excluding 3A) regions. While the high sequence similarity and similar genome organization of the five strains suggested a

single species of "FePV", two potential genotypes may exist based on phylogenetic analysis of VP1 sequences. "FePV" is most closely related to the recently described bat picornaviruses group 1–3 and the genus Sapelovirus, while analysis of G+C content and sequences of P1, P2 and P3 regions showed that it is more closely related to bat picornaviruses, especially bat picornavirus group 3, than to sapeloviruses. "FePV" also exhibited other unique genomic features, including a putative type IV IRES instead of type I IRES in bat picornavirus group 3, different protein cleavage sites and H-D-C catalytic triad in 3Cpro, and the shortest leader protein among picornaviruses.

**Conclusion:** In this study, we identified a novel feline picornavirus from the domestic cat in Hong Kong. Based on its distinct phylogenetic position and genomic features, we believe "FePV" may be more appropriately classified under a new genus separate from Sapelovirus.

**P957** **Group A human rotavirus types circulating over a 25-year period in Palermo, Italy: a single-centre surveillance study**

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**Objectives:** To analyze the molecular epidemiology of human rotavirus (HRVs) in Palermo (Italy) over a 25-year period.

**Methods:** Faecal samples from 5623 children aged <5 years hospitalized with gastroenteritis in Palermo, Italy, from 1985 to 2010 were screened for the presence of rotaviruses by EIA and/or RT-PCR. Positive specimens were G/P genotyped through multiplex nested RT-PCR.

**Results:** HRV infection was detected in 33.3% of the paediatric patients. Yearly rates of infection varied from 11% to 51.6%. Strains with G1–G4 specificities accounted for more than 90% of HRV infections until 1999, when HRVs of the G9 type emerged. HRV strains belonging to the G1 type were the most common and were constantly detected but their relative prevalence varied widely, from a maximum of 88.9% in 2010 to a minimum of 6.4% in 2006. G2 strains were epidemic in 1996–97 but otherwise displayed a low prevalence, except in 2003 and 2007 when they represented 22.6% and 14% of the strains, respectively. G3 rotavirus circulation was limited and accounted generally for no more than 6.1% of the HRV strains, but in 2003 and 2005 they were involved in 17% and 16.9% of the gastroenteritis episodes. G4 epidemics occurred in 1990–1993, 1999–2001 and 2003. G9 HRVs were introduced in Palermo in 1999 when they represented 31.4% of the strains detected. In the following years G9 circulation fluctuated, almost disappearing before raising again, in 2005–2006 and 2008, so that G9 became the second most common G-type in the last 12 years. Human/animal reassortant G3P[9] strains were detected in 1994 and 1996 and a G3P[3] canine rotavirus was isolated in 1997, while G6 strains of animal origin with either a P[9] or P[14] VP4 specificity were sporadically isolated from the late 1980s to 2003. G1P[4] and G2P[8] strains were also sporadically detected.

**Conclusion:** Over the 25-year surveillance period, the circulation of the most common HRV combinations, G1P[8], G2P[4], G3P[8], G4P[8] and G9P[8], was confirmed, but unusual G/P combinations were occasionally found. The prolonged surveillance of HRV infection performed in Palermo allowed to confirm the important role of HRVs as agents of enteritis in the Italian paediatric population and highlighted the dynamics of circulation of the different genotypes.

**P958** **Molecular epidemiology of rotavirus strains in Bulgaria without rotavirus mass vaccination, 2009–2010**

A. Dikova\*, A. Stoyanova, Z. Mladenova (Sofia, BG)

**Objectives:** Rotaviruses are a leading cause of infantile acute gastroenteritis worldwide responsible for a great morbidity and economic losses in developed countries and significant mortality in developing countries. The study aimed to investigate the rotavirus

epidemiology and strain diversity in Sofia-city and Sofia-region, before the introduction of rotavirus vaccination. Methods:

For the period of September 2008 to August 2010 a total of 600 stool samples from children 25 days–12 years old were tested and confirmed as rotavirus-antigen positive using immunoassays. All children were hospitalized exhibiting symptoms of diarrhoea and/or vomiting. The rotavirus strains detected were further characterized using molecular-based techniques—RT-PCR, sequence and phylogenetic analysis toward VP7 and VP4 genome segments.

**Results:** The results revealed that most affected of rotavirus gastroenteritis were children 1–24 months old (70.7%, 424/600). Year-round circulation of rotavirus strains were recorded with a clear winter peak (December–April) and a second peak emerging in August–September. Rotavirus-positive samples which contained a single rotavirus strain accounted for 79.5% of the samples. Of them, the pre-dominant strain was G4P[8] (36.0%, 216/600), followed by G2P[4] (25.0%, 150/600), G1P[8] (15.3%, 92/600), G9P[8] (2.5%, 15/600) and G3P[8] (0.7%, 4/600). Increased incidence of rotavirus human-human reassortants (22, 3.7%) and emergence of rare/uncommon or potentially zoonotic rotavirus strains was seen in the absence of rotavirus mass vaccination. Among rotavirus reassortants the prevalent were strains with G1P[4] (16 samples), followed by G9P[4] (three samples), G4P[4] (two samples) and G2P[8] (one sample). Unusual rotavirus strains with porcine or feline-like characteristics (G3P[9], G4P[6], G5P[6]) along with isolates with G6P[8], G6P[9], G8P[8], G8P[14] and G12P[8] specificity were detected in a single samples. Sequence and phylogenetic analysis revealed that rotaviruses with G1–G4 genotype circulating in Bulgaria were distantly related to G1–G4 strains included in rotavirus vaccines available. Conclusion: Rotavirus immunization would be an effective preventive tool against severe rotavirus gastroenteritis in Bulgaria. Active surveillance and investigations over the fluctuations in rotavirus epidemiology and strain diversity before and after the introduction of rotavirus vaccine will need as basis in attaining success of the rotavirus vaccination program.

**P959** **Norovirus can be detected by PCR in oral swab samples from patients with acute gastroenteritis**

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**Objective:** Norovirus (NoV) can be detected by PCR in samples from stool or vomit in almost all patients with acute NoV-infection. In a recently published study NoV was detected by PCR in mouthwash samples in 24% of patients with acute NoV-enteritis. We have previously shown that rectal swab samples are comparable to traditional stool samples for diagnosis of acute NoV infection. The objective of this study was to investigate if NoV can be detected in oral flocculated swab samples from patients with acute gastroenteritis.

**Methods:** Between October 2010 and April 2011, eighty-six hospitalised patients with suspected viral gastroenteritis who were admitted to the Department of Infectious Diseases, Sahlgrenska University hospital, Göteborg, Sweden were prospectively included. One oral flocculated swab sample, obtained from the oral mucosa of both cheeks, and one rectal swab sample were taken from each patient. Clinical data and symptoms were registered. The samples were analysed with a previously described multiple real-time PCR detecting six different gastroenteritis viruses (NoV genogroup I and II, sapovirus, astrovirus, adenovirus and rotavirus).

**Results:** Forty-eight of the 86 included patients (56%) were positive for NoV genogroup II in the rectal swab sample, and were subsequently included in the analysis. Twenty-eight patients (58%) were female, the median age was 80 (IQR 64–88) years and the median duration of symptoms was 3 (IQR 2–4) days. Twelve out of 48 (25%) were positive in the oral swab sample, and 9 (75%) of these reported vomiting the day before sampling compared to 24 of 36 (67%) patients with negative oral swab samples (ns.). Only two patients reported vomiting on the day of sampling, none of which were positive in the oral swab sample. In the patients with a positive oral swab sample the cycle threshold (Ct) values

were significantly lower in the rectal swab samples than in the oral swab samples (the median (IQR) Ct value was 20.5 (17.9–22.6) and 35 (32.5–40.4), respectively,  $p < 0.001$ ), indicating that the amount of virus was higher in rectal swab samples.

**Conclusion:** NoV could be detected in oral flocculated swab samples in a quarter of patients with NoV-enteritis, which is in accordance with the detection frequency reported for mouthwash samples. The amount of virus in oral swab samples was low.

**P960** **The first, second and third wave of pandemic influenza A (H1N1) in North Denmark region, 2009–2011: a population-based study of hospitalisations**

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**Objectives:** A new Influenza A (H1N1) virus emerged in 2009, leading to a world-wide pandemic. We analysed the epidemiology and clinical characteristics of hospitalized patients in a defined population of North Denmark Region with a mixed urban and rural community of 579 000 inhabitants.

**Methods:** Review of medical records of all hospitalized patients with confirmed Influenza A from July 2009–February 2011.

**Results:** Two hundred and seventy three patients with a positive PCR test for pandemic Influenza A (H1N1) from respiratory tract samples were admitted to hospital: 111 children (0–14 years) and 162 adults (>15 years). The age-related population incidences of hospitalization were, 0–14 years: 111/100 000, 15–64 years: 39/100 000, and >65 years: 17/100 000. During the first wave (July 2009–August 2009) three patients were admitted – none received treatment in Intensive Care Units (ICUs), during the second wave (October 2009–January 2010) 158 patients were admitted – nine received treatment in ICUs, and during the third wave (December 2010–February 2011) 112 patients were admitted – 25 received treatment in ICUs.

The length of hospitalization for all patients was on average 8 days (4 days for children, 11 days for adults).

Thirty of 34 patients treated in ICUs (average of 22 days) received ventilator therapy (average of 19 days), and seven patients received dialysis (average of 19 days).

Eighteen of 111 children (16%) and 114 of 162 adults (70%) were treated with oral oseltamivir.

No children and 14 of 162 adults (9%) were treated with intravenous zanamivir due to clinical failure of oseltamivir. Only two cases of H275Y mutation were observed, and these were not related to therapeutic failure.

Thirty three patients were diagnosed with concomitant bacterial pneumonia, predominantly caused by *Streptococcus pneumoniae* (n = 10), *Haemophilus influenzae* (n = 7) and *Staphylococcus aureus* (n = 3).

Fourteen of 273 patients (5%) died within 30 days of diagnosis, one child (14 years old) and 13 adults (41–76 years).

**Conclusion:** Patients hospitalized with pandemic Influenza A (H1N1) were predominantly children and younger adults, and only a few patients were >65 years. The third wave was the most severe taking the number and percentage of patients admitted to ICUs and 30-day mortality into consideration. We find it remarkable, that the incidence of hospitalization as well as clinical severity among younger adults did not decline from the second to the third wave.

**P961** **Actinobaculum schaalii a part of the normal flora of the genital area**

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**Objective:** To study the habitat of *A. schaalii*, and if it is a uropathogen in patients with kidney or urethra stones.

**Method:** A quantitative real-time PCR assay was used to examine 38 urine samples, 38 groin swabs, 30 faecal samples and 19 vaginal swabs.



**Results:** *A. schaalii* was found by real-time PCR in 14 (37%) urine samples from the patients undergoing extracorporeal shock wave lithotripsy (ESWL). Five (36%) of the urine samples containing *A. schaalii* were stix positive for leukocytes compared to 2 (8%) from urine without *A. schaalii* ( $p = 0.10$ ). *A. schaalii* was found in 7 (18%) of the groin swabs and predominantly from females ( $p = 0.10$ ).

*A. schaalii* was not found in any of the faeces samples but 6 (32%) of the vaginal swabs contained *A. schaalii* predominately from females above 50 years ( $p = 0.06$ ).

**Conclusion:** This study indicates that *A. schaalii* is a commensal on skin in the genitals and can be a cause of UTI. When patients show clinical signs of urinary infection and with leukocytes in the urine but no growth by culture, *A. schaalii* and its requirement in growth medium should be considered.

**P962** **Virological tests on broncho-alveolar lavage fluid have a low yield except in immunocompromised patients with ground-glass attenuations on CT-scan**

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**Objectives:** Broncho-alveolar lavage (BAL) is a major diagnostic tool in selected patients. Although viruses have been incriminated in a wide range of respiratory diseases, the diagnostic yield of virological tests on BAL fluid remains poorly characterized. We aimed to analyze the characteristics of patients in whom viruses were identified in BAL fluid, to better define indications for virological tests during BAL.

**Methods:** All patients who underwent virological analysis on BAL fluid at the Rennes University Hospital – a 1435 bed tertiary care center – during year 2008, were retrospectively studied. Data were extracted using a standardized questionnaire, through the review of medical charts, imaging studies (all with radiologist interpretation), and microbiology laboratory database. The following patients were classified as immunocompromised: (i) patients with chronic immunodeficiency, including HIV; (ii) patients on corticosteroids >10 mg/day for  $\geq 3$  weeks; (iii) patients on any immunosuppressive drugs. We compared characteristics of patients with positive vs negative BAL virological analysis, using nonparametric Student ( $n > 30$ ), or Mann–Whitney ( $n < 30$ ) test for quantitative variables, and Fisher exact test for categorical variables.

**Results:** In 2008, 232 BAL were tested for viruses, in 212 patients (mean age  $54.4 \pm 45.4$  years; female-to-male ratio 0.6). Of these 232 BAL, 70 (30%) were positive, yielding 84 viruses: herpes simplex virus 1 ( $n = 27$ ), cytomegalovirus ( $n = 23$ ), Epstein–Barr virus ( $n = 17$ ), human-herpes virus 6 ( $n = 12$ ), respiratory syncytial virus ( $n = 3$ ), rhinovirus ( $n = 1$ ), and adenovirus ( $n = 1$ ). Pneumonia in immunocompromised patients was the most common indication for virological tests on BAL (55%), with a diagnostic yield of 44% in this group, as compared to 0% in the 17 patients with diffuse infiltrative lung disease. Immunocompromised patients represented 83% of virus-positive BAL. On bivariate analysis, only immunosuppression (82.9% vs 46.9%,  $p < 0.0001$ ), and ground glass attenuations on chest CT-scan (65.6% vs 43.7%,  $p = 0.006$ ), were more common in patients with virus-positive BAL, as compared to patients with virus-negative BAL. There were no significant differences in demographic and clinical criteria (age, gender, fever, dyspnea, cough, hemoptysis).

**Conclusion:** Pneumonia in immunocompromised hosts is the only situation where virological analysis of BAL fluid is of value, especially when chest CT-scan demonstrates ground-glass attenuations.

**P963** **Discrepancies between rubella IgG results: which assay is right, which assay is wrong?**

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Rubella-specific IgG testing is performed either for epidemiological studies, determination of the immune status or confirmation of rubella infection (together with the detection of rubella-specific IgM). Many

techniques have been developed for the detection of rubella-specific IgG but today, enzyme and chemiluminescent automated immunoassays are those most commonly used.

For over 15 years, commercial immunoassays for Rubella-specific IgG have been calibrated with a WHO international standard, and results are reported in IU/mL. The positivity threshold varies according to the assays: 10 IU/mL (recommended in US) or 15 IU/mL (commonly used in Europe). However, expressing results in IU/mL should have improved the correlation between assays. Actually, the antigen(s) used in the assays (total virus, recombinant antigens), the conjugate, the assay format (indirect, sandwich, competition, capture...), differ from one kit to another one. Under these conditions, even though commercial assays are calibrated with the same international standard, large differences in the result(s) obtained may be observed when testing the same sample, depending on the assay used. This situation is very confusing for clinicians and biologists and lead to misinterpretations of serology.

Firstly, we report the results of 10 sub-national quality controls, concerning rubella-IgG, conducted in French laboratories during a 3-year period (2009–2011). Results show that, depending on the assay used, results can differ by a factor up to 10!

A second part of this work focused on discrepant results between Elecsys® rubella IgG assay (Roche) and other assays. Since 2007, 178 serum samples were referred to our laboratory, because of discrepant results between Elecsys® rubella IgG assay (Roche) and another assay. Specificity was controlled on 137 samples, which were positive with the Roche assay, by using an in-house Western-blot. Anti-E1 Rubella antibodies were detected in 133 (97%) samples proving that Elecsys® rubella IgG assay is specific but much more sensitive than many other commercial assays.

Rubella specific-IgG results are often discordant, depending on the assay used even though results are expressed in IU/mL. However, the most sensitive assays are specific, as proved by Western-blot. Due to these discrepancies, consecutive samples should always be tested in the same laboratory using the same assay. Finally, given these conditions, one may question the usefulness of expressing results in IU/mL!

**P964** **Evaluation of serum cytokine levels as severity criteria in CCHF patients**

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**Background:** Crimean–Congo hemorrhagic fever (CCHF) is a potentially fatal disease caused by a tick-borne virus from the Bunyaviridae family. Cytokines plays an important role in the pathogenesis of viral, bacterial, and immunologic diseases. This study aimed to evaluation of serum cytokine levels as severity criteria in CCHF patients.

**Study design:** Sixty patients with confirmed CCHF were recruited for this study. Patients were divided into two groups (severe group: Patients who exhibited hemorrhage during their hospital stay, and non-severe

	Severe CCHF n=22	Non-severe CCHF n=38	P
Age	47,9±19,7	48,8±16,9	0,860
Gender (male/female)	10/12	15/23	0,856
IL-1 alpha	2,12±0,5	2,17±0,6	0,731
IL-1 beta	1,7±0,5	1,8±0,8	0,799
IL-2	2,86±0,48	3,06±1,27	0,836
IL-4	6,4±2,7	8,6±3,7	0,021
IL-6	62,7±40,6	34,2±20,4	0,000
IL-10	6,8±2,6	5,8±0,9	0,024
IL-12	10,2±2,6	11,2±3,3	0,262
IL-15	60,8±96,8	40,9±83,7	0,634
IFN-gama	161,3±135,4	121,5±80,1	0,330
TNF-a Iffa	68,6±49,5	43,4±17,3	0,034
TGF-beta	4,93±2,04	6,54±2,83	0,025
WBC	3090±4100	2657±1119	0,124
PLT	58177±38956	110315±44876	0,000
CRP	3,02±2,9	1,4±1,7	0,032
ALT	228±278	104±98	0,059
AST	564±941	166±214	0,000
CPK	1374±1383	509±651	0,021
LDH	1334±1213	562±469	0,000

group: Patients who displayed no hemorrhage during their hospital stay). Demographic characteristics, laboratory tests on admission of all patients with CCHF were investigated, and serum cytokine levels were measured.

**Results:** A total of 60 patients with confirmed CCHF were investigated. Twenty-two (36.7%) of these patients were in the severe group. In patients with severe CCHF, significantly higher serum levels of IL-6 ( $62.7 \pm 40.6$ ;  $p = 0.000$ ), IL-10 ( $6.8 \pm 2.6$ ;  $p = 0.024$ ), TNF-alpha ( $68.6 \pm 49.5$ ;  $p = 0.034$ ), and significantly lower serum levels of IL-4 ( $6.4 \pm 2.7$ ;  $p = 0.021$ ), TGF-beta ( $4.93 \pm 2.04$ ;  $p = 0.025$ ), were detected, compared with cytokine levels in patients who non-severe CCHF (Table 1). No differences in serum IL-1 alpha, IL-1 beta, IL-2, IL-12, IL-15 and IFN-gama levels between patients who severe CCHF and those who non-severe CCHF were detected ( $p > 0.05$ ).

**Conclusions:** Cytokines, chemokines, and other inflammatory mediators function in a manner, acting on many different cell types to regulate the host's immune response. When cytokines present in high concentrations, they might toxic or even lethal effects. In accordance with this view, In accordance with this view, our study significantly changed serum levels of cytokines in the patients with severe CCHF.

#### **P965** HPV infection linked to increased risk of breast cancer in north of Iran

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**Objectives:** Breast cancer is the most frequently diagnosed cancer and the leading cause of cancer deaths among women worldwide. Viruses including Human papillomavirus (HPV) have been related to the pathogenesis of breast cancer. In the present study, we conducted a case-control study of breast cancer patients and controls and tried to find a viral aetiology by detection of HPV genome, and to evaluate the possible relevance of this factor for breast cancer risk among north female Iranian population from Mazandaran province.

**Methods:** A total of 130 blocks of paraffin-embedded tissue including 79 samples diagnosed as breast carcinomas and 51 non-cancer samples as control were retrieved from archive of Imam Khomeini Hospital, Medicine faculty of Sari city, Mazandaran University of Medical Sciences, Iran between 2002 and 2009. Polymerase chain reaction method with three different primer sets was applied for detection of HPVs in formalin-fixed paraffin-embedded breast cancer and breast fibroadema as case and control tissues, respectively. HPV genotypes were determined by phylogenetic analysis of viral genome sequences.

Phylogenetic analysis of these partial genome sequences was conducted using MEGA version 4.0 along with representatives of the HPV genotypes described previously.

**Results:** Of the 58 DNA extraction quality confirmed breast cancer cases, 25.9% (15 isolates) had positive test results for HPV DNA in contrast to 2.4% of cases (1 out of 41) with non cancer status ( $p = 0.002$ ). The infection of HPV had an OR of 14.247 (95% CI 1.558–130.284;  $p = 0.019$ ). The HPV genotypes in samples of breast cancer patients were 26.67% for HPV-16 (four isolates) and HPV-18 (four isolates), 13.3% for HPV-23 (two isolates) and HPV-6 (two isolates), 6.67% for HPV-11 (one isolate), HPV-15 (one isolate) and HPV-124 (one isolate) and one isolate could not be genotyped compared to HPV reference sequences, while the sole detected HPV in control specimens was HPV-124.

**Conclusions:** Our study reveals that HPV infection and age are the risk factors in breast cancer development in the north part of Iran, Mazandaran province. Meanwhile, confirming an etiologic role for HPV in breast cancer may help develop vaccine strategies for combating this increasingly common cancer. The association between risk of breast cancer development and viral infection is open and deserves further investigation.

#### **P966** Rotavirus, norovirus and adenovirus gastroenteritis in hospitalised children, Turkey

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**Objectives:** This study aims to determine the prevalence and the distribution of Enteric viruses (especially rotavirus, norovirus and adenovirus) responsible for gastroenteritis in children.

**Methods:** A epidemiological study on common diarrheal viruses was conducted in Afyon City, Turkey between January and November 2009. One hundred and fifty faecal samples from children under 6 years of age (mean age,  $2.18 \pm SD 3.64$  years, range: 1–72 months) (negative for the presence of pathogenic bacteria by standard culture methods) were tested by ELISA (Ridascreen and Biomeriux) and RTPCR methods for detection of- Norovirus G1, G2. Stool samples positive for group A rotavirus by commercial enzyme immunoassay were subjected to reverse transcription-polymerase chain reaction based genotyping of the outer capsid antigens, VP7 and VP4, determining G and P type specificities, respectively.

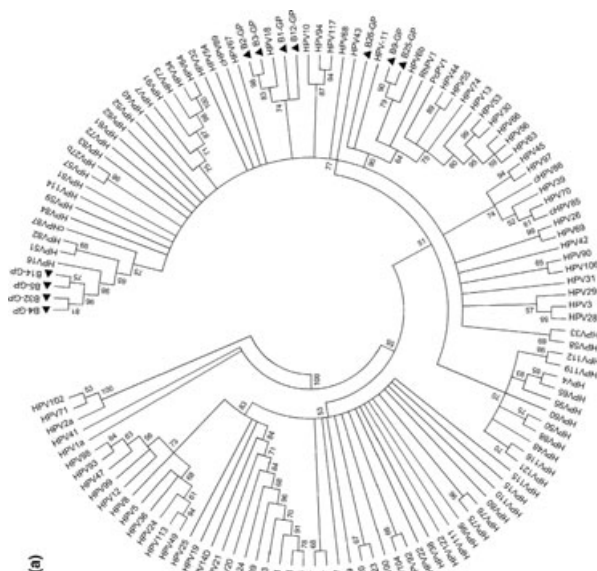
**Results:** Norovirus were detected in 22.8% of 92 children (<6 years of age) and rotavirus and adenovirus were detected in 23.3% and 6% of 150 hospitalized for gastroenteritis in Afyonkarahisar, Turkey, during 2009, respectively; predominant genotypes were GGIIb/Hilversum and GGII.4 Hunter for norovirus. The most common rotavirus strain was G2P[4] ( $n = 16$ ), followed by G9P[8] ( $n = 7$ ). Other strains were G1P[8] ( $n = 3$ ), G2P[8] ( $n = 3$ ), G1 + 2P[8] ( $n = 2$ ), G9P[4] ( $n = 1$ ), G2 + 9P[8] ( $n = 1$ ), G4 + 9P[6] ( $n = 1$ ), and G2P[4 + 8] ( $n = 1$ ). Of children with viral enteritis, 6.5% had a mixed norovirus-rotavirus infection.

**Conclusions:** Rotavirus is still the most common cause for gastroenteritis. Norovirus might be a more frequent agent in patients with vomiting prominent. The severity of infection by norovirus was lower but an increase was observed with rotavirus co-infection.

#### **P967** Analysis of HPV 6 variability in cervical and anal clinical samples

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**Objectives:** Human papillomavirus genotype 6 (HPV6) is the major causative agent of genital warts and the most frequent benign tumours in the anogenital region. HPV 6 is regarded as a low risk HPV genotype and has been included in the recently developed quadrivalent HPV vaccine. On the basis of nucleotide sequence comparisons, HPV6 isolates are usually grouped into prototype



HPV6b-related (prototypic) and HPV6a and HPV6vc-related (non-prototypic). Non-prototypic HPV6 genomic variants seem to predominate in genital warts. The level of genomic diversity has been well established for some of the high-risk HPV genotypes, identifying some viral variants that differ in pathogenicity, transmission, persistence and progression of the clinical disease. Similar knowledge about low-risk HPVs such as HPV6 is scarce and in Italy there are no published papers about variants of HPV6. In this work, we analyzed different isolates from ano-genital samples of HPV6 using nucleotide sequence alignments of L1 region amplified by primer MY09/MY11.

**Methods:** A total of 75 HPV6 positive samples were included in this study, and the variability of the genomic fragment obtained by MY09/MY11 PCR assay (nt 6764–7167), was analysed by sequencing. The identification of HPV 6 genomic variants analysis was done with the ClustalW Multiple alignment program of the BioEdit Sequence Alignment Editor v7.0.9.0 program package identified using the prototype HPV6b genome (GenBank accession no. X00203) as a reference genome.

**Results:** The sequences of 42 cervical samples presented nucleotide substitution from prototype HPV6b in 14% (6/42) of analyzed sequence whereas 33 anal samples presented nucleotide substitution in 27% (9/33). Aminoacid substitutions from the prototype HPV6b L1 sequence were five silent and nine missense (conservative and semi-conservative) mutations (see table).

HPV6	6764	6779	6790	6792	6829	6866	7003	7076	7079	7120	7121	7129	7145	7155	7158	7167
X000203	T	G	C	G	T	A	A	A	G	T	A	A	G	G	A	G
A257c				A					C							
A166c		A						C	C							
A158c								C	C							
28c								C	C			G			T	
68Ac					G	G										
134Bc																
104Va			A													
252Va					G			C	C							
221Va							C									
462Va											G					
254Va																
27a								C	C				G			
190Va															C	
531Va														A		
codon	326	331	334	335	347	356	405	430	431	444	445	447	453	456	457	460
Prototype aa	F	D	R	S	S	K	P	K	E	V	N	K	E	Q	Y	G
Mutated aa	F	N	R	N	S	K	P	Q	Q	V	D	K	K	H	F	G

**Conclusion:** In the present study we have analyzed the nucleotide and amino acid sequence variability of L1 sequence amplified by MY09/MY11 primers, revealing a wider variability than previously reported. This study revealed 12 not previously reported locations where nucleotide substitutions occurred and 6 isolates presented more than one substitutions. Our results support the need analyze the different isolates of HPV6 and investigate about a possible correlation with tissue tropism and clinical disease.

### P968 Varicella-zoster disease of the central nervous system in adults in a university hospital

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**Objective:** The presence of shingles is the clinical clue for suspicion of varicella-zoster virus (VZV) aetiology in CNS infections (CNSI). In the latest months we have observed a cluster of CNSI by VZV. Most of them did not have shingles.

The aim of this study was to know the clinical presentation of CNSI by VZV paying special interest in the presence of skin injuries.

**Methodology:** We have reviewed the clinical histories of all the cases of CNSI by VZV in adults from January 2006 to June 2011 in a university hospital that holds 600 beds. We performed a multiplex nested polymerase chain reaction (PCR) for herpesviridae routinely in all cases of aseptic CNSI.

**Results:** A total of 12 cases were detected, 5 of them in the first semester of 2011, corresponding to five meningitis, five encephalitis and two myelitis. Most cases took place in spring (58.3%) and summer (25%). No seasonal predominance of encephalitis and myelitis was seen but meningitis was predominant in spring (80%).

Mean age was 55.9, being females 58.3%, 83.3% had suffered from varicella in infancy and no patients had received vaccination against VZV. 58.3% presented skin lesions due to VZV, all of them but 1 with shingles. Table 1 shows the clinical presentation, CSF data and comparative study between meningitis and encephalitis. Proportions were compared with Fisher's exact test and continuous variables with Student's *t*-test. Patients with meningitis were younger, had had epidemiological contact with a patient with VZV infection more frequently, presented a lower rate of immune deficiency and did not present skin injury by VZV.

All cases presented CSF alterations, 91.7% pleocytosis, always with lymphocitary predominance. Sixty percent patients with meningitis presented hypoglycorrhachia.

All patients received acyclovir IV (10–21 days) and 25% finished the treatment with oral acyclovir or valacyclovir.

Glasgow outcome scale (GOS) at discharge was "V" in 83.3%. Only patients with myelitis presented neurological sequels corresponding to GOS IV (8.3%) and GOS III (8.3%). There was no mortality.

VARIABLES	MENINGITIS	ENCEPHALITIS	MYELITIS	P
n (%)	(n=5)	(n=5)	(n=2)	Me vs E
Age, median (SD)	40 (19.7)	72.6 (17.4)	54	0.02
Male	3 (60)	2 (40)	0	NS
Immune deficiency or immunosenescent	1 (20)	5 (100)	2 (100)	0.02
Previous chickenpox	4 (80)	5 (100)	1 (50)	NS
Vaccination VZV	0	0	0	NS
VZV contact	3 (60)	0	1 (50)	0.04
Fever	2 (40)	0	1 (50)	NS
Headache	5 (100)	1 (20)	0	0.01
Vomiting, nausea	4 (80)	0	0	0.01
VZV rash	0	5 (100)	2 (100)	0.001
Backneck stiffness	2 (40)	2 (40)	1 (50)	NS
Focal neurologic sign	0	1 (20)	2 (100)	NS
GOS = V	5 (100)	5 (100)	0	NS
CSF WBC (cell/mm3) median (SD)	333.8 (217.3)	44.5 (50.5)	111	0.03
CSF WBC >100	5 (100)	1 (20)	1 (50)	0.01
CSF glucose (mg/dl) median (SD)	48 (16.2)	120.25 (54.5)	65	0.02
CSF glucose < 45	3 (60)	0	0	0.04
CSF protein (g/L) Median (SD)	1.95 (1.6)	0.71 (0.6)	1.52	NS
CSF protein > 1	4 (80)	1 (20)	1 (50)	0.05

**Conclusions:** Patients with meningitis by VZV are younger, healthy, and do not feature shingles. In contrast, patients with encephalitis present immune deficiency and skin manifestations of VZV infection.

The seasonal predominance and the epidemiological contact with VZV in patients with meningitis could be related to a reinfection. In contrast, patients with encephalitis corresponded to the classical reactivation of VZV.

**P969** Evaluation of the new Adeno Respi K-Set in comparison to the Adeno Respi-Strip and cell cultures for the detection of adenoviruses

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**Objective:** To evaluate the performances of the new ready to use immunochromatographic test (ICT), Adeno Respi K-Set (Coris BioConcept, Belgium), in comparison to the Adeno Respi-Strip (Coris BioConcept, Belgium) and cell cultures for the diagnosis of adenoviruses in clinical samples under routine conditions.

**Methods:** from April to June 2011, 492 clinical samples submitted for the rapid diagnosis of adenovirus infection were evaluated with Adeno Respi K-Set.

Results were compared with those of Adeno Respi-Strip and viral cell cultures performed on three cell lines (Vero, MRC5 and LLC-MK2). The samples included 416 nasopharyngeal aspirates, 56 nasopharyngeal swabs, 11 throat swabs, three tracheal aspirations, two bronchoalveolar washes, two sputums and two urines. They were issued from 261 male and 231 female patients aged from 1 month to 90 years (mean age: 10 months). Results were compared with those of cell culture considered as the reference method but home made Adenovirus PCR was used to elucidate discrepant results.

**Results:** of the 492 samples tested, 63 (12.8%) were positive for adenovirus in one or more cell cultures. Sensitivities of the ICT were 74.62% and 74.62% for the Adeno Respi K-Set and the Adeno Respi-Strip respectively. Specificities were 100% and 99.05% for the Adeno Respi K-Set and the Adeno Respi-Strip respectively. The positive and negative predictive values found were 100% and 96.15% and of 92.59% and 96.11% for Adeno Respi K-Set and Adeno Respi-Strip respectively. For Adeno Respi K-Set, no false positive result was observed among samples yielding a positive culture for viruses other than adenovirus (56 parainfluenza, 50 rhinovirus, 21 enterovirus, 5 metapneumovirus, 5 cytomegalovirus, 5 HSV, 4 respiratory syncytial virus, 1 influenza A). There was only one equivocal result with Adeno Respi-Strip in an HSV positive culture. Three of the false negative results with the Adeno Respi K-Set were observed in slimy samples. Those samples were positive with the Adeno Respi-Strip indicating probably slightly better performances in such samples.

**Conclusions:** The new Adeno Respi K-Set is a reliable and easy to use test for rapid diagnosis of adenovirus infection in comparison with methods used in our routine practice. This test could be used for patients for which early adenovirus diagnosis is needed or for epidemiological studies.

**P970** A phase 1, single ascending-dose study of AVI-6003, a combination of two PMOplus™ compounds with activity against marburgvirus

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**Objective:** Marburg hemorrhagic fever (MHF) is a rare human disease caused by marburgvirus, a filamentous single-stranded, negative-sense RNA virus of the family Filoviridae. No vaccine or established effective therapy is currently available for this catastrophic disease. AVI-6003 is an experimental combination of two phosphorodiamidate morpholino oligomers with positive charges (PMOplus™) that specifically target two viral messenger RNAs (mRNAs) encoding marburgvirus proteins. AVI-6003 has demonstrated evidence of protection against lethal infection in experimental mouse, guinea pig and non-human primate models of marburgvirus infection. The objective of this clinical study is to determine the safety, tolerability and pharmacokinetics of

intravenous administration of AVI-6003 in healthy human subjects over a dose range predicted to cover a therapeutic dose.

**Methods:** In this first-in-man study, 30 healthy male and female subjects between 18 and 50 years of age were enrolled in six dose escalation cohorts of five subjects each and received a single intravenous (IV) infusion of AVI-6003 (0.01, 0.1, 1.0, 3.0, 6.0 and 9.0 mg/kg) or matched placebo in a 4:1 ratio. Safety was monitored through adverse event collection, telemetry, oximetry and serial blood tests, urine tests and electrocardiograms. The study was overseen by an independent Data Safety Monitoring Board (DSMB).

**Results:** No significant safety concerns arose upon review of blinded study data from the first 5 cohorts by the independent DSMB. While 10 of the first 25 subjects dosed experienced a variety of adverse events such as headache (n = 3), almost all were mild or moderate in severity. The only exception was one episode of exacerbation of chronic schizophrenia, which was not considered related to study drug. No changes in kidney function related to study drug were observed.

**Conclusion:** Preliminary results of this first-in-man phase 1 study suggest that single IV administrations of AVI-6003 are well-tolerated up to a dose level of 6 mg/kg. Follow-up of subjects enrolled in the 6th cohort (9 mg/kg) is ongoing. Final, unblinded safety and pharmacokinetic results for all subjects will be presented.

ClinicalTrials.gov ID: NCT01353040. This work is being conducted under contract with the Department of Defense Joint Project Manager Transformational Medical Technologies.

**P971** A phase 1, single ascending-dose study of AVI-6002, a combination of two PMOplus™ compounds with activity against ebolavirus

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**Objective:** Ebola hemorrhagic fever (EHF) is a rare human disease caused by ebolavirus, a filamentous single-stranded, negative-sense RNA virus of the family Filoviridae. No vaccine or established effective therapy is currently available for this catastrophic disease. AVI-6002 is an experimental combination of two phosphorodiamidate morpholino oligomers with positive charges (PMOplus™) that specifically target two viral messenger RNAs (mRNAs) encoding ebolavirus proteins. AVI-6002 has demonstrated evidence of protection against lethal infection in experimental mouse, guinea pig and non-human primate models of ebolavirus infection. The objective of this clinical study is to determine the safety, tolerability and pharmacokinetics of intravenous administration of AVI-6002 in healthy human subjects over a dose range predicted to cover a therapeutic dose.

**Methods:** In this first-in-man study, 30 healthy male and female subjects between 18 and 50 years of age were enrolled in six dose escalation cohorts of five subjects each and received a single intravenous (IV) infusion of AVI-6002 (0.01, 0.1, 1.0, 3.0, 6.0 and 9.0 mg/kg) or matched placebo in a 4:1 ratio. Safety was monitored through adverse event collection, telemetry, oximetry and serial blood tests, urine tests and electrocardiograms. The study was overseen by an independent Data Safety Monitoring Board (DSMB).

**Results:** No significant safety concerns arose upon review of blinded study data from the first five cohorts by the independent DSMB. While 14 of the first 25 subjects dosed experienced a variety of adverse events such as headache (n = 4), nausea (n = 3) or fatigue (n = 2), almost all were mild or moderate in severity. The only exception was one episode of severe hypertension, which was not considered related to study drug. No changes in kidney function related to study drug were observed.

**Conclusion:** Preliminary results of this first-in-man phase 1 study suggest that single IV administrations of AVI-6002 are well-tolerated up to a dose level of 6 mg/kg. Follow-up of subjects enrolled in the 6th cohort (9 mg/kg) is ongoing. Final, unblinded safety and pharmacokinetic results for all subjects will be presented.

ClinicalTrials.gov ID: NCT01353027. This work is being conducted under contract with the Department of Defense Joint Project Manager Transformational Medical Technologies.

## Bacterial and viral respiratory infections in the paediatric population

### P972 Epidemiological changes of complicated pneumonia in Spanish children

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**Introduction and objectives:** Pleural effusion is a frequent pneumonia complication in hospitalized pediatric patients. The main etiologic agent is pneumococcus. Since 2001, a Heptavalent pneumococcal conjugate vaccine was introduced in our region (Navarra, North of Spain). Our objectives are to describe the temporal evolution of these pathologies and to evaluate the possible effects that the introduction of the Pneumococcal vaccines has done in these diseases.

**Methods:** We retrospectively analyzed data from 1995 to 2010 of all the admissions in the pediatric ward due to complicated pneumonia with pleural effusion (PE) or empyema (E) in our hospital (the reference pediatric hospital, for approximately a 90 000 population under 15 years). The population figures were obtained from the Navarre Institute of Statistics.

Statistical analyses were performed using Student's t test and Pearson's r, and a binary logistic regression. We considered Pneumococcal pneumonia (NN) those with: positive culture either in blood or in pleural fluid, or positive Ag/PCR in pleural fluid.

**Results:** Two hundred and seventy cases were recorded, estimating a mean age of 52.6 months (DS:40.94). A significant increase of the this pathologies was found over the years ( $p < 0.001$ ): PE (Rp:0.906), E (81, Rp:0.860) and NN (110, Rp:0.834). Comparing the annual incidence of cases in the pre-vaccination years (1995–2001) with post-vaccination years (2002–2010), there was a significant increase ( $p < 0.05$ ) in PE (8.15 vs. 29.14/105 hab. <15 years old per year), E (1.32 vs. 9.46) and NN (2.26 vs. 12). There was a significant increase in the population risk level estimated for PE (OR: 3.70; CI95%:2.67–5.13) and E (OR:7.42; CI95%:3.42–16.11). This incidence increase was significant ( $p < 0.05$ ) in all age groups, but it was more pronounced in small children (2–5 years old) than in infants.

Comparing the initial post-vaccine period (2002–2005: vaccination rate: 20.99%) with the late post-vaccination period (2006–2010 vaccination rate: 53.18%) a increase was found of PE (OR:1.43; CI95%:1.08–1.88) and E (OR:1.94, CI95%:1.16–3.25), but it was only statistical significant in the older patients group (5–15 years) in age's subgroups analysis.

**Conclusions:** During the last 15 years in our country, an increase number of admissions for pneumonia with PE and E has been found. This increase is more pronounced in small children. Even though the vaccination rate has annually increased the admission for complicated pneumonia keeps growing, overall in older children.

### P973 Correlation of viral load of respiratory syncytial virus with disease severity in children hospitalized for bronchiolitis

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**Objectives:** It is well established that factors determining severity of respiratory syncytial virus (RSV) associated bronchiolitis are likely to be determined by a combination of host and viral factors. To this regard the relationship between viral load, disease severity and antiviral immune activation in infants suffering from RSV associated bronchiolitis has not been well identified. The main objective of this study was to determine the existence of any correlation between RSV load and disease severity and also between different clinical markers and mRNA-levels of the interferon stimulated gene (ISG) 56 in infants

hospitalized for bronchiolitis. We also evaluated whether viral load tended to be persistent over the course of the RSV infection.

**Methods:** The levels of RSV-RNA were quantified by using a TaqMan-based real-time PCR technique in nasopharyngeal washings (NPW), collected from 132 infants infected with RSV as a single (90.15%), or as a dual infection with other respiratory viruses (9.85%).

**Results:** There was a distribution of viral load values of several orders of magnitude in infants with bronchiolitis. When the RSV-positive infants were divided on the basis of RSV detection as a single infection or as a coinfection, the viral load was not significantly different between groups. In addition results showed that viral load was positively related to the clinical severity of bronchiolitis, the length of hospital stay, the levels of glycemia and number of ISG56-mRNA copies, whereas an inverse correlation was observed with levels of haemoglobin. We also found that the RSV load significantly decreased between the first and second NPW sample in most single and RSV coinfections.

**Conclusion:** These results suggest that infants with high RSV load on hospital admission are more likely to have both more severe bronchiolitis and a higher airway activation of antiviral immune response.

### P974 The role of viral infections in nasopharyngeal bacterial colonisation in children: a case-control study

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**Objectives:** There is accumulating epidemiological evidence indicating a close association between viral respiratory infections and bacterial superinfections. The present study aims at examining nasopharyngeal bacterial colonization among children with and without a viral respiratory tract infection.

**Methods:** Nasopharyngeal colonization with *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, and nasal colonization with *Staphylococcus aureus* was examined in children <5 years of age with symptoms of viral respiratory tract infection for <5 days, and in controls with no respiratory symptoms during the week prior to enrolment. Both patients and controls had not received any antibiotics for one week prior to inclusion. The presence of respiratory syncytial virus (RSV), influenza virus, parainfluenza virus, rhinovirus and adenovirus was examined with the use of PCR. A preformed questionnaire including demographic and clinical data was completed for each patient. Categorical data were compared with the use of chi squared or two-tailed Fisher's exact test and analysis was carried out with the GraphPad Prism v5 software.

**Results:** During the period February 2009–April 2011, 391 infants and children were recruited, while samples from 36 children were excluded from the study on the basis of inappropriate sampling technique. The average age of the remaining 355 children (217 males) included in the study was 26.4 months, among which, 128 were asymptomatic controls and 227 had respiratory symptoms and one of the following diagnoses: upper respiratory tract infection, bronchiolitis, bronchitis, bronchial asthma, pneumonia, croup, acute otitis media or sinusitis. A higher percentage of asymptomatic subjects was found negative for all bacteria examined in our study protocol ( $p < 0.01$ ). *S. pneumoniae* and *M. catarrhalis* were more frequently isolated ( $p < 0.01$ ) from children with symptoms of respiratory tract infection as compared to asymptomatic children. *S. pneumoniae* plus *H. influenzae* plus *M. catarrhalis* but also the presence of any four bacteria at the same time were more frequently observed among symptomatic children as compared to asymptomatic controls ( $p < 0.05$ ). Colonization of (any) virus positive children with any bacterium or *S. pneumoniae* was higher than in (any) virus negative children ( $p < 0.05$ ).

**Conclusion:** Viral infections influence nasopharyngeal bacterial colonization among children possibly leading to accentuation of disease severity or invasive disease.

### P975 Community respiratory virus infections among hospitalised children with acute respiratory diseases

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**Objective:** To describe the potential role of community respiratory viruses in the natural history of acute respiratory diseases in hospitalized children who have chronic medical conditions or are immunocompromised with the use of nucleic acid detection tests (NATs).

**Method:** From 2007 to 2009, children <14 years of age with chronic medical or immunocompromising conditions who were admitted to King Faisal Specialist Hospital and RC, Riyadh with acute respiratory diseases were included in this study. Clinical and epidemiological data were recorded and respiratory samples including nasopharyngeal aspirate/nasopharyngeal swabs and if available bronchoalveolar lavage were obtained. Respiratory samples were tested by NATs for respiratory syncytial virus (RSV), adenovirus, parainfluenza virus 1–4, influenza virus, rhinovirus, human metapneumovirus (hMPV) and coronavirus (OC43, 229E, & NL63).

**Results:** One or more respiratory viruses were detected in 156 hospitalized children with acute respiratory disease. Sixty-seven percent of patients had chronic medical conditions and 33% of patients had immunocompromising conditions. The median age was 4 years 3 months, 53% of patients were male. Forty-six percent of patient had severe community acquired pneumonia (CAP) with associated mortality of 14.1%. A single virus was identified in 83% of patient. Rhinovirus was the most frequently identified virus (37%) followed by RSV (22%), adenovirus (12%), parainfluenza (12%), influenza virus (8%), hMPV (5%), Coronavirus (4%), and mix viral infection (17%). Respiratory viruses were found to peak during the winter months, such as RSV type-B & parainfluenza 1 (November–January), Influenza A (October–Jan) Influenza B (January–February), and coronavirus (October and January) while there were also viruses detected during summer months such as Parainfluenza 3 (June–August), hMPV detected (March–May). RSV type-A, Adenovirus and Rhinovirus were isolated all throughout the year. Children with rhinovirus, adenovirus and RSV infections needed treatment in the intensive care unit more than often than others. hMPV infection was associated with idiopathic pneumonia and culture negative shock syndrome in immunocompromised children.

**Conclusion:** Respiratory viruses are frequent found in acute respiratory disease in hospitalized children who had chronic medical or immunocompromising conditions. Community acquired viral pneumonia is important cause of morbidity and mortality. Routine testing using NATs for respiratory viruses may be warranted in these high risk patients.

### P976 Varicella and children attending kindergartens in Lisbon

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**Study design:** Based on pathognomonic features of varicella, a cross-sectional study was designed, using random sample of children attending kindergartens in Lisbon 2006. Children's parents were inquired through self-completion questionnaire. Sample size was estimated based on varicella prevalence in children <2 years old was 20% (varicella national seroprevalence was 40% in age group 2–3 years)

**Aims:** To estimate the proportion of children <2 years of age that had varicella, the mean duration of disease, the proportion of children medicated and those who had varicella complications. To analyse parent's knowledge/opinion about varicella vaccine and benefits of vaccination.

**Material and methods:** One thousand two hundred and seventy-five parents with children attending 50 kindergartens were inquired about

previous varicella history of child and siblings and the parent's knowledge/opinion about the varicella vaccine and its benefits.

Descriptive statistical analysis performed with 95% confidence interval. Analysis of variables was performed using Qui-square, Fisher, Exact test. Risk estimated by Odds Ratio. Averages compared using Student's t test. Logistic regression analysis was performed. Significance level was 5%.

**Results:** One thousand three hundred and thirty-three of non-vaccinated children included in the study. Varicella occurred in 559 (41.9%;CI95%:39.27–44.64) children with a 2 years median. Fever occurred in 407 (72.8%;CI 95%:68.92–76.46) cases; 515 (92.1%;CI95%:89.58–94.22) were assist by doctor; 519 (92.8%;CI95%:90.38–94.84) received medication. Duration of disease had a median of 10 days. Hospitalizations due to varicella complications occurred in 20 (3.6%;CI95%:2.06–5.13) children. Children <12 months showed a higher risk of complications (7.1%). Vaccine administration is considering a benefit by 87% of the parents and 90% vaccinated their children against varicella if available through the National Vaccination Programme (NVP).

**Conclusions:** in non vaccinated children attending kindergartens varicella occurs early. Varicella was associated with high morbidity, complication occurred in 3.6% with 55% of hospitalizations in children <3 years. More than 90% of the children had professional health care, 92% received medication. The parent's opinion on benefits of vaccination is high in Portugal; as in other EU countries, varicella has a high social cost due to work loss and medical expenses. Introduction of varicella vaccine in NVP could depend more on the economic impact of varicella than on the severity of the disease.

### P977 Occurrence of pathogenic bacteria in Danish child care centres

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**Background:** Pre-school children in day care are often sick, in average they have 6–8 disease episodes per year and most of these are caused by infections. Infections spread not only by direct contact, but also indirectly via toys, nursing pillows and other surfaces in day care centers. There is little knowledge about the type and prevalence of bacteria on these surfaces. Our objective was therefore to determine the prevalence of human pathogenic bacteria in Danish child care center environments.

**Methods:** Two hundred and twenty Environmental samples from six Danish child care centers in Copenhagen were analyzed during summer 2011. Forty predetermined spots in each center were sampled using 3 M Dip slide™ TV, TSA sampling plates and cotton swabs. After incubation, human pathogenic bacteria were isolated and identified using conventional identification and Maldi-Tof MS. The predetermined spots were situated in the toilet, play room and kitchen and were, among others, toys, tables, nursing pillows and kitchen tables.

**Results:** All samples were polymicrobial, but mostly low-pathogenic bacteria such as *Bacillus* sp., *Acinetobacter* sp. and CNS were grown. Pathogenic bacteria were found in 59 (27%) samples. Coliform bacteria were found in 22 (10%), airway bacteria in 33 (15%) and *S. aureus* in 4 (~2%) samples. The most prevalent coliform bacteria found were *Enterobacter* and *Pantoea* sp., whereas the dominant findings in airway bacteria were Non-hemolytic streptococci. These are all bacteria with low pathogenicity, but can be used as indicators of fecal and nasopharyngeal contamination. We did, however, also find more virulent bacteria such as *S. pneumonia* in five samples and *S. aureus* in four samples. The sites with the highest prevalence of coliform bacteria were the nursing pillows, kitchen tables and sinks. Most airway bacteria were found on toys, tables and pillows in the playroom.

**Conclusion:** Twenty-seven percent of all samples contained pathogenic bacteria, most of which were low-pathogenic coliform and airway bacteria. Coliform bacteria were most frequently found on nursing pillows and in the kitchens whereas the most common site for airway bacteria was on toys, tables and fabric surfaces in the playroom.

These sites appear to be the most important sites for indirect transmission of infectious microorganisms and therefore need special attention when cleaning and disinfecting in the day care centers.

**P978 Eradication of nasopharyngeal carriage of penicillin-non-susceptible *Streptococcus pneumoniae***

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**Objectives:** The high presence of penicillin-non-susceptible pneumococci (PNSP) is a global problem and colonization is mainly seen in young children. The prevalence in Sweden has been low, but increased during the 1990s, especially in Skåne County. After a prolonged carriage of two to 3 months, eradication therapy with rifampicin in combination with amoxicillin, clindamycin or erythromycin was considered. The aim of the study was to evaluate the outcome of eradication therapy and to see if differences in outcome existed between the different antibiotic combinations.

**Methods:** Between the years of 1997 and 2011, 125 children, ages 0–10 years, were referred for eradication therapy due to a prolonged nasopharyngeal carriage of PNSP with a MIC of PcG  $\geq 0.5$  mg/L. All the children were part of the South Swedish Pneumococcal Intervention Project (SSPIP), which aims were to limit the spread of PNSP. When an individual with a clinical infection caused by PNSP was found, nasopharyngeal cultures were taken from the family members and, if the patient was attending day-care, from the other members of the day-care group, to identify asymptomatic carriers. All pre-school children identified as carriers of PNSP were denied attendance to their day-care centre until two consecutive nasopharyngeal cultures yielding no growth of PNSP could be obtained. Eradication was defined as two consecutive negative cultures, with the second one no shorter than 7 days after completed treatment.

**Results:** Of the 125 referred children (most of the excluded patients had resolved the carriage spontaneously), 71 received treatment with rifampicin in combination with amoxicillin (n 44), erythromycin (n 22) or clindamycin (n 5) for 7 days. The primary eradication rate was 91.5%. All of the children whose carriage was not eradicated had been treated with amoxicillin + rifampicin, giving this combination an eradication rate of 86%.

**Conclusion:** Our study indicates that the nasopharyngeal carriage of PNSP can successfully be eradicated by a combination of antibiotics.

**P979 Carriage rates of *Bordetella pertussis* and *Bordetella parapertussis* among school children in China as determined by pooled real-time PCR**

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**Objectives:** Many studies documented that symptomatic old children, adolescents and adults act as the source of pertussis for young infants who have not yet completed their primary immunizations. Objective of this study was to prove that *Bordetella pertussis* and *Bordetella parapertussis* colonized the nasopharynx of 7–15 years old asymptomatic population by designing a cross sectional study using method of pooled real-time PCR for IS481 and IS1001 gene.

**Methods:** Nasopharyngeal (NP) swab samples (n = 629) were collected from same number of 7–15 years old asymptomatic population in the period of 2 months in four counties (one county in each province) of China in 2011. All of NP swabs were inoculated Regan-Lowe agar (Oxiod<sup>®</sup>) supplemented cephalixin within 24 hour in provincial CDC laboratory. After inoculation, swabs were then eluted into 400 microlitre (µL) of PBS. 200 µL of individual elution samples were pooled into groups of 10 samples. The *B. pertussis* and *B. parapertussis* real-time PCR was based on the amplification of the 154 bp IS481 and 186 bp IS1001 gene respectively, and 155 bp human-2-microglobulin target acted as internal control. For pools and

specimens testing, under the strict quality control, any cycle threshold (Ct) value was considered to be a positive test result. Descriptive statistics was performed by using SPSS 13.0 software.

**Results:** There were three positive cultures: two for *B. pertussis* and one for *B. parapertussis*. Sixty-three pools were constructed. Only one supposed positive elution specimen was negative on human-2-microglobulin gene which indicated the loss of nucleic acid extraction as well as inhibition of the real-time PCR. Elution specimens of all three positive cultures also could be detected IS481 and IS1001 target in respective affiliated pools and relevant elution sample. The carriage rates of *B. pertussis* and *B. parapertussis* among studied population were 4.77% (30/628) and 2.07% (13/628). (Details in table).

Group (yr)	No. of Children	No. of positive RT-PCR and percentage		No. of positive Culture and percentage	
		<i>B. pertussis</i>	<i>B. parapertussis</i>	<i>B. pertussis</i>	<i>B. parapertussis</i>
7-9	215*	7(3.26)	2(0.93)	1(0.47)	
10-12	205	13(6.34)	9(4.39)	1(0.49)	1(0.49)
13-15	208	10(4.81)	2(0.96)		
Total	628	30(4.78)	13(2.07)	1(0.32)	1(0.16)

\*Indicate that one specimen was excluded from group due to the negative on human-2-microglobulin gene.

**Conclusion:** Our results indicated that it was feasible to apply a pooled real-time PCR for IS481 and IS1001 target in estimating the carriage rate of *B. pertussis* and *B. parapertussis* in elution specimens of NP swabs. Due to absence of booster pertussis vaccinations for old children and adolescents in China and protection effect of programmed pertussis vaccine of waning as age increasing, asymptomatic old children and adolescents had *Bordetella* organisms in their nasopharynx and could act as the reservoir of pertussis infection.

**P980 Pertussis epidemic in 2011, region of Vallès (Catalonia, Spain)**

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**Objective:** The aim of this study is to describe the epidemic of pertussis based on whopping cough confirmed cases reported during 2011 at the Vallès Occidental and Vallès Oriental regions, with 1.283.000 inhabitants, at the Barcelona northern metropolitan area.

**Methods:** Pertussis is a statutorily reportable disease in Catalonia. Confirmed case was defined as a person with acute cough illness reported by a health professional with isolation of *Bordetella pertussis* or positive polymerase chain (PCR) reaction assay for *B. pertussis*. A confirmed case is also defined as an acute cough illness lasting at least 2 weeks with epidemiological linkage to a laboratory-confirmed case. A descriptive study was performed with demographic data, clinical and vaccine history and epidemiological information.

**Results:** As of November 2011, we have recorded 400 cases of pertussis during this year, a 12-fold increase from 2010. Incidence was higher during summer and on females (67%). The incidence of reported pertussis was 32 cases/100.000 persons. Cases aged between 5 and 9 years were the most frequent (28%), followed by those aged 1–4 years (20%) and under 1 year (17%) but the highest incidence was among <1 year old (405/100 000) followed by the 5–9 years (148/10 000). Around 8% of cases required hospitalization, 50% of those were infants under 2 months. The vaccination status was known for 93% of cases. The 78% of cases under 14 years old had been fully vaccinated with DTaP in accordance with Catalonia's guidelines for DTaP use: <1 year 56%, 1–4 years 78%, 5–9 years 86%, 10–14 years 95%.

**Conclusions:** The infants have the highest reported incidence of pertussis. Whopping cough can occur in children despite being well vaccinated, suggesting that the vaccine does not confer complete immunity against pertussis. Those findings should be taken into account

to develop a new vaccination policy with the aim of reducing the risk of transmitting *B. pertussis* to infants.

**P981** Prevalence of major middle-ear pathogens and adenoid in Iranian children with otitis media with effusion by culture and PCR

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**Introduction:** Otitis media with effusion (OME) is one of the most common childhood diseases and it is the main cause of several otological problems. Although the etiology of OME is still unclear, bacterial and viral infections have an important role in its pathogenesis. The aim of the current study was to determine the common bacterial agents and their susceptibility pattern among both Iranian children with OME and without OME.

**Materials and methods:** PCR and bacterial culture methods were used for detection and isolation of *Alloicoccus otitidis*, *Streptococcus pneumoniae*, *Moraxella catarrhalis* and *Haemophilus influenzae* in 65 middle ear fluid samples and 50 adenoid tissues from 50 OME patients. Fifteen patients were bilaterally affected. Antimicrobial susceptibility of all bacterial isolates was determined by disk agar diffusion (DAD) method.

**Results:** Bacteria were isolated from 47% (n = 30) of the 65 middle ear fluid samples and 79% (n = 38) of the 65 adenoid tissue specimens in OME patients. *A. otitidis* was the most common bacterial isolated from the middle ear fluid 23.8% by culture and 39.7% by PCR method. *S. pneumoniae* was the most prevalent pathogen (35.5% and 31.2% by culture and PCR) in the adenoid tissues of OME patients. Antimicrobial susceptibility pattern showed that most isolates of bacteria were sensitive to amoxicillin, amoxicillin/clavulanate and fluoroquinolones.

**Conclusion:** The present study, being the first report on the isolation of *A. otitidis* by culture method in Iran and Asian countries, shows that *A. otitidis* is the most frequently isolated bacterium in Iranian children having otitis media with effusion. In this study *A. otitidis*, *S. pneumoniae*, *H. influenzae* and *M. catarrhalis* are the major bacterial pathogens in patients with OME and we found that amoxicillin, and amoxicillin/clavulanate have the excellent activity against bacterial agents.

**P983** Prevalence of *Chlamydomydia pneumoniae* in adenoid tissue of children with chronic adenoiditis

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The aim of the study was to determine the presence of *C. pneumoniae* in adenoid tissue and estimate risk factor of *C. pneumoniae* infection in children with chronic adenoiditis.

Two hundred consecutive children aged 2–16 years undergoing planned adenoidectomy between 02.2010 and 05.2011 were enrolled to the study. Eligibility criteria: upper airways obstruction, caused by adenoid hypertrophy and/or with chronic adenoiditis.

Adenoids were analysed for the presence of *C. pneumoniae* DNA by real-time PCR on a LightCycler® 2.0 with LightMix® Kit *Chlamydomydia pneumoniae* (Tib Molbiol GmbH, Germany). DNA from a tissue was isolated with High Pure PCR Kit (Roche Diagnostics, Germany). Smears from the adenoids were cultured on bacteriological media. Additionally, adenoid tissue samples from 22 patients were examined with immunohistochemical methods with application of monoclonal anti-*C. pneumoniae* antibodies (Thermo Scientific) for the presence of chlamydia.

*C. pneumoniae* DNA in the adenoid was present in 11 children (5.5%). *C. pneumoniae* DNA was detected most frequently (24.1%) in children 10–16 years old. Girls were infected more often than boys 63.6% vs 36.26%.

No statistically significant differences were observed between PCR (+) and PCR (-) children as a result of, a cough, recurrent respiratory

infections, otitis media, an asthma, atopic diseases and frequency of antibiotic courses. In PCR (+) children more frequently (54.5%) than in PCR negative children (14.2%) poor standard of living was observed (p < 0.01). *C. pneumoniae* PCR (+) children tend to have rhinitis prolonging ≥3 weeks (90.9% vs 68.7%). Interestingly, *C. pneumoniae* DNA was detected in one case in adenoid removed from siblings.

In nine out of 11 (81.82%) *C. pneumoniae* PCR (+) children other pathogenic bacteria were detected: *S. pneumoniae* (4), *S. aureus* (4), *S. pyogenes* (3) and *H. influenzae* (2). In two of the PCR (+) patients no co-pathogens were found.

Results of immunohistochemical staining showed presence of *C. pneumoniae* in lymphocytes and epithelium of analysed tonsils.

Existence of *C. pneumoniae* in an adenoid tissue suggest participation of the bacteria in adenoid hypertrophy. However, comparison of results of bacteriological culture from adenoid swabs with PCR results suggests co-participation of *C. pneumoniae* in pathogenesis rather than decisive part. This etiology specially concerns school children who are more prone to *C. pneumoniae* infections.

**P984** Aetiology of acute otitis media and pneumococcal serotypes in children from Saint Petersburg

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**Objectives:** *Streptococcus pneumoniae* (Spn), *Haemophilus influenzae* (Hin), *Moraxella catarrhalis* (Mca) and *Streptococcus pyogenes* (Spy) are leading pathogens causing acute otitis media (AOM) in children. Possible role of *Alloicoccus otitidis* (Aot) in the development of AOM is under discussion. Microbiologic diagnosis of AOM requires a sample of middle ear fluid (MEF) and often complicated by antibiotics pretreatment. Molecular methods are more sensitive in the detection of pathogens and allow identification of pneumococcal serotypes from pure cultures and culture-negative specimens.

**Methods:** The study was approved by local ethical committee. Children (>18 years) admitted to the emergency department with AOM and requiring paracentesis were enrolled. Paracentesis was performed by an otolaryngologist. Immediately after incision MEF was collected by the swab applicator and put into transport medium. (ESwab, Copan, Italy). Specimens were refrigerated at 4–8°C and stored for not more than 48 hour before processing. Swab applicator was used for inoculation of agar plates for isolation and identification of Spn, Hin, Mca and Spy. Bacterial DNA was isolated from 100 µL of transport medium using Ribo-Prep kit (Interlabservice, Russia). DNA of Spn, Hin, Mca and Aot were detected in both culture-positive and negative samples by multiplex PCR using primers targeting 16S RNA genes. Identification of serotypes of Spn isolates and of DNA from culture-negative, Spn DNA-positive MEF samples was carried out by multiplex PCR.

Bacteria	n (%)
Total 268 samples	
<i>S. pneumoniae</i>	80 (29.8)
<i>H. influenzae</i>	18 (6.7)
<i>M. catarrhalis</i>	18 (6.7)
<i>S. pyogenes</i>	8 (3)
<i>A. otitidis</i>	12 (4.5)
<i>S. pneumoniae</i> + <i>H. influenzae</i>	15 (5.6)
<i>S. pneumoniae</i> + <i>M. catarrhalis</i>	20 (7.5)
<i>S. pneumoniae</i> + <i>A. otitidis</i>	19 (7)
<i>S. pneumoniae</i> + <i>S. pyogenes</i>	2 (0.7)
<i>S. pneumoniae</i> + <i>H. influenzae</i> + <i>M. catarrhalis</i>	10 (3.7)
<i>S. pneumoniae</i> + <i>H. influenzae</i> + <i>A. otitidis</i>	4 (1.5)
<i>S. pneumoniae</i> + <i>M. catarrhalis</i> + <i>A. otitidis</i>	10 (3.7)
<i>S. pneumoniae</i> + <i>H. influenzae</i> + <i>M. catarrhalis</i> + <i>A. otitidis</i>	3 (1.1)
Total	219 (81.7)
Negative samples (PCR + culture)	49 (18.3)



**Results:** A total of 268 children (180 of them >5 years) were included in the study between December 2010 and November 2011. Data on bacteria and their combinations isolated from MEF or detected by PCR are presented in Table. Among 57 Spn isolates cpsA gene was detected in 54. Among 106 Spn culture-negative, 16S rRNA-positive MEF samples cpsA was detected in 64. In a total of 99 Spn isolates and culture-negative MEF samples serotypes were detected. Serotype 19F was the most prevalent – 37 (37.4%), followed by serotype 3 – 22 (22.2%). Serotypes 9L/N and 23F were represented by 9 (9.1%) each, 6A/B/C – by 6 (6.1%), 9V/A – by 5 (5.1%), 19A – by 3 (3%), 8, 14, and 10A – by 2 (2%) each and 18A/B/C – by 1 (1%), 19 (16.1%) – were non-typable.

**Conclusion:** Spn is the major cause of AOM in children in St.-Petersburg responsible for 60.8% of cases, PCV-7 and PCV-10 are covering 51.8% of circulating serotypes, the broadest coverage – 73.7% is provided by PCV-13.

## Gastrointestinal tract infection in children

### **P985** A clinico-epidemiological comparison study of paediatric acute viral gastroenteritis in South Korea

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**Objectives:** Acute viral gastroenteritis, especially rotavirus, norovirus, enteric adenovirus, and astrovirus is a common disorder in young children. Our aim was to monitor the epidemiologic characteristics of the aforementioned viruses and to determine the laboratory and clinical characteristics of patients associated with these viruses.

**Methods:** From December 2009 to November 2010, 685 stool specimens from patients hospitalized in Chung-Ang University Hospital were tested for aforementioned viruses by using multiplex PCR, and their medical record review was conducted retrospectively.

**Results:** The overall rates of prevalence were 44.8%, and the prevalence for rotavirus, norovirus, enteric adenovirus, and astrovirus were 36.3%, 55%, 7%, and 1.7%, respectively. Mixed virus infections were detected in 37 (5.4%). The highest incidence was in March 2010 (18.9%), in the 13–24 months age group (38.1%) and among males (53.1%). In clinical aspect, chilling was most frequently observed with adenovirus (44.4%), and diarrhea was most frequently observed with rotavirus (93.7%). The incidence of diarrhea was higher in the rotavirus-infected group (93.7%) than in the norovirus-infected group, but leukocytosis (55%) and lymphocytosis (21%) were more common in the norovirus-infected group.

**Conclusions:** Our finding showed prevalence and clinical differences of each gastroenteritis-associated viruses. For a better understanding, further epidemiologic and clinical investigations are essential.

### **P986** Evaluation of infectious agents frequencies and epidemiological features in children with acute gastroenteritis

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**Objectives:** Acute gastroenteritis remains a common illness among infants and children throughout the world. The aim of this study was evaluation of infectious agents frequencies and epidemiological features in children with acute gastroenteritis in and around Konya.

**Methods:** A total of 62 stool specimens obtained from 27 female and 35 male children <14 years who were admitted to an education and research hospital with symptomatic acute gastroenteritis, during February–September 2011 were included to the study. Stools were examined using antibody-based ELISA for rotavirus, adenovirus, norovirus, astrovirus, *Campylobacter* spp, *Salmonella* spp, VTEC, *E. coli* H7, *Clostridium perfringens*, and Shigella.

**Results:** Diarrhea was the most common symptom in children with acute infectious gastroenteritis (95.2%), while the rates of vomiting and fever were 64.5% and 50%, respectively. The positivity rates of

*Campylobacter* spp, *Salmonella* spp, VTEC, *E. coli* H7, *C. perfringens*, and Shigella were 8%, 4.8%, 1.6%, 4.8%, 1.6%, and 1.6%, respectively. Viral antigen positivity was detected in 58.1% of the samples, and the positivity rates of rotavirus, adenovirus, norovirus and astrovirus were 32.3%, 16.1%, 16.1%, and 3.2%, respectively. Rotavirus was the most frequently detected agent in children with viral gastroenteritis (55.6%). Six of 36 patients (16.7%) yielded two at a time viral antigen positivity in their stool samples. Rotavirus + norovirus (33.3%), adenovirus + norovirus (33.3%), rotavirus + adenovirus (16.7%), and adenovirus + astrovirus (16.7%) associations were detected coinfections. The difference between the rates of rotavirus positivities in age groups was found statistically insignificant ( $p > 0.05$ ). Rotavirus infections were mainly detected in winter season (50%). Adenovirus positive cases were detected in winter (21.4%) and in spring (20.6%). Norovirus infections were mainly detected in spring (20.6%) and summer (16.7%). The duration of hospitalization was found as 35.5% in lesser than a day group, 29% in 1–7 days group, and 3.2% in more than 7 days group.

**Conclusions:** More than half of the childhood gastroenteritis cases (58.1%) were due to viral agents. Rotavirus is most common viral enteric agent. The antigens of the viral agents may guide the clinical approach to the patients with acute gastroenteritis among ≤5 years of age, especially in the winter and spring months.

### **P987** Temporal trends in rate of hospitalisation for rotavirus gastroenteritis in the paediatric population in Italy: cross-sectional study utilising national hospital discharge database

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**Study design:** This was a retrospective observational study, conducted using hospitalization data from national Hospital Discharge Database (HDD) available on the website of the Italian Ministry of Health.

**Objective:** The primary objectives were to estimate the frequency of hospitalization for Rotavirus gastroenteritis (RVGE) for the period 2002–2005 in children aged 0–14 years in the different regions of Italy, and to analyze temporal trends of hospitalization rates (HR) for geographical location.

**Methods:** In Italy there are no specific surveillance systems for RV disease in place, so HDD are one mean by which diseases trends among hospitalizations may be observed. Cases of RVGE were identified using ICD9-CM code 00861. Three aged groups are studied: 0–1, 1–4, and 5–14 years. In order to estimate HR, we used population for each study year published by the National Institute of Statistic website. Data analysis was performed using STATA 12. The nptrend command was used to examine for significant trends in temporal HR ( $p < 0.05$ ), based on the Wilcoxon rank-sum method.

**Results:** In the study period, a total of 21 300 RVGE diagnosis were collected in children. The following HR for rotavirus were recorded: 71/100 000 residents in 2002, 49/100 000 in 2003, 51/100 000 in 2004 and 66/100 000 in 2005. RVGE accounted for the highest percentage of admission in children of 1–4 years of age. The respective HR were found to be: 2002:181/100 000; 2003:157/100 000; 2004:161/100 000; 2005: 204/100 000. The region with higher HR is Trentino Alto Adige while Calabria, while Valle D'Aosta and Sardegna present the lowest values. The analysis of temporal trends by geographical location shows that in the North, the average HR reached a peak in 2002, before declining in 2003 and 2004 and return to a high value in 2005. A similar trend, was observed for the Centre. In the South and Islands observed a different trend, indeed the HR varies from 39/100 000 in 2002 to 31/100 000 in 2003. In the following years, HR substantially increases, going to 48/100 000 residents. The analysis of temporal trend in the HR in the 4 years examined showed no statistically significant differences ( $p > 0.05$ ).

**Conclusions:** The highest HR observed in the Northern compared to the rest of Italy could reflect actual geographic variability in incidence

of rotavirus infection or disease, or could be due to differences in the sensitivity of the hospital discharge databases for detecting RVGE.

#### **P988** Rotavirus surveillance: genotyping and epidemiological evaluation in hospitalised children in Milan

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**Objective:** Concerning the magnitude of the rotavirus infection worldwide this study was designed to assess the most prevalent circulating rotavirus genotypes and to devise strategies for intervention in pediatric children admitted with acute gastroenteritis to the L. Sacco University Hospital (Milan, Italy).

**Methods:** A 41 months study (2008–2011) involving 700 children aged between 1 and 204 months (40 mos, males 57%), was carried out. ImmunoCardSTAT Rotavirus test (Meridian, USA) was performed as screening; for G- and P-typing RT-PCR of VP7 and VP4 genes was used. A subset of rotavirus strains was characterized by nucleotide sequencing of PCR products and compared with the NCBI data bank, using DNASIS Max software (Hitachi Software Engineering Company, Alameda, CA, USA).

**Results:** Rotavirus was the cause of acute gastroenteritis in 97 children (14%); 84 out of 97 samples (86.6%) were available for genotyping, 13 (13.4%) proved negative to RT-PCR.

Sequence analysis of the VP4 and VP7 genes were carried out on 13 strains out of 84 that could not be G- and P- typed at first by PCR. After sequencing 9 out of 13 resulted G1, 3 were identified as G9 and in one case infection was due to G1 and G9 strains together.

The distribution of the different rotavirus Group A genotypes detected in our hospital showed a prevalence of the G1 genotype, 81% (68/84). Other major strains reported in this epidemiological evaluation were G9, 8.4% (seven cases), followed by G4, 3.6% (three cases), G2 and G3, one case each (1.2%). Also the rarer G12 and G10 were reported in one case each (1.2%) and two mixed infections with G1 and G9 types (2.4%) were detected. Among the P-types P[8] accounted for the total of the samples tested. Sequences analysis of VP4 and VP7 genes of typed samples has confirmed the presence of the human G and P types described, and revealed a close relationship between strains isolated in the rest of Europe reported in GenBank (97% to 99% sequence similarity).

**Conclusion:** In the present study rotavirus surveillance genotyping reveals new emerging rotavirus genotypes sporadically detected G9P, G10 and G12.

Given the unbalance in the representation of the genotypes any detailed comparison between genotypes occurrence or properties was not possible, and only a representative set of study cases was available for clinical parameters of G1.

#### **P989** Norovirus gastroenteritis in a paediatric population

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**Objectives:** Noroviruses (NoVs) are recognized as an important cause of epidemic and sporadic acute gastroenteritis in humans of all ages worldwide. In Greece, there is not surveillance system for viral gastroenteritis. The aim of the present study was to give our experience about gastroenteritis due to NoVs in children in the area of Athens, Greece.

**Methods:** A total of 846 stool specimens, obtained from an equal number of children (56% boys) under 14 years of age hospitalized or referred to outpatient clinic with gastroenteritis from November 2009 to October 2011, were tested by using an immunochromatographic test (RIDA QUICK Norovirus Test<sup>®</sup>, R- Biopharm, Germany) for determining genogroup I and II NoVs. Cases were studied by

reviewing the medical charts for clinical and epidemiologic characteristics. NoVs were not covered by routine analysis during summer months July and August (cost effective criteria), so these months were not included in the study.

**Results:** Children infected by NoVs comprised 27 out of 846 (3.2%) enrolled patients. Of these, two were suffering from chronic diarrhea, and the remaining 25 cases were presented with acute diarrheal syndrome. Other than diarrhea, vomiting and abdominal pain were the most common symptoms among the 25 norovirus-positive patients, reported for 15 patients (60%), following by fever (>37.5°C) reported for 10 patients (40%). Sixteen patients were boys (59%), with median age 12 months (range 27 days to 14 years). The majority of NoVs infections (n = 21, 78%) occurred in children up to 2 years of age. The highest incidence (82%) was recorded in winter and spring months from February through May. Outbreak was not identified, but person-to-person transmission in different hospital wards was detected in eight cases from March to May 2010 and one case in January 2011. Of note, eight cases (29.6%) with a dual infection were found; co-infection with rotavirus was found in four patients and viral – bacterial co-infection in a further four cases: *Escherichia coli* (2), *Salmonella enterica* (1), *Campylobacter jejuni* (1). During the study period, the incidence for rotavirus was 15% and for adenovirus 2.7%.

**Conclusion:** NoVs were the second most common agent responsible for viral gastroenteritis in Greek children, especially under 2 years. Co-infection viral-viral or viral-bacterial was common. Virus transmission in hospital wards occurred in a high rate. Strict hygiene measures must be taken when caring for patients with gastroenteritis.

#### **P990** Outbreak of *Salmonella* Poona among infants during 2011 in the Vallès region, Catalonia, Spain

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**Objective:** In 2010 the National Centre of Epidemiology (Instituto de Salud Carlos III, Madrid) detected an increase of *Salmonella enterica* serotype Poona (SP), mostly on infants aged under 1 year suggesting a transmission from formula milk, but until February 2011 it was not informed state wide. *Salmonella* isolates are only reported for some laboratories and on a voluntary basis. The aim of this study is to describe the epidemiological investigation of the SP outbreak in the Vallès Occidental and Vallès Oriental region, with 1.283.000 inhabitants at the Barcelona northern metropolitan area.

**Methods:** A case was defined as an infant with symptoms compatible with a *Salmonella* infection and SP isolate from a clinical specimen. The health centres were alerted and encouraged for the ESUVV to inform about any presumptive case. Data about demographic, clinical and food consumption was collected. *Salmonella* strains from patients and formula milk recipients provided by their parents were analysed by pulse-field gel electrophoresis (PFGE) by the PHAC laboratory since February 2011.

**Results:** Until April 2011, 28 cases were reported to ESUVV: 2 in 2010 and 26 in 2011, 75% of the cases were under 5 months. All the cases had diarrhoea (67% bloody diarrhoea), 5 were hospitalised, one case needed intensive care. All cases had consumed the same batch of a milk brand. SP was cultured from two milk cans. All SP strains had the same PFGE. It was the first time during the outbreak that SP was isolated from a not manipulated recipient. These findings let the Spanish food safety authorities recall the batch of milk, source of the infection. The last case was in April 2011.

**Conclusions:** The investigation of this outbreak shows the usefulness of information management and active surveillance at regional level. Microbiological reporting should be mandatory, together with the establishment of a *Salmonella* data bank in order to identify and investigate clusters of cases.

**P991** The occurrence of *Helicobacter pylori* specific genotypes in symptomatic children and evaluation of immune response to infection

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**Objectives:** *Helicobacter pylori* (*H. pylori*) infection is acquired in early childhood and its progress seems to be associated with bacterial virulence factors, environmental factors and host determinants, including immune response to pathogen. Analysis of the relationship between these factors can contribute to a better understanding of the course of *H. pylori* infection. The aim of the study was to establish the prevalence of *H. pylori* cagA, vacA, iceA, babA genotypes in a cohort of pediatric patients and to analyze the association between the presence of IgG antibodies against specific *H. pylori* antigens with strain genotype and clinical outcome.

**Methods:** The retrospective study was performed on one hundred and two (n = 102) *H. pylori* strains and 102 serum samples collected in years 2007–2010. The strains were isolated from gastric biopsies of children aged 4–18 years, diagnosed and treated for: chronic gastritis (CH) (n = 69), peptic or duodenal ulcer disease (UD) (n = 14), gastroduodenal reflux disease (GERD) (n = 19). Genotyping of virulence factors cagA, vacA, iceA, babA was performed by multiplex PCR. Serum antibodies reactivity against *H. pylori* specific proteins was tested by Western blot.

**Results:** The high frequency of IgG antibodies against 95 kDa (VacA) and 120 kDa (CagA) was detected in 73% and 80% of examined children. The highest prevalence of anti-CagA and anti-VacA antibodies was observed in youngest children (81%). In children with CH the percentage of anti-CagA and anti-VacA antibodies amounted 61.4% and 37.6% whereas in subjects with UD 92.3% and 82%, respectively. In patients with UD the most prevalent *H. pylori* genotypic profile was: vacA s1m2, cagA+(28.5%) whereas in patients with GERD: vacAs2m2, cagA-, iceA+ genotype (31.5%), respectively. In children with CH the most frequently detected *H. pylori* genotype was vacAs2m2, cagA-, iceA+ (17.3%).

**Conclusions:** The specific antibodies against CagA and VacA proteins are observed more frequently in children with peptic and duodenal ulcer than in patients with chronic gastritis. A high prevalence of the presence of anti-CagA and anti-VacA antibodies in youngest children might be associated with the high rate of infection caused by CagA+, VacA+ *H. pylori* strains. The high frequency of serological response to VacA and CagA antigens well correlates with high prevalence of vacA s1, cagA+ *H. pylori* genotype. Grant support: Ministry of Science and Higher Education, No3230/P01/2007/32, Poland.

**P992** Sero-epidemiology of *Helicobacter pylori* in children in Greece. Is there any difference between allergic and nonallergic individuals?

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**Objectives:** The prevalence of *Helicobacter pylori* infection in asymptomatic children varies greatly between countries worldwide. It has been suggested that the lack of exposure to *H. pylori* is an important risk factor of asthma in childhood. Thus, *H. pylori* seropositivity has inversely related to allergic disorders in children. The aim of this study is to determine the antibody status against *H. pylori* in a paediatric population in Greece and the correlation of seropositivity with total IgE serum levels.

**Methods:** Serum samples from 270 outpatients or hospitalized children [159 boys (59%) and 111 (41%) girls] from January to August 2011, aged 2–15 years old, were analysed. This pediatric population did not present any peptic or other chronic disease. The children were distributed in three groups according to age, each age group represented a similar number of samples with normal and abnormal levels of IgE: group a; 2–5, group b; 6–10, group c; 11–15 years old.

All samples were tested for IgG and IgA *H. pylori* antibodies by ELISA (Dia.Pro Diagnostics Bioprobes). Total IgE levels were measured using a FEIA method (Immunocap 250-Pharmacia). Statistical analysis was performed using a chi-square test.

**Results:** Out of 270 samples, 45 (16.7%) were found positive for *H. pylori* IgG or/and IgA antibodies. Of these, 11 (24.5%) children with negative IgA results were found to have detectable anti-*H. pylori* IgG, while in 16 (35.5%) with negative IgG results determined exclusively IgA antibodies. There was no significant difference in positive samples between boys and girls (15.7% vs 18.0%). In these three study groups the seroprevalence was 10.8% (10/93), 13.3% (13/98), and 27.8% (22/79) in group a, b and c, respectively. As concerning as the correlation of seropositivity for *H. pylori* with the high IgE levels (>150 kU/L) suggesting allergic status, there was not found statistically significant correlation (p > 0.05) between healthy and allergic children [20.2% (26/129) vs 13.5% (19/141)]. However, in the latter group the proportion of positive for *H. pylori* results was inversely associated with IgE levels [151–500 kU/L; 15.20% (7/46), 501–1000 kU/L; 13.10% (8/61) and >1000 kU/L; 11.80% (4/34)].

**Conclusions:** Healthy children infected with *H. pylori* are prevalent in our country. The positive *H. pylori* results increase with age. Contrary to other studies, the inverse relation of seropositivity with allergic disorders in childhood has not been detected in our study.

## Serological diagnosis of infections

**P993** Evaluation of rapid immunofiltration assays for the diagnosis of Epstein–Barr virus infectious mononucleosis

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**Objectives:** The aim of this study was to compare two rapid immunofiltration assays (IMFAs) (BIO-RAD, France), requiring no specific instrumentation, one to detect Epstein–Barr virus (EBV)-VCA IgM (RDT EBV IgM) and the other for the simultaneous detection of EBV-VCA IgG and EBNA-1 IgG (RDT EBV IgG), with the methods used in our laboratories, for the diagnosis of EBV infectious mononucleosis (IM).

**Materials and methods:** A total of 107 serum samples, grouped in two panels, were studied. Panel A included 76 samples from IM patients (median age 13 years old) received for routine EBV-specific antibody testing, showing antibody profiles compatible with acute primary EBV infection (presence of VCA-IgM and -IgG in absence of EBNA-1-IgG antibodies [62] or single VCA-IgM positive result [14]). Panel B included 31 samples from EBV seronegative individuals. All samples were tested with chemiluminescent immunoassays (CLIAs) for VCA-IgM, VCA-IgG and EBNA-1-IgG (Liaison®, DiaSorin, Italy) and for heterophile antibodies (HA) (Monogen®, Biokit, Spain). All assays were performed according to the manufacturer's instructions. Samples showing discrepant results were retested and classified according to indirect immunofluorescence (IIF) for VCA-IgM and -IgG and anticomplement immunofluorescence (ACIF) for EBNA antibodies (Merifluor®, Meridian Bioscience, USA).

**Results:** Out of the 76 sera from Panel A, 70 were reactive for VCA-IgM by CLIA and IMFA. Six samples were only reactive by CLIA; 5 of them were IIF positive. No positive results were detected by IMFA in samples from Panel B. Thus the figures for sensitivity, specificity and agreement were 93.3%, 100% and 95.3%, respectively. Forty-four samples from Panel A were positive for HA, all of them showing positive result in IMFA for VCA-IgM. For VCA-IgG, 56 samples were positive and 43 negative by both assays. After testing discrepant samples by IIF, six samples were classified as false negative and 2 as false positive by VCA-IgG IMFA. The values for sensitivity, specificity and agreement, were 90.3%, 95.6% and 92.5%, respectively. Finally, eight samples showed false positive result in IIF for EBNA-1-IgG, as referred by ACIF (92.5% for both specificity and agreement).

**Conclusion:** RDT EBV assays were accurate for the diagnosis of acute EBV IM. The IgM assay was found as especially useful in patients with

no HA response. Both IMFA assays are easy to perform, include ready-to-use reagents and they are suitable for point-of-care use.

#### **P994** Evaluation of three immunoassays for the determination of Epstein–Barr virus IgG and IgM specific proteins

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**Objective:** Epstein Barr (EBV) is the etiologic agent of infectious mononucleosis (IM). Diagnosis of infectious mononucleosis is based upon clinical manifestations in conjunction with serological and haematological findings. Serologically, heterophile antibodies can be detected as well as antibodies against EBV IgG and IgM specific proteins. The aim of the present study was to evaluate the performances of three immunoassays: Immulite 2000 (Siemens), Liaison® (Diasorin) and Vidas (Biomérieux).

**Methods:** The first group of our study consists of sera from 41 patients (both sexes, aged 5–45 years old) with positive heterophile antibodies and clinical findings. The second group consists of 63 samples from healthy individuals. All samples were tested for EBV IgG and IgM antibodies using Immulite 2000 Siemens (CLIA), Liaison® Diasorin (CLIA) and Vidas Biomérieux (ELFA). Samples with discordant results were also tested for Epstein Barr nuclear antigen IgG (EBV NA IgG). Indirect immunofluorescence (IIF) was used as a reference method. The SPSS 17.0 was used for the interpretation of the results.

**Results:** The specificity of Immulite, Liaison® and Vidas for EBV IgM was 90.8%, 93.8% and 92.3% respectively. The sensitivity of EBV IgM were 95.1% for Immulite, 95.3%, for Liaison 97.6% and 95.3% for Vidas. In the IgG immunoassays the specificity of Immulite was 89.4%, for Liaison® was 94.4% and for Vidas was 94.4%. The sensitivities of EBV IgG were 98.9%, for all immunoassays. The agreement of EBV serology profile between Immulite and Liaison® was 94.3%, between Liaison® and Vidas 93.3% and between Immulite and Vidas 92.4%.

**Conclusion:** In conclusion, our results indicate that the three immunoassays have similar performances. Thus, any of the above immunoassays can be used for routine detection of EBV IgG and IgM specific proteins in serum samples. To distinguish EBV primary infection from seronegative or past infection EBV nuclear antigen can be very useful. In addition, the combination of EBV IgG and IgM specific proteins and EBV NA IgG can help us resolve false-positive testing.

#### **P995** Evaluation of serological assays on the Vidas (bioMérieux) platform for delineation of Epstein–Barr virus serostatus

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**Background:** Epstein–Barr virus (EBV) persistently infects over 90% of adults. Whilst primary infection in childhood is generally asymptomatic, it may cause infectious mononucleosis (IM) in adulthood. Immunosuppression following solid organ transplantation (SOT) reduces T cell-mediated immune control of EBV which is an oncogenic virus that may drive formation of post-transplant lymphomas.

**Objective:** The aim was to compare performance of Vidas (bioMérieux, France) with Liaison (Diasorin, USA) enzyme immunoassays (EIAs) and immunofluorescence (IF; MBL-Bion, USA) in the IM and SOT setting.

**Methods:** For each study population, 100 stored serum samples were analysed by EBV VCA IgM, VCA (or VCA/EA) IgG and EBNA IgG EIAs and IF. For each assay platform, delineation of EBV serostatus was based on all three test results [“no evidence of infection”, “acute infection” (VCA IgM+ve), “recent infection” (VCA IgM+ve and VCA IgG+ve) or “past infection” (EBNA IgG+ve)].

**Results:** The study assessed three patient populations (n = 100 each): (i) patients suspected clinically of IM [37 males; age range 7–50 years (mean (SD) 24.6 (9.7) years)]; (ii) SOT (live kidney) donors [51 males; age range 20–86 years (mean (SD) 51.2 (12.2) years)]; (iii) SOT (cadaveric kidney) recipients [70 males; age range based on 99 patients 17–77 years (mean (SD) 51.0 (14.5) years)]. Overall, the proportional agreement between individual Vidas and Liaison EBV tests ranged from 88% to 100% (median 96%) demonstrating similar performance of these assays as reflected further in overlapping confidence intervals. Furthermore, EBV serostatus (based on all three tests) determined on the Vidas platform mirrored that shown by Liaison. Similar results were obtained when VCA IgG or EBNA IgG results on Vidas or Liaison were compared with IF. In contrast, the proportional agreement between individual Vidas or Liaison VCA IgM tests and IF ranged from 48% to 72% (median 52%) which resulted in discrepancies in EBV serostatus between Vidas or Liaison and the IF assay.

**Conclusion:** Overall, the Liaison and Vidas assays gave similar performance. The same applied to the IF assay – with the notable exception that the VCA IgM IF test often gave discrepant results between the Vidas and Liaison platforms. The main reason for this discrepancy was likely inherent operator subjectivity of IF assessments which can be avoided by using automated EIA platforms.

#### **P996** Comparison of three testing algorithms for staging of Epstein–Barr virus infection by the new ARCHITECT EBV prototype panel

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**Objectives:** Staging of EBV infection and differentiation from other illnesses with similar symptoms are the major goals of serological EBV testing. The most routinely used serological test panels consist of three assays: EBV viral capsid antigen (VCA) IgM, EBV VCA IgG and Epstein–Barr virus (EBV) nuclear antigen (EBNA) IgG. In this study we compared three testing algorithms using the ARCHITECT EBV VCA IgM, VCA IgG and EBNA-1 IgG assays currently in development. Purpose was to validate the testing algorithms regarding their ability to stage of Epstein–Barr virus (EBV) infection.

**Methods:** Five hundred and forty-seven diagnostic specimens, including presumed acute (n = 49) and prospective (n = 498) specimen, were evaluated by three testing algorithms: (i) Initial testing by VCA IgM/IgG; follow up testing with EBNA-1 IgG (VCA M/G screening), (ii) Initial testing with EBNA-1 IgG; follow up testing with parallel VCA IgM/IgG testing (EBNA screening) and (iii) Parallel testing of all three assays. Acute phase specimens had been selected based on a positive heterophile antibody test, prospective specimens were collected from daily EBV routine serology. Specimens were staged as EBV negative, suspected acute, acute, transient phase, past infection or rated as unresolved (VCA IgG only or EBNA IgG only reactive). Follow-up testing is required if specimens were staged as suspected acute, transient phase or unresolved. Calculation was performed both with grayzone (GZ) results considered unresolved, or using an algorithm that evaluates GZ results for one marker based on the outcome of the other two markers (GZ resolved).

**Results:** The EBNA screening algorithm required follow up tests in 4% of patients, while VCA M/G screening as well as testing of all assays in parallel necessitated follow up tests in 7% of patients. The GZ resolved evaluation as well reduced the need for follow-up testing. Forty-two of 49 heterophile antibody positive specimens staged acute independent of testing algorithm. Six were either Epstein–Barr virus (EBV) serologically negative or identified as past infection. Six specimens staged acute in the prospective specimens independent of testing algorithm.

**Conclusions:** When using the new ARCHITECT EBV prototype panel with an EBNA screening and GZ resolved approach, only 4% of patient results required further follow-up testing to resolve the state of EBV infection, while presumed acute Epstein–Barr virus (EBV) infection was still detected with high sensitivity. With these features the

ARCHITECT EBV panel might improve workflow and time to result in the diagnostic laboratory.

**P997** A comparative analysis on five different fully automated anti-rubella IgG immunoassays reporting results in IU/mL

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**Objectives:** Since the 1980s rubella IgG assays have been calibrated against the same World Health Organization (WHO) international standard and the test results reported in International Units per milliliter (IU/mL).

The aim of this study was to compare in term of sensitivity and specificity five fully automated anti-rubella IgG immunoassays that report results in IU/mL: ACCESS<sup>®</sup> Rubella IgG (Beckman), LIAISON<sup>®</sup> Rubella IgG (DiaSorin), ARCHITECT<sup>®</sup> Rubella IgG (Abbott), IMMULITE<sup>®</sup> 2500 Rubella IgG (Siemens Healthcare Diagnostics) and ELECSYS<sup>®</sup> Rubella IgG (Roche Diagnostics).

**Methods:** The study was performed in 482 selected samples from pregnant women with different level of IgG anti-rubella antibodies. Samples that showed discordant results between systems were further investigated by resolution with immunoblot (Mikrogen).

Analytical performances were calculated vs. expected results obtained using a combination of the consensus and immunoblot classification.

**Results:** At first evaluation 88 samples were negative by all methods, 175 positive and 219 discordant. After immunoblot analysis on discordant samples, 217 were classified as positive and 2 as negative. The classified 90 negative and 392 positive samples were used to determine the sensitivity and specificity of each assay. When equivocal results were interpreted as positive, the sensitivity of the immunoassays ranged from 71.4% to 99.5% and the specificity ranged from 97.8% to 98.9%.

When equivocal results were interpreted as negative, the sensitivity of the immunoassays ranged from 47.7% to 85.7% and the specificity ranged from 97.8% to 100%. (Table1).

Assay	Sensitivity (%) [95% confidence limits [%]] with equivocal results assigned as:		Specificity (%) [95% confidence limits [%]] with equivocal results assigned as:	
	Positive	Negative	Positive	Negative
ACCESS*	71.4(66.7-75.9)	47.7(42.9-53.0)	97.8(92.2-99.7)	100(96.0-100)
LIAISON*	73.2(68.5-77.5)	68.9(64.0-73.4)	98.9(94.0-100)	98.9(94.0-100)
ELECSYS*	85.7(81.9-89.0)	85.7(81.9-89.0)	97.8(92.2-99.7)	97.8(92.2-99.7)
ARCHITECT*	95.2(92.5-97.1)	68.9(64.0-73.4)	97.8(92.2-99.7)	98.9(94.0-100)
IMMULITE*	99.5(92.2-99.9)	84.9(81.0-88.3)	97.8(92.2-99.7)	97.8(92.2-99.7)

**Conclusions:** Even though all assays reported results in IU/mL this study showed only a moderate correlation, particularly in term of sensitivity. Further investigations have been performed to evaluate sensitivity and specificity of assays using a cut off with wider grey zone, which may be useful in population where vaccination is commonplace.

**P998** Usefulness of rubella IgM detection: evaluation of serological samples from patients with acute rubella infection following a rubella outbreak in southern Italy

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**Objectives:** Rubella virus infection causes a generally benign exanthema disease but it is particularly disastrous if contracted during the first 4 months of gestation. If not immunologically protected, women infected during pregnancy run a high risk of embryo fetal damage. Congenital rubella causes a wide range of severe defects, many of which are permanent and adversely affect later development. Pathological consequences on the fetus or newborn depend on teratogenicity of the virus and on the time of pregnancy when the infection has been contracted.

The aim of this study was to assess the performance of four automatic systems for the serodiagnosis of primary acute infection.

**Methods:** The study was performed upon 68 samples of symptomatic patients who were infected by rubella virus during the course of an epidemic in Southern Italy (Cosenza 2008). All of the patients had fever and were affected by lymphadenopathy, a short lasting rash and joint pain. To evaluate the sensitivity of the tests paired sera were also assessed.

The sera were screened for the presence of anti-rubella IgM using LIAISON<sup>®</sup> Rubella IgM (DiaSorin), ARCHITECT<sup>®</sup> Rubella IgM (Abbott), IMMULITE<sup>®</sup> 2500 Rubella IgM (Siemens Healthcare Diagnostics) and ELECSYS<sup>®</sup> Rubella IgM (Roche Diagnostics) assays.

**Results:** On 68 symptomatic patients LIAISON<sup>®</sup> showed 55 IgM positive results, IMMULITE<sup>®</sup> 47, ARCHITECT<sup>®</sup> 42 and ELECSYS<sup>®</sup> 38.

At first evaluation of paired samples all patients were symptomatic with high level of IgM antibodies. After 3–5 days seroconversion of IgG was observed as confirmation of clinical signs.

**Conclusions:** Statistical analysis of the findings showed a good sensitivity for all the systems and confirmed the usefulness of serological Rubella IgM detection to diagnose an acute rubella infection.

**P999** The challenges of serological prediction of chronic Q fever

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**Introduction:** After primary infection with *Coxiella burnetii*, 1–5% of patients develop chronic Q fever. PCR and culture have low sensitivity for detection of chronic Q fever. Hence diagnosis relies mainly on serologic tests, of which immunofluorescence assay (IFA) is the most commonly used. Cut-off titers for phase I IgG to detect chronic infection are matter of debate, but are estimated between 1:800 and 1:1600. To improve diagnostic work-up, we studied the serological profiles in patients with established chronic Q fever.

**Methods:** We selected all patients included until September 2011 in the Dutch National Database of Chronic Q Fever Patients. According to Dutch consensus, patients were categorized as proven, probable or possible chronic Q fever. This classification ranks the probability of having chronic Q fever based on PCR results, serology, clinical parameters, imaging studies and pathology. We examined phase I IgG antibody titers (IFA) at time of positive blood PCR, at diagnosis, and at peak levels and compared these between the three chronic Q fever subgroups.

**Results:** We evaluated 200 patients, of whom 56 (28.0%) had possible chronic Q fever, 51 (25.5%) probable chronic Q fever, and 93 (46.5%) proven chronic Q fever. Of the patients with proven chronic Q fever, 52 patients (55.0%) had a positive *C. burnetii* PCR in blood, 10 (10.8%) in tissue and 13 (13.9%) in both blood and tissue. High phase I IgG levels did not negatively influence positive blood PCR results. Median phase I IgG titers at time of diagnosis and peak titer in patients with proven chronic Q fever were significantly higher (both 1:8192,  $p < 0.05$ ), compared to patients with probable (1:2048 and 1:4096, respectively) and possible chronic Q fever (both 1:2048). Test characteristics of

Phase I IgG titer	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
1:1024	97.8%	nd	62.2	nd
1:2048	94.6%	21.4%	66.7	70.6
1:4096	80.6%	38.9%	76.3	64.7
1:8192	60.2%	83.9%	86.2	60.0
1:16384	47.3%	94.6%	93.6	52.0
>1:16384	25.8%	96.4%	94.4	47.8

PPV, positive predictive value; NPV, negative predictive value; nd, not determinable

different phase I IgG titers, comparing proven cases and possible cases are displayed in table 1.

**Conclusion:** Our study shows that high phase I IgG titers are strongly associated with proven chronic Q fever, especially when exceeding 1:4096 (PPV >86%). However, due to low sensitivity of these titers (<60%) and high morbidity and mortality of untreated chronic Q fever, increasing the current diagnostic phase I IgG cut-off is not recommended. Our study emphasizes that, in case of chronic Q fever, serology is not a diagnostic tool on its own. Therefore, serologic results should, in the absence of a positive PCR, be interpreted in combination with clinical parameters.

#### **P1000** Evaluation of three immunoassays for serodiagnosis of human *Mycoplasma pneumoniae* infection

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**Objectives:** Serology is still the most widely used method to diagnose the infections. Several immunoassays are available for the detection of specific *Mycoplasma pneumoniae* IgG and IgM. The aim of this study was to evaluate a new commercial chemiluminescent assay based on recombinant capsid proteins derived from baculovirus for diagnosis of *M. pneumoniae* infection. This assay has been adapted by Savyon® to be fully automated on LIAISON® instrument (DiaSorin) using chemiluminescence detection system. We have compared this assay with enzyme immunoassays from Savyon® and Medac® performed on the Eti-max® 3000 (DiaSorin) analyser.

**Methods:** A total of 355 sera from different patients from routine daily practice (142 for both IgM and IgG, 100 for IgM and 113 for IgG) were tested with *M. pneumoniae* IgM and IgG kits from Savyon®, Medac® and DiaSorin®. Discordant results were solved by Virion® complement fixation (CF) testing. Furthermore clinical data and PCR results when available were also used.

Intra-assay and inter-assay imprecision based on samples tested in replicates have been performed for both IgM and IgG.

Agreement for at least two results or confirmation with CF assay established reliable diagnosis.

**Results:** Sensitivity was 97.9%, 92% and 93.3% and specificity was 77.2%, 95.9% and 95.2% for *M. pneumoniae* IgM from Savyon®, Medac® and DiaSorin® assays respectively; accuracy was 86.2%, 94.5% and 94.3% respectively.

The degree of agreement between DiaSorin® and Savyon® was 85.1% and 84.1% for IgM and IgG respectively, and between DiaSorin® and Medac® was 86.3% and 88.7% for IgM and IgG respectively.

All discordant results were close to the equivocal range values of each assay.

Intra-assay and inter-assay imprecisions were <10% coefficient of variation for both positive IgM and IgG samples.

**Conclusion:** According to our evaluation, DiaSorin® and Medac® *M. pneumoniae* IgM and IgG assays have similar analytical and clinical performances. Moreover, these assays discriminate sick from healthy patients well, show good specificity, and detect less residual IgM and IgG. *M. pneumoniae* DiaSorin® assays appear to be a valid alternative for the detection of *M. pneumoniae* antibodies. These assays performed on the LIAISON® instrument combine robust analytical and clinical performance with all the advantages of a fully automated, random access instrument system.

#### **P1001** Evaluation of a new chemiluminescent assay for detection of *Mycoplasma pneumoniae* infections

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**Background:** *Mycoplasma pneumoniae* is an important etiologic agent of tracheobronchitis and primary atypical pneumonia in children and adults. The diagnosis of *Mycoplasma pneumoniae* infection is commonly confirmed through nucleic acid amplification or serologic testing since bacterial culture of this organism is slow and lacks sensitivity.

**Aim:** In this study, the performance of two new assays, LIAISON *Mycoplasma pneumoniae* IgG and IgM (DiaSorin, Saluggia, Italy), was compared to that of six ELISA assays: *Mycoplasma pneumoniae* IgG, IgM (Ani LabSystems, LabSystems, Helsinki, Finland), *Mycoplasma pneumoniae* IgG, IgM (MEDAC, Hamburg, Germany) and SeromP Recombinant IgG and IgM (Savyon Diagnostics, Ashdod, Israel).

**Methods:** Group I: 136 selected paired samples from 68 patients with primary infection of *M. pneumoniae* (46 children and 22 adults); group II: 122 selected samples from 27 children and 95 adults with respiratory infections, other than those caused by *Mycoplasma pneumoniae*.

**Results:** The results of the seroconversion panel (number of positive samples to total number of samples) is as follows: 71.3% (for Ani LabSystems IgG), 90.4% (for Ani LabSystems IgM), 62.5% (for LIAISON IgG), 72% (for LIAISON IgM), 62.5% (Savyon IgG), 69.9% (Savyon IgM), 65.4% (MEDAC IgG) and 71.3% (MEDAC IgM). On evaluation of samples from the group II panel Ani LabSystems gave higher IgG seroprevalence (87.7%) when compared with that LIAISON (23.8%), Medac (34.4%) and Savyon (18.9%). IgM seroprevalence was 5.7%, 6.6%, 12.3% and 39.3% for LIAISON, Medac, Savyon and Ani LabSystems respectively. An update version of the Ani LabSystems assays is available whereby the cut off of the IgG kit is increased (from 30 EIU to 45 EIU) and IgM kit cut off 1.1 as opposed to 0.5 (index). Calculation of the results based on these new cut offs would lead to Ani LabSystems score in group I of 64% for IgG and 79.4% for IgM. In group II, the IgG and IgM seroprevalence will be 84.4% and 12.3% respectively.

**Conclusion:** LIAISON *Mycoplasma pneumoniae* IgG and IgM, the first fully-automated assay, showed a good concordance with MEDAC and Savyon assays.

## STD and other infections in women

#### **P1002** Prevalence of *Streptococcus agalactiae* colonisation in pregnant women and antimicrobial resistance profiles

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**Background:** Group B Streptococcus (GBS) is a cause of early neonatal morbidity and mortality. Maternal vaginal colonization with GBS is a risk factor for invasive disease in the first week of newborn life.

**Objectives:** To study the prevalence of vaginal colonization by GBS in pregnant women and to determine the antibiotic susceptibility pattern of the isolates.

**Material and methods:** During a 2 year period from October 2009 through October 2011, 2793 pregnant women attending the department of Obstetrics and Gynecology of "Alexandra" Hospital of Athens were examined for GBS colonization as a part of routine culture of vaginal swabs for common bacterial and fungal pathogens. A vaginal swab obtained in Stuart transport medium was cultured onto Columbia CNA blood agar with colistin and nalidixic acid for Streptococcus isolation. A rapid latex agglutination test was performed for identification of Lancefield A, B, C, D, F and G group antigens of streptococci. Antibiotic susceptibility testing was performed by disc diffusion technique on Mueller-Hinton agar with 5% sheep blood according to CLSI recommendations and MICs were determined by Etest (AB Biodisk, Solna, Sweden).

**Results:** During the study period 93 (3.33%) out of 2793 pregnant women were found positive for GBS. Susceptibility testing of 93 isolates to penicillin, ampicillin, erythromycin, clindamycin, tetracycline, levofloxacin, vancomycin and linezolid showed the following resistance rates: 0% (MICs of 0.094–0.12 µg/mL), 0% (MICs of 0.023–0.032 µg/mL), 26.88% (MICs >256 µg/mL), 19.36% (MICs >256 µg/mL), 92.48% (MICs of 8–12 µg/mL), 3.23% (MICs of 12–24 µg/mL), 0% (MICs of 0.75–1 µg/mL), and 0% (MICs of 0.75–1.5 µg/mL) respectively. The susceptible isolates to penicillin can be considered susceptible to all beta-lactams (cefazolin cephalothin, cefuroxime, ceftriaxone, cefotaxime, cefepime, imipenem) according to CLSI guidelines. Inducible clindamycin resistance was not detected by D-test.

**Conclusions:** (i) The prevalence of GBS in pregnant women of our study is low. (ii) Penicillin or ampicillin remain the drugs of choice for intrapartum antibiotic prophylaxis as isolates with increasing MICs to both agents were not detected. (iii) Clindamycin, the drug of choice for penicillin-allergic women at high risk for anaphylaxis, demonstrates a significant resistance rate. (d) GBS show an unusually very high resistance rate to tetracycline.

**P1003** Epidemiological survey of *Streptococcus agalactiae* isolates from university clinics, Budapest, Hungary

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**Objectives:** *Streptococcus agalactiae* (GBS) remains the leading cause of serious neonatal infections, but can also cause diseases in adults. The aim of the present study was to perform an epidemiological survey of GBS isolates in Hungary. The actuality of the topic is emphasized by the fact that obligatory screening of pregnant women was introduced this year in the country.

**Methods:** Two hundred and ninety-two GBS strains, isolated at the Central Diagnostic Laboratory and the Dermatology Department of Semmelweis University, between March 2009 and May 2011, were involved in the study. PCR was used to detect the *dlrR* gene for strain identity and to determine the prevalence of the ST-17 clone. To distinguish serotypes I, II and III, the Pastorex latex agglutination test was used. Antibiotic sensitivity was determined by disc diffusion.

**Results:** 57.2% of the strains derived from the Obstetric and Gynaecology Clinics, 20.2% from Dermatology, 8.2% from Urology, 5.8% from Surgery, 4.5% from Internal Medicine, and 4.1% from others (e.g. Orthopedics). The types of specimen were as follows: 49.3% female genitals (cervix, vagina), 16.1% newborn colonisation (ear, nose, anus), 12.0% urine, 9.9% male genitals, 9.3% skin, wound or abscess, 1.7% haemoculture and 1.7% respiratory tract. The serotype distribution was: type III (49.7%), type I (23.0%), type II (4.8%), and non-I-II-III (22.6%). Half of the type I strains derived from the Obstetric Clinics. One third (30.1%) of the isolates belonged to the hypervirulent ST-17 clone, and 2/3 of these derived also from the Obstetric Clinics, in many cases from newborn colonisation. Most of the strains were sensitive to both erythromycin and clindamycin, 33% resistant to both, and three strains showed only clindamycin resistance. In five cases we could link the specimens of the newborn and the mother. These isolates were genetically identical, macrolide resistant (ermB+), and three of the pairs were ST17+ (type III).

**Conclusions:** Although serotype III was most prevalent, especially in asymptomatic colonisation, but a higher variation of types among the different specimens was observed, compared to previous data from Hungary. The high prevalence of the ST17 clone in Hungary, which can lead to development of invasive neonatal infections, support the necessity of regular screening during pregnancy. Furthermore, a relative high proportion of adult infections was observed, indicating the increasing pathogenic significance of this bacterium.

**P1004** Susceptibility of vaginal group B *Streptococcus* isolates to penicillin and erythromycin

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**Objectives:** Group B *Streptococcus* (GBS) is capable of causing invasive infections in neonates and pregnant women. Antibiotic intrapartum prophylaxis with penicillin G, or macrolides in allergic patients is mandatory. However, lately erythromycin resistance rates are increasing, leading to severe complications in newborns.

**Aim:** To study the vaginal GBS for susceptibility to antimicrobials including those recommended for prophylaxis.

**Methods:** A total of 50 vaginal GBS isolates from pregnant patients in Skopje were analyzed. Isolation and identification were performed with

conventional microbiology techniques. Mueller Hinton agar with 5% defibrinated sheep blood was used for antimicrobial susceptibility testing to erythromycin (E), azithromycin (AZM), clarithromycin (CLR), penicillin (P), clindamycin (CL), and co-trimoxazole (SXT). Broth dilution technique and M.I.C Evaluator (M.I.C.E.) Strips (Oxoid, UK) were used to determine erythromycin and penicillin MICs and MBCs.

**Results:** Disc diffusion technique revealed resistance of CL and SXT in 19.7% and 100.0% of the strains, respectively. Macrolides showed similar susceptibility, i.e. 16.7% and 13.3% strains were resistant to AZM and CLR, respectively. ERY resistant were 12 (24%) strains with MIC >8 mg/L and MBC ≥16 mg/L. The majority of the GBS strains, 37 (74%) had ERY MIC ≤1 mg/L out of which 27 had MBC 4–8 mg/L; the remaining 10 strains showed tolerance towards ERY (MIC = 1 mg/L and MBC = 16 mg/L). All 50 GBS isolates were susceptible to penicillin MIC ≤0.03 mg/L. There was no significant difference in results between the data obtained by conventional broth dilution and commercial MIC Evaluator.

**Conclusion:** Our study shows that erythromycin resistance is rather high in our environment and antibiotic prophylaxis must be guided by susceptibility testing of each GBS isolate, where commercial methods are both reliable and time efficient.

**P1005** Symptomatic and asymptomatic infections of the lower genital tract in pregnant and non-pregnant women of reproductive age

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**Objectives:** To investigate the prevalence of bacterial and fungal pathogens in pregnant and non pregnant women of reproductive age over a 2-year period.

**Materials and methods:** Between October 2009 and October 2011, 8403 pregnant and non-pregnant women aged 16–42 years attending the department of Obstetrics and Gynecology of “Alexandra” Hospital of Athens were tested for bacterial and fungal pathogens of the lower genital tract. Combined cervical and vaginal or vaginal and urethral swabs were obtained from non-pregnant and pregnant women respectively. The specimens were cultured onto chocolate agar, chromogenic sabouraud dextrose agar, Columbia CNA blood agar with colistin and nalidixic acid and A7 Mycoplasma agar. *Chlamydia trachomatis* were detected in cervical or urethral specimens by immunofluorescence assay. Vaginal swabs were used for wet mount microscopy (detection of yeasts, *Trichomonas vaginalis*, polymorphonuclear leucocytes, clue cells) and preparation of Gram stain. Bacterial vaginosis (BV) was diagnosed based on at least three Amsel criteria and Nugent scoring system.

**Results:** Pathogens were identified in 2282 (40.68%) out of 5610 non-pregnant and 1039 (37.20%) out of 2793 pregnant women examined. The frequency of *Candida albicans*, non-*C. albicans*, BV, group B *Streptococcus* (GBS), group A *Streptococcus* (GAS), *Chlamydia*, *M. hominis*, *U. urealyticum*, *T. vaginalis*, and mixed infection (more than one pathogen) was 29.14%, 17.88%, 16.87%, 10.08%, 0.44%, 8.28%, 0.22%, 3.47%, 1.70%, 11.92% in non-pregnant and 41.48%, 24.54%, 7.80%, 8.95%, 0%, 1.64%, 0%, 3.95%, 1.73%, 9.91% in pregnant women respectively. *Neisseria gonorrhoeae* was not detected in any specimen. Leukocytes were detected in 33.17% of non-pregnant and in 31.18% of pregnant women found positive for pathogens. For a total number of non-pregnant (5610) and pregnant women (2793) studied, the prevalence of GBS was 4.09% and 3.33% respectively. Statistical analysis by chi squared test showed statistically significant: (i) higher frequency of BV and *Chlamydia* infection in non-pregnant and (ii) higher rate of *Candida* infection in pregnant women.

**Conclusions:** *Candida* spp is the most common pathogen in both groups with higher infection rate in pregnant women. The frequency of BV and *Chlamydia* infection is higher in non-pregnant than in pregnant women, whereas there is no statistically significant difference in GBS prevalence among the two groups.

**P1006** Increased quinolone-resistant *Ureaplasma urealyticum* and *Mycoplasma hominis* isolated in reproductive-age women with vulvovaginitis

S. Baka, I. Tsirmpa, I. Tsouma, E. Logothetis, E. Kouskouni\* (Athens, GR)

**Objectives:** *Ureaplasma urealyticum* (Uu) and *Mycoplasma hominis* (Mh) are members of a unique group of microorganisms commonly found in the genital system of reproductive age women. However, these mycoplasmas have been associated with a plethora of genital tract infections with different clinical manifestations. We aimed to study the prevalence and the susceptibility to antibiotics of Uu and Mh isolated from the vaginal secretions in a group of reproductive age women with vulvovaginitis.

**Methods:** Vaginal samples from 3702 symptomatic women of reproductive age, presenting with signs and symptoms of vulvovaginitis to the Outpatient Clinic of Aretaieion University Hospital from January 2007 to October 2011, were studied. For the isolation and susceptibility testing of both mycoplasmas the commercial kit Mycoplasma IST2 (BioMerieux, France) was used. After inoculation onto the respective nutrient medium, the samples were incubated at 36–37°C for 48 hour in aerobic conditions.

**Results:** Out of the 3702 samples Uu was isolated in 769 (20.8%) samples while Mh in 70 (1.9%), always in association with Uu. Only concentrations of  $>10^4$  CFU/mL at 48 hour were included as positive samples in the study. As for the susceptibility testing to antibiotics, we considered both resistance and intermediate susceptibility as resistance and the data is presented as such. In the isolates studied, doxycycline and tetracycline displayed the lowest percentages of resistance (1.0% and 3.0%, respectively). Among the macrolides, the same percentages for clarithromycin, azithromycin and erythromycin were 12.2%, 17.6% and 18.1%, respectively. In contrast, the genital mycoplasmas tested showed decreased susceptibility to quinolones. Specifically, 86.1% and 53.7% of the mycoplasmas were resistant to ciprofloxacin and ofloxacin.

**Conclusions:** The isolates studied were highly resistant to quinolones, due to the increased and irrational use in the last years. As a result, it is imperative to stop the empirical treatment of genital mycoplasmas and clinicians must adjust the therapeutic approach to the results of the in vitro susceptibility testing.

**P1007** Vaginal microflora in postpartum females after vaginal delivery

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**Objectives:** In postpartum period, the composition of vaginal microflora changes due to wash-out of microorganisms with amniotic fluid and blood, traumatizing the birth channel, contamination of the vagina with intestinal microflora. These changes are temporary, and gradually the vagina is populated with bacterial species characteristic for this ecological niche. Timely restoration of normal vaginal microbiota is necessary for successful postpartum period. The study aimed to assess the duration of the period after vaginal delivery during which vaginal microflora is restored.

**Methods:** Cultural investigations of lochia from 43 postpartum females who had vaginal delivery were performed on days 1, 3, 5, and 15 postpartum.

**Results:** On day 1, in 23.2% of the females no microorganisms were isolated. By day 5, different bacterial communities were detected in most subjects (93.0%), due to intense migration of microorganisms from the anogenital area to the vagina. On days 1–5, enterococci, bacteria of the family Enterobacteriaceae, epidermal staphylococci, and non-fragilis Bacteroides were present in 27.9–37.2%, 34.9–44.2%, 27.9–51.6%, and 44.2–60.5% of the females, respectively. The frequency of detection of corynebacteria from lochia in the puerperas during the period from day 1 to day 5 increased three-fold. Yeasts were

isolated rarely (in 4.6–7.0% of the females). Lactobacilli on days 1–5 were detected in 7.0–11.6% of the females. By day 15, lactobacilli were present in the majority of the subjects.

**Conclusion:** Vaginal microbiota on the first days after delivery is characterized by predominance of anaerobic microorganisms belonging to the genus Bacteroides (non-fragilis Bacteroides). Complete restoration of normal vaginal microflora (predominance of lactobacilli) occurs in two weeks after delivery.

**P1008** Microbiological features of vulvovaginitis in prepubertal age

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**Background:** The aetiology of most cases of vulvovaginitis in prepubertal age is not specific. However in some girls the symptoms are caused by specific bacterial or fungal pathogens.

**Objectives:** To evaluate the prevalence of various pathogens involved in the pathology of vulvovaginitis in girls of prepubertal age over a 2-year period.

**Materials and methods:** Three hundred eighty (380) prepubertal girls of age from 1 to 10 years, presenting at the paediatric gynaecology clinic from October 2009 to October 2011 with symptoms and signs of vulvovaginitis (vaginal discharge, erythema or pruritus), were tested for bacterial and fungal pathogens. Swabs of vaginal secretions were obtained and placed in normal saline for wet preparation and in Stuart transport medium for culture. The wet preparation was examined for yeasts, Trichomonas and polymorphonuclear leucocytes. The swab in transport medium was cultured onto chocolate agar for *Haemophilus influenzae* growth, Columbia CNA blood agar with colistin and nalidixic acid for Streptococcus isolation, chromogenic sabouraud dextrose agar for *Candida* isolation and species identification and MacConkey agar for isolation and differentiation of Enterobacteriaceae, and non-Enterobacteriaceae. Urea-Arginine broth for specimen collection and A7 Mycoplasma agar for culture of *U. urealyticum* and *M. hominis* were not routinely used. A rapid latex agglutination test was performed for identification of Lancefield A, B, C, D, F and G group antigens of streptococci (SLIDEX® Strepto-Plus, bioMérieux SA Lyon, France).

**Results:** Causative agents were isolated from vaginal secretions in 48 (12.63%) out of the 380 study girls, whereas in 332 (87.37%) girls non-specific pathogens were identified. Of the 48 culture-positive cases, group A streptococcus (*S. pyogenes*), group B streptococcus (*S. agalactiae*), non-*Candida albicans*, *Candida albicans*, *Haemophilus influenzae*, *Escherichia coli*, *Ureaplasma urealyticum* and *S. pyogenes* with non-*C. albicans* (mixed infection) were isolated in 23 (6.05%), 13 (3.42%), 6 (1.58%), 2 (0.53%), 1 (0.26%), 1 (0.26%), 1 (0.26%), and 1 (0.26%), girls respectively.

**Conclusions:** (i) In most cases of vulvovaginitis in prepubertal girls non-specific bacterial or fungal pathogens are identified as causative agents. (ii) The most common infectious cause of vulvovaginitis in prepubertal age is *S. pyogenes* followed by *S. agalactiae* and *Candida* spp.

**P1009** Pathogens implicated in cases of vulvovaginitis in prepubertal and pubertal girls

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**Objectives:** Vulvovaginitis is recognized as the most common gynecological problem in prepubertal and pubertal girls. However, the clinical significance of the pathogens isolated from the vaginal cultures performed in children can be interpreted only after taking into consideration clinical information and possible risk factors, if any. This study aimed to evaluate the vaginal pathogens isolated in a group of prepubertal (PP) and pubertal (P) girls with vulvovaginitis.

**Methods:** A total of 441 girls aged 2–18 years, presenting at the outpatient clinic for pediatric and adolescent gynecology of our hospital



during January 2008 to June 2011 with signs and symptoms of vulvovaginitis (vaginal redness and discharge and/or itching) were included. Cases were divided into two groups: 198 PP (age 2–11 years) and 243 P (age 12–18 years). Vaginal samples were collected using a sterile newborn suction catheter carefully inserted into the vagina. Samples were inoculated onto blood agar, MacConkey, Mannitol Salt, Sabouraud Dextrose agar, Gardnerella agar and Wilkins-Chalgren agar as well as Thayer-Martin and chocolate agar followed by incubation in aerobic, anaerobic or CO<sub>2</sub> atmosphere at 37°C for 24 or 48 hours, as appropriate. Wet mount and Gram stain preparations were examined to assess the presence of leukocytes and the type of bacteria present. The identification of isolated strains and their susceptibility test to antibiotics were carried out with the API System and the automated system VITEK 2 (BioMérieux, Marcy l'Etoile, France).

**Results:** Bacterial pathogens were isolated from 105/198 (53.0%) of PP girls and 191/243 (78.6%) of P girls. Interestingly, leukocytes were observed only in samples where pathogens were retrieved. Isolated bacteria in the PP and P groups included 21/198 (10.6%) and 143/243 (58.8%) Gardnerella vaginalis and anaerobes, 45/198 (22.7%) and 15/243 (6.2%) Gram-positive cocci, 39/198 (19.7%) and 20/243 (8.2%) Gram-negative rods, respectively. *Candida* species were isolated in 13/243 (5.3%) only in the P group. Finally, in 93/198 (47.0%) in PP group and 52/243 (21.4%) in P group no pathogen was isolated.

**Conclusion:** The presence of leukocytes in vaginal samples increases the likelihood of finding pathogens which require specific treatment. In the PP girls predominantly Gram-positive cocci and Gram-negative rods were isolated while in the P girls, Gardnerella vaginalis and anaerobes. *Candida* species were found only in the P group.

#### **P1010** Asymptotic co-infection of candidiasis and vaginal trichomoniasis among pregnant women in southeastern Nigeria

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**Objective:** Morbidity and mortality rate among pregnant women in rural setting in third world countries are ever increasing. This condition is often caused by infections that may occur at sub-clinical level but may progress to acute clinical condition in the course of pregnancy.

**Methods:** A total of one thousand five hundred (1500) pregnant women of ages between 25 and 40 years, attending antenatal clinic at Federal Medical Centre Abakaliki between March and October 2010 were enlisted for this study. Personal biometric data and trimesters of the gestation were considered. Wet preparation, culture and germ tube test were used to examine high vaginal swab specimen.

**Result:** The result showed that a total of 326 (22%) women had asymptomatic co-infection of *Candida* and trichomonas while 412 (275%) and 355 (24%) women had asymptomatic candidiasis and trichomoniasis as single infection respectively.

**Conclusion:** This study therefore underscores the need to screen pregnant women regularly for candidiasis and trichomoniasis especially considering its public health importance in the transmission of HIV/AIDS and other undesirable pregnancy outcome such as low birth weight and preterm delivery.

#### **P1011** Is bacterial vaginosis associated with cervical (pre)neoplasia in asymptomatic reproductive-age women?

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**Objectives:** Early detection of cervical cell abnormalities by Papanicolaou (Pap) smear has reduced the risk of cervical cancer development. It has been suggested that other factors besides human papillomavirus (HPV) infection, might be involved in the malignant progression. Cervical inflammation has been proposed as one of the cofactors in cervical carcinogenesis, because disturbance of the vaginal microflora is associated with an increased risk of acquisition of HPV

infection. Bacterial vaginosis, which sometimes can be asymptomatic, is a clinical entity quite common in women all over the world and is characterized by an increased production of N-nitrosamines by Gardnerella vaginalis, which are carcinogenic. We aimed to evaluate a possible association between the presence of *G. vaginalis* in asymptomatic reproductive age women with cervical (pre)neoplasia.

**Methods:** Asymptomatic women with inflammatory changes on routinely performed Pap smear and recalled for cultures in the last year were included in the study. Vaginal and cervical cultures were performed under standard conditions. A wet mount as well as a gram-stained smear were examined under microscope to obtain valuable information about the microorganisms present and to apply Nugent criteria for the diagnosis of bacterial vaginosis.

**Results:** For this preliminary study we included 77 women with bacterial vaginosis and 41 women with negative cultures (normal flora present), who served as controls. In the bacterial vaginosis group cervical cytology was normal in 64 (83.1%) and abnormal in 13 (16.9%) cases. Epithelial cell abnormalities included seven cases of atypical squamous cells of undetermined significance (ASCUS), five cases of low-grade squamous intraepithelial lesions (LGSIL) and one case of high-grade squamous intraepithelial lesions (HSIL). In the controls, normal cervical cytology was present in 38 (92.7%) and abnormal in 3 (7.3%), including two cases of ASCUS and one LGSIL. All patients with epithelial cell abnormalities were referred for colposcopy and managed according to standard practices, while women with bacterial vaginosis were treated with metronidazole

**Conclusions:** The higher prevalence of abnormal cytology in the group of women diagnosed with bacterial vaginosis stresses the need for cervical cytology screening in these patients. Further research on women with bacterial vaginosis is needed to study the causal relationship between *G. vaginalis* infection and cervical (pre)neoplasia.

#### **P1012** Prevalence and antibiotic susceptibility of *Ureaplasma urealyticum* in genital samples between 2007 and 2011

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**Objectives:** The genital mycoplasmas constitute a group of microorganisms commonly found in the genitourinary tract. The most frequently isolated species among them, *Ureaplasma urealyticum*, is an important opportunistic pathogen, which has been associated with various urogenital and intrauterine infections.

**Methods:** We assayed the positive cultures for *U. urealyticum* detected from vaginal, cervical, male urethral and ejaculatum samples at our Institute from 01. January 2007 to 30. June 2011. For screening, enumeration, identification and antimicrobial susceptibility testing, the Mycoplasma IST 2 kit (bioMérieux) was used. Only samples containing mycoplasmas above the pathological threshold were regarded as positive.

**Results:** During the examined period 1144 specimens were found to be positive for *U. urealyticum*. The vast majority of the strains (1044) were cultured from vaginal samples, while 17, 56 and 27 strains originated from cervical, male urethral and ejaculatum samples, respectively. Because of the routine screening of pregnant women, the number of vaginal specimens was much higher than those of the other types of samples. Altogether, 5677 vaginal specimens from pregnant women were tested for *U. urealyticum*, among which 450 proved to be positive (7.93%). In 19.77, 18.22% and 8.22% of these cases, *U. urealyticum* was detected in co-infection with *Candida* spp., *Streptococcus agalactiae* and *E. coli*, respectively. Antimicrobial susceptibility was tested against doxycycline, josamycin, ofloxacin, erythromycin, tetracycline, ciprofloxacin, azithromycin, clarythromycin, and pristinamycin. Nearly all of the strains cultured proved to be susceptible to doxycycline (99%), tetracycline (98%), josamycin (99%), pristinamycin (99%) and clarythromycin (91%). Eighty-four percent and 68% of the strains were susceptible to azithromycin and erythromycin, respectively. At the same time, only a few strains were susceptible to ofloxacin (26%) and ciprofloxacin (13%); moreover, 51% of the strains proved to be resistant to ciprofloxacin.

**Conclusion:** Our results indicate that only the detection of mycoplasmas in genital samples is not sufficient for successful therapy, and highlight the need for accurate susceptibility tests.

#### P1013 Sexually transmissible infections among young adolescents in Milan areas: a multicentre study

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**Objective:** Sexually transmitted infections (STIs) are a major health problem affecting mostly young people, the exact magnitude of STIs is frequently unknown due to lack of country surveillance systems.

Aim of this study was to determine the prevalence of STIs and relative risk factors among and adolescents in Milan areas, Italy.

**Methods:** From May to October 2011, 117 adolescents (63 female, 54%), median age 15 years, attending hospitals from the north-western areas of Milan, Italy, were enrolled. All subjects completed a questionnaire and provided a urine sample, which was tested for *Neisseria gonorrhoea*, *Chlamydia trachomatis*, *Mycoplasma hominis*, *Mycoplasma genitalium*, *Ureaplasma urealyticum/parvum*, *Trichomonas vaginalis*, *Treponema pallidum*, *Streptococcus agalatae*, Haemophilus ducrey, Citomegalovirus (CMV), Herpes Simplex Virus 1 (HSV1) and Lymphogranuloma venereum by a multiplex PCR assay: Seeplex® STI Master ACE Detection (Seegene, Seoul, Korea).

Forty three out of 117 adolescents (36%) were HIV-1 positive, 63% (74/117) were without any underlying infectious disease.

**Results:** Fifty seven (48.7%) out of 117 adolescent were sexually active (SA), 20 out of 57 (35%) had STIs as follow: 24.5% (14 cases) *U. urealyticum/parvum*, 7% (four cases) *C. trachomatis*, 1.7% (one case each) *M. genitalium* and *N. gonorrhoea*.

Thirty-two (56%) out of 57 SA adolescents were HIV-1 positive and infected with *U. urealyticum/parvum* (37.5%, 12 cases) and *C. trachomatis* (6.2% two cases). A single case (3.1%) of mixed infection due to *C. trachomatis*, *N. gonorrhoea* and *U. urealyticum/parvum* was observed.

Six out of 60 (10%) sexually inactive (SI) adolescents resulted positive for *U. urealyticum/parvum* (three cases), *C. trachomatis* (two cases) and *N. gonorrhoea* (one case). Eleven out of 60 were HIV-1 positive and among this group one case of *C. trachomatis* and *U. urealyticum/parvum* infection was reported. For *T. vaginalis*, *T. pallidum*, *S. agalatae*, H.ducrey, CMV, HSV1 and Lymphogranuloma venereum any infection was reported.

**Conclusion:** STIs as expected were higher in SA adolescent than in SI and in HIV-1 positive patients ( $p < 0.025$ ). Twenty-two percent of SA adolescents resulted positive for at least one STIs.

A prevalence of 14.5% (17/117) for *U. urealyticum/parvum*, was detected in the adolescents studied, even if its clinical significance has yet to be assessed. Findings suggest that surveillance and screening programs should be implemented to prevent sequels on this vulnerable population.

#### P1014 Prevalence and risk factors for *Chlamydia trachomatis* infection in adolescents and university women from north Portugal

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**Objectives:** Worldwide, Chlamydia is a common bacterial sexually transmitted disease (STD) in younger women. Our goal was to characterize *Chlamydia trachomatis* infection status in female students from university and pre-university schools resident in the northern region of Portugal.

**Methods:** Gynaecological samples were obtained from 435 young women (14–30 years) by self-collection using a commercial kit. Demographic, behavioural, and clinical factors were accessed through

an anonymous questionnaire. *C. trachomatis* were detected by Polymerase Chain Reaction (PCR) using CP24/CP27 primers.

**Results:** The results revealed that the median age of participants was 17.0 years; the frequency of sexually active women in the group was of 63.7% ( $n = 277$ ) with a median age of first sexual intercourse of 16.0 years; a median number of years after first sexual intercourse of 2.0 years and 33.9% ( $n = 94$ ) of the sexually active women had two to five sexual partners and 96.4% ( $n = 267$ ) had no STD history. *C. trachomatis* prevalence was 8.7%, among sexually active women. Statistical significant differences were observed when comparing *C. trachomatis* distribution to median age (OR = 4.87;  $p = 0.006$ ), median age of first sexual intercourse (OR = 3.74;  $p = 0.004$ ), education level (OR = 2.90;  $p = 0.011$ ) and Human Papillomavirus vaccination (OR = 0.24;  $p = 0.017$ ) (Table1).

	p	OR	IC95%
<b>Education level</b>			
University vs Pre-university	0.011	2.90	1.24 – 6.813
<b>HPV Vaccination</b>			
Vaccinated vs Non-Vaccinated	0.017	0.24	0.07 – 0.84
<b>Median age</b>			
≤17 vs >17	0.006	4.87	1.42 – 16.81
<b>Median age first menarche</b>			
≤12 vs >12	0.350	1.49	0.64 – 3.46
<b>Median age first sexual intercourse</b>			
≤16 vs >16	0.004	3.74	1.43 – 9.73
<b>Nº Sexual Partners</b>			
1 vs 2-5	0.670	1.21	0.50 – 2.91
<b>Nº years after first sexual intercourse</b>			
≤2 vs >2	0.989	1.01	0.41 – 2.45
<b>Contraception use</b>			
No vs yes	0.251	1.10	1.06 – 1.15
<b>Smoking status</b>			
Smoker vs Non-smoker	0.268	0.50	0.14 – 1.75

p, Significance level; OR, Odds Ratio; CI, Confidence interval.

**Conclusion:** In young Portuguese women, *C. trachomatis* infection is common in asymptomatic women, university students and who initiated sexual activity over the 16. Furthermore, these results revealed that *C. trachomatis* detection can be performed in self-collected samples. The knowledge of *C. trachomatis* profile in young women may be important to appraise better preventive measures within different populations.

#### P1015 Emergence of macrolide resistance for *Mycoplasma genitalium* in France

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**Objectives:** *Mycoplasma genitalium* is a sexually transmitted organism associated with non gonococcal urethritis (NGU) in men and several inflammatory reproductive tract syndromes in women such as cervicitis, pelvic inflammatory disease and infertility. A dose of 1 g of azithromycin is commonly used for NGU and cervicitis treatment but is responsible for 13–33% of treatment failures. Resistance to macrolides has been recently identified in *M. genitalium* in Scandinavia, Australia, New Zealand and Japan and is associated with point mutations in 23S rRNA. Our goal is to identify such a resistance and to determine its prevalence in France.

**Methods:** A retrospective study conducted in France over a period of 8 years (2003–2010) has selected 156 urogenital clinical samples positive for *M. genitalium* from 136 patients. Detection of mutations in the domain V of 23S rRNA of *M. genitalium* was performed by amplification and sequencing. The genotyping of the mutated strains was performed by studying the single nucleotide polymorphisms of the *mgpB* gene directly from specimens and clinical data and treatment of the patients were collected.

**Results:** A substitution in the 23S rRNA was found in 10.6% (13/123) of the patients infected with *M. genitalium*. No mutation was found

before 2006, they have been detected in samples from 2006 at a rate between 10% and 15% of patients per year. Ten mutations at position 2059, two A2058G mutations, mutations A2062T and C2038T were identified in *M. genitalium* (*Escherichia coli* numbering). These patients had treatment failure with azithromycin in 75% (6/8) of cases. For one patient, genotyping showed the selection of a mutation during treatment with azithromycin 1 g.

**Conclusion:** We describe for the first time the emergence of macrolide resistance in *M. genitalium* in France. This resistance seems to have appeared in 2006 and its epidemiological surveillance is needed to adapt the treatment of *M. genitalium* infections, given the high rate of treatment failure with azithromycin 1 g in case of resistance.

**P1016** Prevalence and risk factors associated with *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Mycoplasma genitalium* infections in French pregnant women

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**Objectives:** In France, screening for sexually transmitted infections (STIs) is recommended in at-risk populations to the exclusion of pregnant women, for whom screening is performed only if the patient is symptomatic. The objectives of this study were to estimate the prevalence and to identify risk factors of *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG) and *Mycoplasma genitalium* (MG) infections in pregnant women consulting at the Bordeaux University Hospital.

**Methods:** Were included in the study pregnant women aged more than 18 years old (yo) for which a vaginal swab was realized during pregnancy follow-up. Patients received a note written information and gave their oral consent. Patients treated within 3 weeks by macrolides or beta-lactams were excluded from the study. Clinical, sexual behavior and sociodemographic patient's characteristics were collected. Real-time PCRs were performed on vaginal swabs by using the Roche Cobas® 4800 CT/NG test for CT and NG detection and an in-house Taqman assay for MG detection.

**Results:** A total of 1070 patients were eligible. Among them, 42 patients refused to participate in the study, one patient did not understand the objectives of the study, 10 patients had antibiotics before and in 12 cases, the vaginal swab was not performed. Of 996 patients included, the prevalence of CT and MG infections were 2.5% (25/996) (95% CI: 1.6–3.7) and 0.8% (8/996) (95% CI: 0.6–1.6), respectively. Increased infection rates were found in patients aged 18–24 year, with 7.9% for CT and 2.4% for MG. No NG infection was found and no dual CT/MG infection was diagnosed. Most infected patients were asymptomatic (81.8%, 27/33), 9.1% were symptomatic of urogenital infection (3/33) and 9.1% were hospitalized for premature rupture of membranes or threatened premature delivery (3/33). CT infection was associated with the following independent risk factors: age  $\leq 24$  year ( $p = 0.001$ ), being single ( $p = 0.01$ ) and having more than five sexual partners in life ( $p = 0.0001$ ). A younger age ( $p = 0.001$ ), a history of abortion ( $p = 0.01$ ) and having first sexual intercourse after 20 years ( $p = 0.01$ ) were associated with MG infection.

**Conclusions:** Our study shows that pregnant women aged 18–24 year, mainly asymptomatic, represent a population at risk of CT infection. A systematic test screening for CT infection for pregnant women aged under 25 year could be recommended.

**P1017** Epidemiology and clinical characteristics of gonorrhoea in northern Denmark region, 2000–2010

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**Introduction:** An increase in the incidence of gonorrhoea has been reported in several European countries, along with a concomitant rise in the level of fluoroquinolone resistance. To ascertain temporal trends in

*Neisseria gonorrhoeae* infections in the North Denmark Region (population 580 000 inhabitants in mixed urban and rural communities), we conducted a population based study from 2000 to 2010.

**Materials and methods:** We reviewed medical records for all incident episodes of microbiologically confirmed *N. gonorrhoeae* infections.

**Results:** A total of 296 incident episodes from 285 patients were included, with the majority of infections observed in men (71%). The majority of patients (275/285 = 96%) had only 1 diagnosed episode of gonorrhoea during the study period, whereas nine patients had two episodes and one patient had three episodes. The annual incidence increased five-fold during the study period, from 2–4 per 100 000 inhabitants in 2000–2007 to 17 per 100 000 inhabitants in 2009. Most infections were acquired in Denmark: 87% for males vs. 98% for females ( $p = 0.003$ ). Median age was 22 years for females and 26 years for males ( $p < 0.0001$ ).

In 10% of male episodes and 23% of female episodes ( $p = 0.004$ ) swabs were performed from anatomical sites according to recommendations from the National Board of Health (NBH), with highest accordance in the latter part of the study period ( $p = 0.02$ ). Swabs from non-genital sites were performed in 15% of males and 34% of females ( $p = 0.001$ ). Follow-up swabs were performed in 60% of male episodes and 78% of female episodes ( $p = 0.003$ ), and were performed according to recommendations from NBH in 51% of male episodes and 36% in female episodes. In 79% of episodes a concurrent chlamydial swab was performed, 18% of these were positive with no gender-difference.

The level of fluoroquinolone resistance fluctuated between 13% and 93%, with the highest level in 2009. No isolates were resistant to ceftriaxone. All episodes of fluoroquinolone resistance were treated with ceftriaxone i.m. or (in two cases of allergy) with gentamicin i.v. We observed no cases of treatment failure, although not all patients returned for follow-up examination.

**Conclusions:** We have described a recent increase in *N. gonorrhoeae* infections in North Denmark Region and an increasing level of fluoroquinolone resistance. Our study highlights the importance of microbiological confirmation, treatment, and follow up of gonorrhoea in accordance with national guidelines.

**P1018** Syphilis – the peak of the iceberg

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**Background:** Recent epidemiological data show an increased trend of incidence and prevalence for syphilis (S) infection in the general population. *Treponema pallidum* invades the central nervous system early in the course of disease and causes persistent infection in a subset of infected persons. The manifestations of neurosyphilis (NS) can be asymptomatic or symptomatic (acute meningitis, meningovascular S, parietic NS and tabes dorsalis). The incidence of NS is increasing, some reports being of 0.4/100.000 in west European country.

**Objectives:** The aim of this study is to characterize from epidemiological point of view the patients diagnosed with S in the last 4 years in Matei Bals National Institute for Infectious Disease (INBI) – Bucharest, Romania and focused on NS.

**Methods:** This is a retrospective analysis based on reviewing data of patients diagnosed with S in INBI, comparing two consecutive periods: 2008–2009 (I) and 2010–2011 (till 01.10) (II). All patients were serology screened using the Venereal Disease Research Laboratory (VDRL) and confirmed with T pallidum hemagglutination test (TPHA). The staging of the disease was based on clinical presentation: Primary S – painless chancre with/without lymphadenopathy; Secondary S – systemic manifestations (malaise, fever, myalgias, arthralgias, lymphadenopathy) and rash or condylomata lata and patchy alopecia; Tertiary S – neurological or cardiac involvement; Latent S – asymptomatic; NS: asymptomatic or symptomatic with CSF abnormalities (pleocytosis, elevated protein, decreased glucose, and positive VDRL test).

**Results:** In the studied period (2008–2011) the total patients (pts) number was 165, medium age of 31.2 years, 103 males and 62 females. Congenital S – 15 cases (9%); 16 NS cases (9.7%): one meningitis, eight meningovascular S, four general paresis and four latent S. Out of 165, 47 pts (28.5%) were S-HIV-coinfected.

Regarding the two periods, (I) with 93 pts and (II) with 72 pts, there are 2 vs 3 cases for primary S (2 vs 3 cases), 16.2% vs 19.4% secondary S, 8.6% vs 11.1% tertiary S; for latent S 61.6% vs 56.9%; for NS 8.6% vs 11%.

**Conclusions:** In our study there was a high number of latent S reminding the need for S screening especially among HIV infected patients, pregnant women and neonates. There was also a trend of increasing in NS proportion, mostly meningovascular S, so any neuropsychiatric patient or vascular thrombotic neurologic accident and meningitis without a clear etiology should be evaluated for S.

#### P1019 Cost-effectiveness of azithromycin for the treatment of pelvic inflammatory disease in a multi-field hospital in Russia

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**Objectives:** Pelvic inflammatory disease (PID) is a major source of gynaecological morbidity, infertility, ectopic pregnancy and abscess formation, which makes it a very important and expensive problem of healthcare system. Azithromycin (AZI) has potent in vitro activity against main PID pathogens. Two randomised controlled trials showed similar overall clinical success rates for AZI as monotherapy or combined with metronidazole (MET) as well as amoxicillin/clavulanic acid (AC) combined with doxycycline (DOX) (97.1% vs. 98.1% vs. 94.6%), whereas the duration of treatment courses was different (7 vs. 12 vs. 21 days) [Bevan C.D. et al. J Int Med Res. 2003]. We aimed to assess the cost-effectiveness of above mentioned treatment strategies in patients with PID hospitalised into a multifield hospital in Russia.

**Methods:** A cost-minimisation model was developed from the perspective of the Russian National Healthcare System as similar efficacy was assumed between comparators. Only direct medical costs were considered. Drug costs (for original ones where possible) were extracted from Pharmindex database (www.pharmindex.ru). The length of hospital stay was calculated to correspond treatment course duration and average one in Russia (14 days). Uncertainty was explored in two-way sensitivity analyses.

**Results:** The respective total healthcare costs per patient are listed in the table. The results were insensitive to drug cost and length of hospital stay changes.

Treatment regimen	Total costs per patient, €	
	Length of hospital stay corresponds treatment course duration	Length of hospital stay corresponds real practice (14 days for AZI+MET, 21 days for AC+DOX)
AZI IV day 1, AZI orally day 2-7	276.78	534.0
AZI IV day 1-2, AZI orally day 3-7	284.3	541.5
AZI IV day 1, AZI orally day 2-7, MET IV day 1, MET orally day 2-12	467.7	541.2
AZI IV day 1-2, AZI orally day 3-7, MET IV day 1-2, MET orally day 3-12	479.6	553.0
AC IV day 1-5, AC orally day 6-21, DOX orally day 1-21	865.8	865.8

Doses: AZI IV – 500 mg QD, AZI orally – 250 mg QD, MET IV – 500 mg TID, MET orally – 500 mg TID, AC IV – 1.2 g TID, AC orally – 625 mg TID, DOX orally – 100 mg BID

**Conclusion:** AZI as monotherapy or with MET is the most cost-effective regimen for the treatment of PID in a multifield hospital in Russia regardless of rout of administration and hospital stay length.

#### P1020 Nonpuerperal breast infection: epidemiology and predictors for recurrences

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**Background:** Nonpuerperal breast infection (NPBI) is an infection arising within 1 cm of the areola in the nonlactating breast. Although a rare entity, due to its recurring nature, often leads to development of mammary duct fistulae, repeated surgical manipulations and disfigurement of the breast. The aim of the study was to evaluate prognostic factors leading to relapses.

**Methods:** All patients with NPBI referred to our Outpatient Clinic, treated and prospectively followed-up, were evaluated. Epidemiological and clinical features were recorded. Cure was defined as normal ultrasound study and clinical presentation, and improvement as only normal clinical presentation, before discontinuation of treatment. Statistical analysis was made by Kaplan–Meier estimator, Cox proportional hazards model and Prentice–Williams–Peterson model.

**Results:** The 149 patients evaluated, had the following characteristics: median age 39 years old (IQR 33, 46), female gender 96.6%, menopause 14.5%, smokers 74.6%, fibrocystic breast disease 10.1%, chronic infection 91.3%, previous surgery 62%, fistulae 54.1%, and median number of mastitis episodes before recruitment 3 (IQR 2, 5). Twenty-nine patients were excluded from further evaluation due to failure of first treatment or lost to follow up. Most common pathogens were coagulase-negative staphylococci (15.8%), *Proteus mirabilis* (12.5%), *S. aureus* (7.5%), and anaerobes (4.2%).

Amox/clav, moxifloxacin, clindamycin, cotrimoxazole, ciprofloxacin or metronidazole alone or in combination were mostly used for a median duration of 8 weeks. Cure was reported in 104 (86.7%) patients and improvement in 16 (13.3%). Median time until relapse was 1.43 (0.82–3.93) years. When only the first relapse was considered, cure as a result was protective (HR 0.406 95%CI: 0.203–0.811 p = 0.011), whereas the presence of fistula was associated with a significantly increased rate of relapse (HR 1.804 95%CI: 1.055–3.86 p = 0.031). When all relapses were considered cure as a result remained significantly protective (HR 0.44 95%CI: 0.240–0.810 p = 0.008) whereas the presence of fistula was no longer a significant factor (HR 1.48 95%CI: 0.999–2.192 p = 0.051). Surgical intervention didn't protect from recurrences (HR 1.116, p = 0.614).

**Conclusions:** The presence of fistula was associated with a higher relapse incidence. Sonographic evaluation is mandatory, since confirming cure was proved to be a major protective factor.

## Vaccines

#### P1021 Evaluation of immune responses against fused p24 and gp41 genes of human immunodeficiency virus type 1 by using two recombinant vectors as a vaccine candidate

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**Objective:** To date, 60 millions persons have been infected with HIV-1 from which 22 millions have lost their lives due to AIDS. Therapeutic expenses for an AIDS infected patient are about \$20 000 per year. Antiretroviral medications can not completely destroy the virus, and in addition, they cause the appearance of mutant variants. An effective vaccine represents the best hope to curtail the HIV epidemic. It is now widely believed that an HIV vaccine strategy must both a strategy humoral and as well as cell-mediate immune response. DNA vaccine induce conformational – dependent humoral and cellular responses and mimic live vaccines without their pathogenic potential.

**Methods:** In this study, two recombinant plasmids, pcDNA3.1/Hygro (lacking the signal sequence) and pSecTag2/HygroA (with signal sequence), containing immunogenic sequences of p24-gp41 were produced and the expression of p24 and gp41 proteins was confirmed by immunofluorescence techniques. Induction of effective immune responses against the designed vectors as DNA vaccine candidate in Balb/C mice was evaluated. Levels of total antibodies, IgG isotypes (IgG2a and IgG1); IFN-gamma and IL4 were measured by ELISA. MTT assay was used to evaluate the lymphoproliferative activity of lymphocytes.

**Results:** Analysis of results of experiments showed that suitable immune responses can be induced against both vaccines. However, a

significant difference was observed in total antibody and IL4 production in the vector containing signal sequence.

**Conclusions:** The use of pSecTag2/HygroA vector seems to be more suitable in future research. More research needs to follow up to confirm this.

**P1022 Immune response after booster vaccination in HIV-infected patients who had previously received primary rabies immunisation**

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**Objective:** Current WHO recommended pre- and post exposure rabies immunizations have been reported not be able to induce adequate immune response in HIV-infected individuals with low CD4+ T lymphocytes, remained the controversial issue for rabies prophylaxis in these vulnerable people. So the principle of priming immunity which thereafter booster injection induced accelerated potential immune response was applied to our prospective study in order to evaluate the immunogenicity after rabies booster strategy in HIV-infected patients.

**Method:** HIV-infected adults who were identified either prior primary rabies immunized with cell-culture rabies vaccines or ever had rabies neutralizing antibody (RNab) titers >0.5 IU/mL received simulated intramuscular post-exposure rabies booster vaccination with purified Vero cell rabies vaccines on day 0 and 3. Serum samples were taken on day 0, 7, 14 and 28 for serological analysis of RNab by the rapid fluorescent focus inhibition test (RFFIT). CD4+ counts were determined before vaccination.

**Results:** Total 29 HIV-infected adults enrolled (median age 43 years, range 20–56 years). 96.5% were receiving highly active antiretroviral therapy (HAART) with median CD4+ count was 460 cell/mm<sup>3</sup> (range 94–913 cell/mm<sup>3</sup>). Average duration from primary rabies vaccination to this study was 3.6 years (range 2 months–17 years), 62.0% of participants had longevity persistence of acceptable RNab level. After booster injection, all except one recipient who had CD4+ count <100 cell/mm<sup>3</sup> elicited rapid satisfactory anamnestic response by day 7 with at least protective antibody threshold was reached. Patients whose CD4+ count >500 and 200–500 cell/mm<sup>3</sup> had statistically significant higher GMTs on day 7 than patients whose CD4+ count <200 cell/mm<sup>3</sup>. An AIDS patient who previously failed to seroconvert could evoke the immunologic memory response after quantitative CD4+ count recovery. No serious adverse reaction among all subjects.

	GMTs of RNab titers (IU/ml) on days (range)			
	Day 0	Day 7	Day 14	Day 28
CD4+ > 500 cell/mm <sup>3</sup> (n = 13)	1.99 (0.09-183.4)	11.75 (1.24-239.4)	23.34 (1.83-369.21)	36.57 (8.05-297.3)
CD4+ 200 - 500 cell/mm <sup>3</sup> (n = 13)	1.48 (0.17-29.54)	7.72 (1.31-41.77)	22.09 (3.83-219.53)	13.34 (0.71-1354.2)
CD4+ < 200 cell/mm <sup>3</sup> (n = 3)	0.35 (0.16-0.96)	0.75 (0.68-0.81)	9.55 (0.88-54.04)	5.21 (0.96-17.56)

**Conclusion:** Booster rabies vaccination in HIV-infected patients was immunogenic and safe thus its advantages of omitting Rabies Immunoglobulin demand and shortening the vaccination course after subsequent exposure could be beneficial in developing countries where rabies and HIV infection were coexistent. However, particular concern still was needed among severe immunodeficiency subjects (CD4+ counts <100 cell/mm<sup>3</sup>) by which HAART-induced immune reconstitution might be a resolving option. ClinicalTrials.gov identifier: NCT01286493

**P1023 Varicella vaccination in HIV-1-infected children after immune reconstitution**

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**Background:** HIV-1-infected children have an increased risk of severe chickenpox. However, vaccination is not recommended in severely immunocompromised children.

**Objective:** Can the live-attenuated varicella zoster virus (VZV) Oka strain be safely and effectively given to HIV-1-infected children despite previously low CD4 T-cell counts?

**Methods:** VZV vaccine was administered twice to 15 VZV-seronegative HIV-1-infected children when total lymphocyte counts were >700 lymphocytes/μl, and six HIV-negative VZV-seronegative siblings. Weekly clinical follow-up and sampling were performed.

**Results:** None of the children developed any clinical symptom or serious adverse reaction after immunization. Only nine (60%) of the HIV-1-infected children had VZV-specific antibodies after two immunizations, whereas 100% of the siblings seroconverted. Age at baseline was negatively correlated with the VZV IgG titre at 6 weeks after the second vaccination in HIV-1-infected children. VZV-specific antibody titres after two immunizations were at a similar level to those found after wild-type infection in non-vaccinated HIV-1-infected patients, but significantly lower than in HIV-negative siblings. Importantly, VZV-specific T-cell responses increased after vaccination and were comparable in both groups over time. Documented wild-type VZV contact in three vaccinated patients did not result in breakthrough infections.

**Conclusion:** VZV vaccination of previously immunocompromised HIV-1-infected children was safe. Vaccination induced specific immune responses in some of the vaccinated HIV-1-infected children, suggesting that previously immunocompromised individuals are protected against severe forms of varicella.

**P1024 A new chromatographically purified Vero cell rabies vaccine: results of a comparative trial with purified Vero cell rabies vaccine in intradermal post-exposure treatment**

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The Thai Red Cross intradermal (TRC-ID) regimen with purified Vero cell vaccine (PVRV) for rabies post-exposure treatment (PET) has been proven to be immunogenic, effective and safe. It represents a significant saving in vaccine cost in several developing countries. The chromatographically purified Vero cell rabies vaccine (CPRV), produced by Sanofi Pasteur, was assessed in many post-exposure clinical trials for the immunogenicity and safety profile. However, this vaccine was never commercially sold in the market. The new CPRV is obtained by rabies virus infection of a continuous Vero-cell line propagated on microcar-

Rabies vaccination with TRC-ID	GMTs of rabies Nab titers (IU/ml) on days (range)				
	D0	D7	D14	D28	D90
Group A: new CPRV/without ERIG (n=30)	negative	<0.5	12.69 (2.18-49.35)	6.03 (1.35-33.42)	6.95* (2.00-22.63)
Group B: PVRV/without ERIG (n=31)	negative	<0.5	8.97 (1.09-28.10)	4.58 (1.61-13.45)	2.73 (0.50-8.72)
Group C: new CPRV/with ERIG (n=33)	negative	<0.5	9.98** (1.14-69.79)	6.95** (0.74-49.35)	2.84** (1.09-20.75)
Group D: PVRV/with ERIG (n=20)	negative	<0.5	4.36 (0.62-19.87)	2.67 (0.40-16.00)	1.18 (0.35-7.34)

\*p < .05, Gr. A VS gr. B

\*\*p < .05, Gr. C VS gr. D

riers in bioreactors. This vaccine doesn't contain adjuvant, and is purified by column chromatography. We therefore conduct a prospective study to compare the immunogenicity of new CPRV vs. PVRV in the TRC-ID regimen with or without rabies immunoglobulin (RIG).

**Methods:** In the first stage, 70 healthy veterinary students were randomized and received two 0.1-mL intradermal doses of either new CPRV (SPEEDA<sup>®</sup>, Liaoning Cheng Da Biotechnology, China; Lot no. 200904012-3; antigenicity 6.4 IU/vial: gr. A) or PVRV (VERORAB<sup>®</sup>, Sanofi Pasteur, Lyon, France; Lot no. D0998; antigenicity 10.6 IU/vial: gr. B) on days 0, 3, 7, and 28 in simulated PET. In the second stage, 70 patients with low-risk rabies exposure were enrolled and received two 0.1-mL intradermal doses of either new CPRV (gr. C) or PVRV (gr. D) on days 0, 3, 7, and 28 concurrently with equine RIG. Rabies neutralizing antibody (Nab) was determined by rapid fluorescent focus inhibition test on day 0, 7, 14, 28, and 90 after vaccination. Nab titers of at least 0.5 IU/mL were considered "acceptable" for protection.

**Results:** In both stages of study, post-immunization Nab titers in the new CPRV groups with or without ERIG reached 0.5 IU/mL or greater in all subjects by day 14 and remained above that level through day 90. Geometric mean titers of Nab in subjects receiving new CPRV without ERIG, had no significant difference compared with PVRV group on day 7, 14, and 28 ( $p > 0.05$ ). However, subjects receiving new CPRV with ERIG (gr. C) had significantly higher Nab titers than PVRV group (gr. D) on day 14, 28 and 90 (as Table 1;  $p < 0.05$ ). No serious adverse reactions were detected. However, 23% and 43% of subjects in the new CPRV gr. A and 19% and 55% in the PVRV gr. B experienced pain and itching respectively.

**Conclusion:** The new CPRV is safe and immunogenic when given in TRC-ID regimen for PET.

#### **P1025** Measles immunity and measles vaccine acceptance among healthcare workers in Paris, France

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**Background:** A measles outbreak is ongoing since 2008 in Europe, including France. Unprotected healthcare workers (HCWs) may contract and spread the infection to patients. The objective of this study was to evaluate HCWs' measles immunity and vaccine acceptance in our setting.

**Methods/results:** In a survey-based study conducted in three university hospitals in Paris (France), 353 HCWs were included between April 27, and June 30, 2011. The following data were collected at inclusion: age, hospital unit, occupation, history of measles infection, measles vaccination (number of doses and dates), previous measles serology and acceptance of a measles vaccination in case of seronegativity. Sera were tested for the presence of specific anti-measles IgG and IgM antibodies using the "Captia<sup>®</sup> measles" ELISA assay (Trinity biotech, USA).

The mean age of the participating HCWs was 36 years old (range 18–67) and 280 (79.3%) were women. One hundred and five persons (29.8%) declared a history of measles, and 90 (25.5%) declared never having received a measles vaccination. Among the 353 HCWs included in the study, 324 (91.8%) were immunized against measles (IgG above 90 mIU/mL). The risk factors for being unprotected were age (18–24 years, OR 11.8, 95% CI [2.4–58.4] and 25–34 years, OR 8.4, 95% CI [1.8–38.4] compared >35 years), absence of history of measles and absence of vaccination. The global acceptance rate for a measles vaccination, before knowing their results was 80.8%.

**Conclusions:** In this cohort of HCWs, 8.2% were susceptible to measles, mostly among the youngest (<35 years). Vaccine acceptance against measles was high. Vaccination campaign in healthcare settings should target specifically healthcare students and junior HCWs.

#### **P1026** Antibody profiles in response to vaccination against influenza A virus (H1N1) 2009 measured by a protein microarray

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**Objectives:** The emergence of a new influenza A virus (H1N1) variant in 2009 led to a world-wide vaccination program. In this prospective, longitudinal study we tested if antibody responses induced by a 2009 Influenza A (H1N1) Monovalent MF59-Adjuvanted vaccine (Focetria<sup>®</sup>, Novartis) differ between healthy individuals with or without a history of seasonal influenza vaccination.

**Methods:** We collected three serum samples from 98 subjects; prior to the first vaccination against Influenza A virus (H1N1) 2009, before the second vaccination (three weeks later) and before the vaccination with trivalent seasonal influenza vaccine (5 weeks after the second vaccination). Forty subjects (40.8%) were yearly vaccinated against seasonal influenza virus and 58 subjects were never vaccinated. Antibody titres were determined by a protein microarray containing hemagglutinin 1 (HA1) proteins from a range of recent and historic seasonal H1, H2 and H3 influenza viruses, the A(H1N1) 2009 pandemic influenza virus, and three avian influenza viruses.

**Results:** The median age of the subjects was 49 years (range, 25–66), 64.3% of them were women. Subjects with a history of seasonal vaccination in general had a higher baseline geometric mean titre (GMT) against the various HA antigens than subjects without a seasonal vaccination history. Furthermore, the increase of the HA specific antibodies was less pronounced in the seasonally vaccinated group than in the never vaccinated group. For example, for the antigen H1N1 (2009), the GMT at baseline was 25.25 for the seasonally vaccinated and, 37.86 for the never vaccinated group ( $p 0.096$ ; 95% CI:  $-0.82$  to  $0.06$ ). After the first vaccination the GMT was 241.84 for the seasonally vaccinated and 92.57 for the never vaccinated group ( $p 0.001$ ; 95% CI:  $0.41$ – $1.55$ ). After the second vaccination, the GMT was 215.75 for the seasonally vaccinated and 138.29 for the never vaccinated group ( $p 0.056$ ; 95% CI:  $-0.01$  to  $0.98$ ).

**Conclusion:** A history of seasonal influenza vaccination diminished the antibody response against the various HA antigens elicited by vaccination against H1N1 (2009). More research is needed to better understand the immune responses after vaccination in subjects with a history of seasonal vaccination.

#### **P1027** Comparison of the side effects seen after rabies postexposure prophylaxis with two different verocell vaccines

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**Objective:** In our country, rabies prophylaxis has been implemented according to the guideline of Turkish Ministry of Health (Either Zagreb regimen with 2-1-1 vaccination protocol alone or Essen regimen with five doses vaccination with rabies immunoglobulin (RIG). Although human rabies cases were apparently declined in recent years, more than a hundred thousand of people have been receiving postexposure prophylaxis per year because of rabies- risk-contact in our country. In this study, the side effects of rabies vaccines were investigated and compared according to the schedules and brand names.

**Methods:** Rabies vaccine applied patients for postexposure prophylaxis at our center in 2010 were included into the study. All patients were followed up to the end of schedule. The adverse events were compared according to the age, gender of the patients, the type of the vaccine and the schedule used.

**Results:** A total of 1685 patients (1089 male, 596 female) were vaccinated. The Essen regimen with equine RIG was applied to 265 patients (15.7%) and Zagreb regimen was applied to 1420 patients (84.3%). A verocell vaccine, Verorab (Sanofi Pasteur) was used in 761 (45.2%) patients and Abhayrab (Indian Immunologicals Ltd.) also a verocell vaccine was used in 924 patients (54.8%). All side effects were higher in female patients. The patients were divided in three groups, according to ages as; the first group 0–15 years, the second 15–60 years and above 60 years. Whereas fever (21.2%), vomiting (2.4%) and coughing (2.1%) were significantly higher in the first group, headache (8.8%) and joint pain (6.7%) were significantly higher in the second group compared to the other two groups ( $p < 0.05$ ). All side effects were more frequent with first doses of each type of vaccine and those were more frequent after the first doses of 2-1-1 regimen. All side effects were higher after Zagreb regimen than Essen ( $p < 0.05$ ). Fever (15.8% vs 10.6%), local pain (6.6% vs 2.6%), headache (7.5% vs 3%), fatigue (10.2% vs 4.5%) and arthralgia (4.9% vs 1.5%) were the most common complaints, respectively. Fever (23.8% vs 4.8%), fatigue (13.6% vs 4.6%), headache (9.7% vs 3.1%), local pain (8.9% vs 2.5%) and arthralgia (6.8% vs 3.1%) were significantly higher with Abhayrab than Verorab, respectively.

**Conclusion:** As a result, each of the vaccines seemed safe but although they are both verocell vaccines, side effects seen may vary according to the product and regimen.

#### **P1028** Mandatory measles vaccination – are healthcare workers really safe?

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**Objectives:** Mandatory vaccination against measles in Slovenia was introduced in 1968 and since 1978, all children should receive two doses of vaccine. Compliance rate is more than 95%. This resulted in eradication of the disease from our country for more than 10 years. In 2010 one case was introduced to Slovenia from Ireland and this year 22 cases were reported; four imported from abroad and 18 secondary transmissions; 5 of them were healthcare workers (HCW). All patients were treated in University medical centre Ljubljana (3 hospitalized, 19 ambulatory). Because measles pose a risk of epidemic, we tried to establish if our HCW are really protected against the disease.

**Methods:** From June to October 2011 we conducted a survey among HCW employed in University medical centre Ljubljana regarding protection against measles. HCW born before 1960 were excluded. We demanded from HCW written proof of vaccination or having the measles. If they did not provide that, we performed serologic testing.

**Results:** Data from 3424 HCW were collected. Only 1609 (47%) employees provided a written record about vaccination; 608 (38%) received two doses, and 68 (2%) had the disease. For 1747 (51%) no data were available. In this group serologic testing determining IgG measles antibodies was performed using ELISA. One thousand four hundred and seven (80%) had positive IgG and 340 (19%) had IgG under the positive reference value; 131 (7%) were completely negative. Also 49 HCW with written record of two-dose vaccination against measles in childhood were tested. Interestingly, only 31 (63%) had adequate IgG levels. Five (10%) were completely negative. Analysis of 172 serological negative HCW according to their birth year showed, that 10 (5%) were born before measles vaccination was introduced to Slovenia, 110 (64%) were born between 1968 and 1977 when some children received only one dose and 52 (30%) were born after 1978 when all children should have received two doses of measles vaccine.

**Conclusions:** Our serologic data represent one of the largest epidemiologic studies about protection of HCW against measles. It shows that in spite of mandatory vaccination in Slovenia for the last 40 years, HCW are not protected enough. There is clearly evidence that too many of them did not receive two mandatory doses of vaccine. Among fully vaccinated we found 10% with negative measles IgG antibodies and further 26% with IgG under the positive reference value.

It is questionable, whether they are really protected when in contact with measles.

#### **P1029** Serotype distribution among bacteraemic pneumococcal pneumonia in adults in Germany

M. van der Linden\*, M. Imöhl (Aachen, DE)

**Objectives:** *Streptococcus pneumoniae* remains a leading cause of pneumonia, sepsis and meningitis and disproportionately affects young children and the elderly. In July 2006, vaccination with pneumococcal conjugate vaccine was generally recommended by the German Health authorities for all children up to the age of 24 months. In this study, we present the serotype distribution among adults with bacteraemic pneumococcal pneumonia before and after the start of childhood vaccination.

**Methods:** The National Reference Center for Streptococci has monitored the epidemiology of invasive pneumococcal disease (IPD) in adults in Germany since 1992. Cases of IPD in adults are reported by a laboratory-based surveillance system, including 265 laboratories throughout Germany. The present analyses include only bacteraemic pneumococcal pneumonia cases documented between 2002 and 2011. Species confirmation was done by optochin testing and bile solubility testing. All isolates were serotyped using the Neufeld Quellung reaction.

**Results:** In the first pneumococcal season following recommendation, 2006–2007, the most prevalent serotypes among bacteraemic pneumococcal pneumonia in adults were serotypes 14 (19.1%), 3 (10.9%), 1 (8.6%), 7F (6.7%) and 9V (6.5%). In the most recent season, 2010–2011, serotypes 3 (14.9%), 7F (13.9%), 19A (13.1%), 1 (11.4%) and 22F (8.4%) were most prevalent. The serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19F, 19A and 23F, covered by PCV13, caused 66.3% of all bacteraemic pneumonia cases in 2010–2011.

**Conclusions:** The burden of pneumococcal pneumonia among German adults is considerably high. The most prevalent serotypes have changed following recommendation of childhood vaccination and are currently serotypes 3, 7F, 19A, 1 and 22F.

#### **P1030** Incidence of invasive pneumococcal disease in adults

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**Objectives:** Vaccination with the 23-valent polysaccharide pneumococcal vaccine (PPV23) was implemented in Galicia (in the Northwest of Spain) in adults aged  $\geq 65$  years from year 2000 on, with vaccine coverage reaching around 60% the first year and approximately 20% of new targeted population (those reaching 65 years of age) and 5% of previously unvaccinated targeted population vaccinated per year in the following years. This study analyses evolution of invasive pneumococcal disease (IPD) pre- and post-PPV23 vaccination.

**Methods:** IPD incidence was calculated using data (1998–2009) on isolation of *S. pneumoniae* from blood and cerebrospinal fluid (CSF) at clinical laboratories of the Sistema Gallego de Saúde and population data from the annual census of inhabitants. Incidence was aggregated by triennial periods and expressed as cases/100000 inhabitants per year. The relative risk for the last period (RR) was estimated from changes in incidence between the first and last triennia. Isolates from last triennia were serotyped by Quellung and/or dot blot assay at the Spanish

	IPDs (blood & CSF)				Meningitis (CSF only)			
	1998/00	2001/03	2004/06	2007/09	1998/00	2001/03	2004/06	2007/09
45-64 yrs.	6.1	7.3	10.4	11.7	1.3	0.9	0.7	1.1
$\geq 65$ yrs.	19.4	16.1	20.8	26.6	2.3	1.1	1.1	1.7

reference laboratory for pneumococci. Prevalences of serotypes included in PPV23 were determined and 95% confidence intervals were calculated.

**Results:** The table shows per triennia evolution of IPD incidence by age group (45–64 years and ≥65 years) separating cases of meningitis from other IPDs. For IPDs, in the ≥65 years population, after an initial decrease there was an increase (RR = 1.4) while in the 45–64 years population there was a continuous increase (RR = 1.9). For meningitis, in both age groups there were initial decreases, more marked in the ≥65 years group, followed by increases, with final values not reaching those of the first triennia (RR = 0.7 for ≥65 years and RR = 0.8 for the 45–64 years group). In the last triennia 77% (95% CI: 70–84%) of serotypes isolated from the group of ≥65 years, and 87% (95% CI: 80–94%) from the group of 45–64 years were included in the 23v-PPV.

**Conclusion:** The evolution of IPD incidences over the study period and prevalences of serotypes included in PPV23 in the last triennia in both age groups suggests the absence of significant and maintained impact of PPV23 vaccination.

#### **P1031** Serotype distribution of *Streptococcus pneumoniae* isolates from invasive disease in adults aged ≥50 years in Galicia, Spain

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**Objectives:** To determine serotype distribution and susceptibility of *S. pneumoniae* isolates causing invasive pneumococcal disease (IPD) in adults aged ≥50 years in Galicia, Spain.

**Methods:** A total of 140 isolates (one per IPD) collected in the period January to October 2011 were studied. Isolates were serotyped by latex agglutination and Quellung reaction, and susceptibility to penicillin, erythromycin and levofloxacin was determined by broth microdilution following CLSI recommendations.

**Results:** Of the 140 isolates, 88 (62.9%) came from IPDs in males. Origin of samples was: blood in 126 (90%) cases, cerebrospinal fluid in 6 (4.3%), pleural fluid in 4 (2.9%), and other samples in 4 (2.9%) cases. A total of 30 different serotypes were found, with 71.5% of isolates belonging to seven serotypes: 3 (24.3%), 7F (13.6%), 19A (12.9%), 14 (6.4%), 11A (5.7%), 4 (4.3%) and 6C (4.3%). These seven serotypes represented 69.0% isolates in patients ≥65 years. The table shows by age group distribution of serotypes included in 23v-PPV (23-valent polysaccharide pneumococcal vaccine), in PCV13 (13-valent pneumococcal conjugate vaccine) and of serotypes with ≥4 isolates not included in one or both vaccines.

Penicillin MIC for all isolates was ≤2 µg/mL, only one isolate (serotype 8) was resistant to levofloxacin (MIC ≥8 µg/mL) and 25% isolates were resistant to erythromycin (MIC ≥1 µg/mL), being 40% of them serotype 19A.

	50-64 yrs.	65-74 yrs.	≥75 yrs.	Total
n	24	32	84	140
23v-PPV	20 (83.3)	25 (78.1%)	67 (79.8)	112 (80.0)
PCV13	19 (79.2)	23 (71.9)	52 (61.9)	94 (67.1)
11A <sup>a</sup>	1 (4.2)	1 (3.1)	6 (7.1)	8 (5.7)
6C	1 (4.2)	1 (3.1)	4 (4.8)	6 (4.3)
15A	1 (4.2)	1 (3.1)	2 (2.4)	4 (2.9)

<sup>a</sup>Included in 23v-PPV

**Conclusions:** A total of 82.8% IPDs in adults ≥50 years occurred in patients ≥65 years, with 79.3% cases caused by serotypes included in 23v-PPV, the vaccine currently used in elderly vaccination campaigns. Since 64.7% (75 out of 116) IPDs in adults ≥65 years were caused by serotypes included in PCV13, this new conjugate vaccine, recently approved by European Commission for use in adults, could offer advantages in preventing IPDs in the elderly.

#### **P1032** Effects of childhood vaccination on serotype distribution and coverage of PCV13 among adults in Germany

M. van der Linden\*, M. Imöhl (Aachen, DE)

**Objectives:** *Streptococcus pneumoniae* remains a leading cause of pneumonia, sepsis and meningitis and disproportionately affects young children and the elderly. The serotype distribution for invasive pneumococcal disease (IPD) among adults has always been different from that among children. In July 2006, vaccination with a pneumococcal conjugate vaccine was generally recommended by the German Health authorities for all children up to the age of 24 months. In this study, we report on the effects of childhood vaccination on the serotype distribution among adults with IPD (herd protection effect) and the implications on the current coverage of adult pneumococcal vaccines.

**Methods:** The National Reference Center for Streptococci has monitored the epidemiology of IPD in adults in Germany since 1992. Cases of IPD in adults are reported by a laboratory-based surveillance system, including 265 laboratories throughout Germany. The present analyses include IPD documented between 1992 and 2011. Species confirmation was done by optochin testing and bile solubility testing. All isolates were serotyped using the Neufeld Quellung reaction.

**Results:** Prior to the introduction of childhood vaccination (1992–2006), the most prevalent serotypes among adults with IPD were 14, 3, 7F, 4, 23F, 1 and 9V. In contrast, serotypes 3, 7F, 19A, 1 and 22F were most prevalent in the most recent season 2010–2011. While in the first period 40–45% of IPD among adults were caused by PCV7 serotypes, these serotypes were less detectable in the following years. In 2010–2011, only 11.8% were PCV7 serotypes indicating a herd protection effect among adults. The serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19F, 19A and 23F, covered by PCV13, caused 58.8% of all IPD cases 2010/2011 in Germany.

**Conclusions:** Following the general recommendation for pneumococcal conjugate vaccination, a clear reduction in IPD was observed among children. The reduction of PCV7 serotypes among IPD in adults indicates a herd protection effect.

#### **P1033** Invasive *Streptococcus pneumoniae* isolates between 2008 and 2011 in Hungary: antimicrobial susceptibilities and serotype distribution

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**Objectives:** At 1st September 2008, a new IPD survey has started in Hungary. In this project, every laboratories were permitted of sending *S. pneumoniae* isolates to the National Center for Epidemiology for serotyping and to determine the antimicrobial susceptibility.

**Methods:** The identity of the isolates was confirmed by optochin sensibility test, bile solubility test and *lytA* PCR. Serotyping was performed by Neufeld's Quellung reaction using antisera according to manufacturers' instructions. Minimal inhibitory concentrations (MICs) of penicillin, ceftriaxon, erythromycin, clindamycin and meropenem were determined by gradient MIC test. The results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.

**Results:** A total number of 649 isolates were evaluated until 1st of October 2011. Only 11 strains of them proved not to be *S. pneumoniae*. Fifty nine percent of the pneumococcal strains isolated from blood, CSF or other normally sterile site and the other 41% derived mainly from middle ear, sputum and nose. Of the 350 non-duplicated invasive pneumococcal isolates 48 were originated from children under age 5 years (Risk group 1, RG1) and 206 from adults older than 50 years (Risk group 2, RG2). Except two nontypeable strains, in the RG1 and in the RG2, 17 different serotypes and 33 different serotypes were recognized, respectively. The most prevalent serotypes in RG1 were 3 (19%), 19A (19%) and 7F (12.5%) and in RG2 were 3 (33%), 14 (6%), 19A (6%) and 19F (6%). Strains showing intermediate susceptibility (MIC 0.12–1 mg/L) to penicillin (PEN) were found in 17%, and high-level resistance (MIC ≥2 mg/L) in 2% of IPD strains. Twenty three percent of the isolates revealed resistance (MICs ≥1 mg/L) to



erythromycin and more than a half of these isolates showed non susceptible phenotype to PEN (MICs  $\geq 0.12$  mg/L).

**Conclusion:** In Hungary the 7-valent pneumococcal conjugate vaccine (PCV7) was released in December 2005. Compared to our earlier findings our results show changes in the distribution of prevalent serotypes over the time before PCV7 introduced. PCV13 was licensed in the summer of 2010 therefore further distribution shift is expected. Continuous monitoring is needed to predict the effect of vaccines and will be essential to the management of pneumococcal disease.

**P1034** Differences in nasal pneumococcal carriage of vaccinated and non-vaccinated children, during the first 2 years of wide-spread PCV uptake in Hungary

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**Objectives:** Infections caused by *Streptococcus pneumoniae* often originate from children who carry this bacterium in the nasopharynx. Conjugate vaccines (PCVs) are available for children to prevent not just infections, but also carriage. In Hungary, Prevenar (first PCV7, later PCV13) was inserted into the national vaccination calendar as an optional but recommended and free vaccine in April 2009. The aim of the present study was to observe the effect of PCV vaccination on pneumococcal carriage of children attending DCCs in Hungary.

**Methods:** Nasal specimens were collected from 751 children (aged 3–6 years) from 18 DCCs in different parts of Hungary, from February 2009 to December 2010. Serotyping of the isolated pneumococci was done using antisera and a PCR-based method, antibiotic sensitivity was determined by agar dilution, and the genetic relatedness of the strains was examined by PFGE.

**Results:** Out of 751 children, 281 were carriers (=37.4%), no difference in genders. 18.1% of all children (=136) were vaccinated with PCV7, and 55 of them were carriers. We could determine the serotypes of 232 strains. The most prevalent types among the vaccinated children were: 11A (n = 18), 6B (n = 7) and 19F (n = 6); while among the non-vaccinated ones: 19F (n = 22), 6B (n = 21), 15B (n = 20), 23F (n = 16), 14 (n = 16). The strains (especially the new types, e.g. 11A, 15B, 3) were generally very sensitive to antibiotics, except for macrolides (R = 18%). The close genetic relatedness of the isolates from a given DCC group was obvious by the PFGE results, indicating the intense exchange of bacteria between children.

**Conclusions:** During the examined period the PCV vaccination rate increased, but there were differences between nurseries. The carriage rate was nearly the same in the vaccinated and non-vaccinated population, but there was a clear difference in the serotype distribution. For example, while serotypes 14 and 23F were found almost entirely in the non-vaccinated group, rare types such as 11A or 15B emerged rapidly in the entire population. It seems so that even if only a certain percentage of children within a group is vaccinated, it helps in the elimination of the PCV types. Interestingly 19F and 6B remained frequent. The coverage of PCV13 over the non-vaccinated carriers would be 52.5%, while that of the vaccinated ones only 34.5%. This could be explained with the emergence of the new types, but it indicates the need for newer vaccine composition in Hungary.

**P1035** Serotypes causing invasive pneumococcal disease in young adults prior to the use of new conjugate vaccines

A.N. Horácio\*, J. Diamantino-Miranda, S.I. Aguiar, M. Ramirez, J. Melo-Cristino and the Portuguese Group for the Study of *Streptococcal Infections*

**Objectives:** The 13-valent pneumococcal conjugate vaccine (PCV13) currently used in children was also recently approved by EMA for use in adults aged  $\geq 50$  years old. In this study we evaluated the effect of a high uptake (75%) of PCV7 in children and estimated the potential effect of the most recently licensed conjugate vaccine PCV13 in young adults (18–64 years old).

**Methods:** A total of 425 isolates were recovered from invasive disease in 2008 and 2009: 249 (58.6%) from adults aged 18–49 years and 176 (41.4%) from adults aged 50–64 years. All strains were serotyped and characterized for their antimicrobial susceptibility profiling.

**Results:** Among the 425 isolates, 43 different serotypes were detected. The most frequent, accounting for 51% of all infections, were serotypes 1, 3, 7F, 14 and 19A. While in adults aged 18–49 years, serotype 1 was the most common (21%), in adults aged 50–64 years, serotypes 1 and 3 were equally frequent (14.2%).

PCV7 serotypes accounted for 18.1% of young adults' IPD, which represents a marked decline from the pre-vaccine period (30.9%,  $p < 0.05$ ). PCV13 serotypes were responsible for 65.6% of all infections. In adults aged 50–64 years, where PCV13 vaccination is approved, 68.2% of all infections could have been prevented with this vaccine.

Although PCV7 serotypes were detected in only a small proportion of isolates, 60.6% of penicillin non-susceptibility (PNS) and 43.5% of erythromycin resistance (ER) were caused by these serotypes. Since PCV13 includes serotype 19A, 80.3% of PNS and 77.4% of ER were covered by this vaccine. Among the 30 serotypes not included in any conjugate vaccine, 16 presented isolates resistant to some of the antimicrobials tested.

**Conclusion:** Children's high PCV7 uptake resulted in a herd effect in young adults. PCV13 will have the possibility to significantly affect pneumococci responsible for IPD in young adults and may also reduce infections caused by resistant serotypes. PCV13 use in adults may further and more rapidly reduce the infections caused by the serotypes included in this vaccine.

**P1036** Invasive pneumococcal disease in elderly: serotype distribution and antimicrobial resistance before 13-valent conjugate vaccine introduction in Portugal

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**Objectives:** Elderly have an increased risk of pneumococcal infections and the 23-valent pneumococcal polysaccharide vaccine (PPV23) is recommended for this age group. We have previously shown that the serotypes covered by the 7-valent pneumococcal conjugate vaccine (PCV7) decreased not only in children but also in adults after the introduction of this vaccine in 2001 in Portugal. However, there was no reduction in penicillin and erythromycin resistance in adults. PCV13 replaced PCV7 in paediatric vaccination in December 2009 and the European Medicines Agency has recently authorized the use of this vaccine also in adults with  $\geq 50$  years of age.

To evaluate the effect in the elderly of the continued use of PCV7 in children and to anticipate the potential benefits of PCV13, we characterized the pneumococcal population right before PCV13 introduction in Portugal.

**Methods:** A total of 399 pneumococcal invasive isolates were recovered from elderly patients ( $\geq 65$  years of age) in 2008–2009 in Portugal. Serotyping and antimicrobial resistance testing were performed. A comparison was done with data from 1999–2003, which we previously considered the pre-vaccination period since no effect of PCV7 was seen in adults.

**Results:** The most frequent serotypes were 3 (15%), 7F (13%), 19A (12%), 14 (10%) and 1 (6%), together accounting for more than half of the infections. It was not seen any effect of PPV23, probably due to low uptake of this vaccine. However, there was a decrease in the PCV7-serotypes (30–20%), confirming that the herd effect continued to occur. It was also seen an increase of the non-PCV7 serotypes 7F and 19A. Serotype 1 was the most prevalent in invasive infections of children  $\geq 2$  years and adults 18–64 years of age in 2006–2007, but in the elderly it was not so frequently found. Penicillin and erythromycin resistance was 20% and 21%, respectively. Serotype 19A accounted for a significant fraction of resistant isolates. The potential coverage of PCV13 was 68% of all infections and 79% of infections caused by

multi-resistant strains. An association between multidrug resistance and two non-PCV13 serotypes – 6C and 15A – was noted.

**Conclusion:** PCV7 use in children continued to benefit the elderly and the same is expected from the ongoing vaccination with PCV13. Direct vaccination of the elderly with PCV13 may have an increased beneficial impact due to its high coverage. An increase of non-PCV13 serotypes can be expected and they may be associated with resistance.

**P1037** Recent changes in the proportion of piliated pneumococci: consequences of vaccine use and future prospects

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**Objective:** Evaluate the recent changes of the prevalence of the two pilus islets (PI-1 and PI-2) among *Streptococcus pneumoniae* recovered from invasive infections after 7-valent vaccine (PCV7) use.

**Methods:** Pilus-like structures have been recently recognized in pneumococci, implicated in virulence and suggested as potential vaccine targets. However, these structures are not universally distributed among pneumococcal strains. We had previously demonstrated that carrying PI-1 was a clonal property of *S. pneumoniae* and that only 27% of the invasive strains carried the rlrA islet. Furthermore, 83% of these piliated strains expressed vaccine serotypes. Similar findings were reported by others for the PI-2 islet which was shown to be present in 16% of a convenience sample and associated with serotypes 1, 2, 7F, 19A, and 19F.

To evaluate the distribution of pili in invasive pneumococci we determined the presence of the two pilus islets in a collection of invasive isolates recovered from children and adolescents (<18 years) in Portugal between 2003 and 2009 (n = 623) and analyzed their association with capsular serotypes, antimicrobial resistance and clusters defined by PFGE and MLST. We also evaluated the impact of PCV7 in pili distribution by comparing with the datasets of the pre-vaccine period (1999–2002).

**Results:** Overall, 49% of the strains presented one of the pilus islet. A high correspondence between serotype, PFGE and presence and type of pili was observed (Wallace coefficient,  $W > 0.8$ ). The rlrA islet was identified in 15.6% of the strains, most of them expressing serotype 6B, 9V, 14, 19A and 19F, as seen previously. In contrast, the PI-2 islet, was found in 37.6% of the pneumococcal strains and was found mainly among serotypes 1 and 7F, two serotypes not included in the current PCV7 formulation but covered by the currently used PCV13.

**Conclusion:** A decrease in the presence of the PI-1 islet among invasive pneumococcal strains was observed in the post-PCV7 period. However, an increase of the proportion of strains carrying the PI-2 islet was observed which suggests that expression of pilus-like structures may be important for the ability of pneumococci to cause IPD. Yet, since most of the strains carrying pili presented serotypes that are included in current conjugate vaccine formulations, their potential use in a vaccine would offer limited additional benefits.

**P1038** Cloning, expression and purification of autolysin from methicillin-resistant *Staphylococcus aureus* as vaccine candidate

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**Objective:** *Staphylococcus aureus*, a major human pathogens is of increasing importance due to the spread of antibiotic resistance. Novel potential targets for therapeutic antibodies are products of Staphylococcal genes expressing during human infection.

The atl is an autolysin gene in *S. aureus*. The gene product, ATL, is a unique, bifunctional protein that has an amidase and a glucosaminidase domain. It undergoes proteolytic processing to generate two extracellular peptidoglycan hydrolases, a 51-kDa endo-b-N-acetylglucosaminidase and a 62-kDa N-acetylmuramyl-L-alanine amidase, involved in the separation of daughter cells after cell division. The objective of this

study was to Clone, express and purification of atl with the prospect of constructing Staphylococcal vaccine candidate.

**Methods:** The 1120 bp fragment of the atl gene was amplified via PCR which was extracted from *S. aureus* COL strain (methicillin-resistant *S. aureus*) and then cloned into prokaryotic expression vector pET-24a. For expression of recombinant protein, pET24a-atl plasmid was transformed into competent *E. coli* BL21 (DE3). Recombinant protein was overexpressed with isopropylthio-beta-D-galactoside (IPTG) and was exposed to affinity purification by Ni-NTA agarose. SDS-PAGE and western blotting were performed for protein determination and verification.

**Results:** The atl clone was confirmed by colony-PCR and enzymatic digestion as well as sequencing. SDS-PAGE analysis showed that the constructed prokaryotic expression system pET24a-atl Origami efficiently produced recombinant protein target with molecular weight of 43 kDa. The recombinant atl was overexpressed as inclusion bodies by the use of 1.0 mmol/L IPTG.

**Conclusion:** This prokaryotic expression system provides a simple method for producing recombinant atl and may also be useful for the production of other bacterial surface proteins for vaccine studies. This protein purified in high concentration and good conformational structure to be used as Staphylococcal vaccine candidate after further studies.

**P1039** Pertussis: characteristics of hospitalised and outpatient cases

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**Objectives:** Pertussis is a respiratory disease caused by *B. pertussis*. In Catalonia (a region in the Northeast of Spain with >7 million inhabitants), pertussis generates high public health costs due to disease-related hospitalizations. The objective of the study was to evaluate differences between hospitalized and outpatient cases of pertussis, the determinants of hospitalization and strategies to avoid admissions.

**Methods:** The study included cases reported to the Department of Health of Catalonia during 2004–2008. Only confirmed cases were included (laboratory-confirmed or epidemiologically-linked). Cases were classified in 2 groups: hospitalized and outpatients.

**Results:** A total of 555 confirmed cases were reported, of which 222 (40%) were hospitalized 333 (60%) outpatients. Distribution by sex was the same in hospitalized and outpatient cases: 54% female and 46% male. The age distribution was 211 (95.0%) <1 year; 10 (4.5%) 1–14 years and 1 ≥15 years in hospitalized cases, and 103 (30.9%) <1 year; 134 (40.2%) 1–14 years and 96 (28.8%) ≥15 years in outpatients.

Only 3 (1.4%) hospitalized cases were diagnosed by epidemiological link compared with 88 (26.4%) outpatient cases ( $p < 0.001$ ).

Clinically, 136 (61.3%) hospitalized patients and 277 (83.2%) outpatients had cough for more than two weeks at the time of diagnosis ( $p < 0.001$ ). Eighty (36.0%) hospitalized patients and 38 (11.4%) outpatients had apnoea at the time of diagnosis ( $p < 0.001$ ).

Only 24.8% of hospitalized cases were fully vaccinated according to age, compared with 48.6% of outpatients ( $p < 0.001$ ). Table 1 shows the proportions of hospitalized and outpatient cases fully vaccinated according to age.

A total of 157 (70.7%) hospitalized cases had received no dose of vaccine compared with 85 (25.5%) outpatients ( $p < 0.001$ ).

Fully vaccinated according to age	Hospitalized cases (n=222)	Outpatient cases (n=333)
<1 year	50 (23.7%)	63 (62.2%)
1-14 years	5 (50.0%)	88 (65.7%)
≥15 years	0 (0%)	10 (10.4%)

**Conclusions:** The percentage of hospitalized cases fully vaccinated according to age, although lower than that of outpatients cases, shows that vaccination coverage should be improved in children. New strategies such as cocooning pertussis vaccine to prevent cases in infants should be considered.

**P1040** Surveillance of invasive meningococcal disease and vaccination strategy in the Czech Republic

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**Objectives:** The aim of this study is to assess the actual epidemiological situation of invasive meningococcal disease (IMD) in the Czech Republic with the purpose of updating the vaccination strategy accordingly.

**Methods:** Nation-wide enhanced surveillance of IMD was implemented in 1993. The case definition is consistent with the European case definition. Culture and PCR are used for confirmation of cases. Notification is compulsory and *Neisseria meningitidis* isolates from IMD cases are referred to the National Reference Laboratory for Meningococcal Infections to be characterized by serogrouping, PorA and FetA sequencing (<http://neisseria.org/nm/typing/>), and multilocus sequence typing (<http://pubmlst.org/neisseria/>).

**Results:** The IMD incidence has been stable since 2005 (ranging 0.6–0.9/100 000 annually), with an average case fatality rate of 8.4%. The disease has been caused mainly by serogroup B meningococci (ranging 56.7–71.3% annually), followed by serogroups C (decreasing trend, ranging 28.9–5.8% annually) and Y (increasing trend, ranging 1.1–6.0% annually). The following clonal complexes have been most frequently associated with IMD: cc11, cc18, cc41/44, and cc32. The highest age-specific morbidity rates have been observed in the lowest age groups, i.e. 0–11 months and 1–4 years and have been associated with high prevalence of serogroup B. The involvement of serogroup Y in IMD cases has recently increased, causing the highest serogroup-specific case fatality rate. New vaccination guidelines for IMD were issued by the Czech National Immunisation Committee in 2010: vaccination of children aged 2–6 years with conjugate meningococcal vaccine C; revaccination of adolescents aged 11–14 years with tetravalent conjugate meningococcal vaccine A, C, Y, W235. Vaccine effective against *N. meningitidis* B is needed for infants.

**Conclusions:** Detailed surveillance of invasive meningococcal disease including molecular epidemiology is essential for updating the vaccination strategy. New vaccination guidelines for IMD were issued by the Czech National Immunisation Committee in 2010.

**Acknowledgement:** This work was supported by research grant NT11424-4 of the Internal Grant Agency of the Ministry of Health of the Czech Republic and made use of the Multi Locus Sequence Typing website (<http://pubmlst.org/neisseria/>) sited at the University of Oxford and funded by the Wellcome Trust and European Union.

**P1041** Recombinant outer membrane secretin PilQ406-770 as a vaccine candidate for serogroup B *Neisseria meningitidis*

F Haghi\*, S. Najar peerayeh, H. Zeighami, S. Siadat (Zanjan, Tehran, IR)

*Neisseria meningitidis* is a major causative agent of bacterial meningitis in human. Prevention of serogroup B meningococcal disease represents a particularly difficult challenge in vaccine development. PilQ is an antigenically conserved outer membrane protein which is present on most meningococci. This protein naturally expressed at high levels and is essential for meningococcal pilus expression at the cell surface. A 1095 bp fragment of C-terminal of secretin pilQ was amplified by PCR from serogroup B *N. meningitidis* and cloned into prokaryotic expression vector pET-28a. Recombinant protein was overexpressed with IPTG and affinity-purified by Ni-NTA agarose. BALB/c mice were immunized subcutaneously with purified rPilQ406-770 formulated with either an outer membrane vesicle of serogroup B *N. meningitidis* or Freund's adjuvant. Serum antibody responses to serogroup A and B *N. meningitidis* whole cells or purified rPilQ406-770 and functional activity of antibodies were determined by ELISA and SBA, respectively. SDS-PAGE analysis showed that our constructed prokaryotic expression system pET28a-rPilQ406-770-BL21 efficiently produces target recombinant protein with molecular weight of 43 kDa. The output of rPilQ406-770 was approximately

50% of the total bacterial proteins. Serum IgG responses were significantly increased in immunized groups with PilQ406-770 in comparison with control groups. Antisera produced against rPilQ406-770 demonstrated strong surface reactivity to serogroup A and B *N. meningitidis* tested by whole-cell ELISA. Surface reactivity to serogroup B *N. meningitidis* was higher than serogroup A. The sera from PilQ406-770 immunized animals were strongly bactericidal against serogroup A and B. The strongest bactericidal activity was detected in sera from immunized group with PilQ406-770 formulated with OMV. These results suggest that rPilQ406-770 formulated with an outer membrane vesicle is a potential vaccine candidate for serogroup B *N. meningitidis*.

**P1042** Recombinant system for expression of cholera toxin B subunit in *Escherichia coli*

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**Background and objectives:** Cholera toxin (CT) is the key virulence factor of *Vibrio cholerae*, which is encoded by the ctxAB operon, which resides in the genome of a filamentous bacteriophage (CTX) that specifically infects *V. cholerae*. The symptoms of cholera are mainly caused by cholera toxin (CT), B subunit of which binds to the GM1 ganglioside and promote the endocytosis of CT. The aim of this study was to clone and express ctxB and to purify the recombinant protein using pAE as an efficient expression vector.

**Materials and methods:** The recombinant pAE-CTB was transformed to the competent *E. coli* BL21 to express CTB protein. The system was induced by IPTG after which cells were harvested from LB medium by centrifugation and analyzed by 15% SDS-PAGE. Western blotting performed using cholera toxin-specific antibody. Recombinant CTB was expressed in this system with 6XHis tag at N-terminus and was purified through Ni<sup>2+</sup>-charged column chromatography. Concentration of protein measured with Bradford assay. The functionality of the CTB pentamers was assessed by GM1-ELISA assay.

**Results:** SDS-PAGE analysis showed the expression of rCTB in the system and western blot analysis confirmed the presence of recombinant CTB in blotting membranes. Recombinant CTB was able to bind GM1 in a dose-dependent manner. Some part of rCTB may be expressed in the inclusion bodies so we also lubricated the inclusion bodies.

**Conclusion:** Our results confirmed that although expressed in the inclusion bodies, 6XHis-tagged rCTB was properly refolded, easily purified, and as expected was free of possible CTA contaminants. This will enable us to study CTB immunological properties, oral tolerance, its use as mucosal adjuvant or in vaccine development.

**P1044** Efficacy of *Candida albicans* di- and tri-saccharide vaccine conjugates in an animal model and observation of complement binding to reduce bio burden

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**Objective:** This study was undertaken to determine if candidate *Candida* vaccine compounds would elicit an antibody response in a rabbit model of invasive candidiasis and to determine if opsonised yeasts would bind complement to support clearance of invasive infection in the model.

**Methods:** Neutropenia was induced in New Zealand White rabbits with cyclophosphamide and triamcinolone. *Candida* beta-mannan antigens, conjugated with tetanus toxoid were administered twice (tri-saccharide) or four times (di-saccharide) prior to infection. *C. albicans* ATCC3153A (10<sup>4</sup> CFU/mL) was inoculated intravenously. A control group immunized with tetanus toxoid was included. The animals were followed for 7–10 days and then were euthanized. Necropsy tissues were collected for colony counts from liver, spleen, kidney and lung. Complement fixation was performed by incubating diluted tri-

saccharide rabbit serum from immunized animals with the appropriate yeast culture. Complement (1:100 dilution) was added; then polyclonal goat anti-complement C3 was added. Fixation was detected with rhodamine-labeled anti-goat antibody. Appropriate controls were included.

**Results:** Control animals showed an increase in colony counts for all sites cultured from  $10^6$  to  $10^9$  CFU/g of tissue. The highest counts were in kidney. For the tri-saccharide vaccine, colony counts were reduced from control animals by approx. one-half log in lung and kidney, but were increased in liver and spleen. For the di-saccharide vaccine, colony counts were reduced by approximately 2 logs in liver and spleen, and by one-half to one log in kidney and lung compared to controls. No infection in brain was noted. Further, antibody opsonized *C. albicans* cells also induced the deposition of complement C3 component. This is consistent with the findings of others for protective monoclonal antibody C3.1

**Conclusions:** The efficacy of these synthetic *Candida* conjugate vaccines was observed to reduce the bioburden of invasive *Candida* in the neutropenic animal model. The model suggests that there are a few differences in efficacy between these di- and tri-saccharide vaccines. In these experiments, di-saccharide No. 2 reduced the expansion of organism loads by 2 logs in liver and spleen. *Candida* is often found in these two organs in serious infections in susceptible hosts. The observation of complement fixation by opsonised *Candida albicans* cells provides further evidence to support the protective effect of these vaccine candidates.

#### **P1045** Study of the APC gene function in the mouse APC+/APC1638N model

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Familial adenomatous polyposis (FAP) is an autosomal dominant disease characterized by the presence of many of polyps in the colon is induced by germline mutation in the APC gene. If not removed prophylactically they represent a risk of developing colon cancer with an almost 100% penetrance.

One of the possibilities of cancer prevention could be an alternative gene therapy using recombinant bacteria as vectors for delivery of therapeutic APC protein. The first step of this work was cloning of a complete APC gene into the vector for expression in bacterial cells. For this purpose the vector pET24a+ was used and expression was performed in *E. coli* BL21 (DE3) pLysS. After transformation, accuracy of the complete APC gene was tested by sequencing. Expressed APC protein was identified by Western blotting using monoclonal and polyclonal antibodies. Recombinant bacteria were orally applied into transgene mice APC+/APC1638N carrying a mutated APC gene resulting in the production of nonfunctional protein and consequently formation of intestinal tumors. Potential reduction of intestinal tumor formation after application were analyzed.

The whole gastrointestinal tract was investigated macroscopically, microscopically and immunohistochemically with the use of polyclonal rabbit antibody against APC protein. Also we analysed cytokines (IL-1b; IL-2; IL-4; IL-5; IL-10; IFN-gamma; TNF-alpha) in mouse serum using Bio-Plex ProTM Assay.

All four transgenic mice without therapy developed adenomatous polyps in the gastrointestinal tract. Six transgenic mice treated by oral administration of bacteria expressing the APC gene, developed polyps in 33.3% of two cases. The remaining four mice 66.7% were without polyps development and immunohistochemistry confirmed in all parts of the gastrointestinal tract positive APC protein more or less strong intensity.

We observed positive effect of this therapy at mouse model. The expression of APC protein by non-pathogenic bacteria may be suitable for clinical use as a potential drug.

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#### **P1046** Using recombinant *E. coli* as a novel vaccine against foot and mouth disease virus

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**Background:** Foot-and-mouth disease (FMD) is a highly contagious disease of livestock that causes severe economic loss in susceptible animals and it may lead to a new outbreak of FMD because of either incomplete inactivation of Foot and Mouth Disease Virus (FMDV) or the escape of live virus from vaccine production workshop. Thus, it is urgent to develop a novel FMDV vaccine that is safer, more effective and more economical. We have designed a peptide-based vaccine for FMD effective in livestock. Display of heterologous proteins on the surface of microorganisms, enabled by means of recombinant DNA technology, has become an increasingly used strategy in various applications in microbiology, biotechnology and vaccinology.

**Methods:** Several different FMDV peptides containing the immunogenic regions of vp1 were fused to the OMPA of Salmonella and transferred into *E. coli*. After induction the expression was shown by SDS PAGE and to confirm the presence of this fused protein on the surface of *E. coli*, fractionation method performed. By ELISA method the activity of the epitopes approved and the lyophilized bacteria was inoculated to the mice feed and the immunogenicity was evaluated.

**Results:** The immunogenicity of these recombinant bacteria was tested by immunizing the mice. Ten days after the last inoculation, the animals were bled and the sera analyzed to evaluate the presence of Antibody against FMDV by ELISA and Western blot. The results show extra stimulation in the immune system of the mice which the recombinant bacteria were inoculated in their daily feed.

**Conclusion:** These result suggested that designing a recombinant peptide vaccine would be a good and possible way to gain high levels of immunity in veterinary medicine but there is still a strong need for additional studies.

#### **P1047** The live attenuated *B. pertussis* vaccine strain BPZE1 subverts the immune functions of RSV-infected human dendritic cells

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**Objectives:** The respiratory syncytial virus (RSV) is a leading cause of lower respiratory tract infection in infants. The immune response to RSV is still not fully understood and many data suggest an activation of innate immunity mainly focussed on the induction of a cytokine storm and a consequent tissue damage. Despite several efforts to develop effective vaccines, none have been successful to date. Recently, BPZE1, a live attenuated *Bordetella pertussis* vaccine strain, has been developed and entered a phase I safety trial ([www.ChildINNOVAC.org](http://www.ChildINNOVAC.org)). Immunization with BPZE1 protects mice challenged with influenza virus, suggesting potent adjuvant properties. To support the potential application of BPZE1 as adjuvant in a future RSV vaccine, we evaluated the immunomodulatory properties of BPZE1 in a model of human dendritic cells (DC) infected with RSV.

**Methods:** GM-CSF and IL-4 monocytes-derived DC (mDC) were exposed to RSV and/or to BPZE1. Functions of infected mDC were assessed, including cytokine production and polarization of T helper cells in co-culture experiments with allogeneic T cells. The phosphorylation of mitogen-activated protein kinases (MAPKs) p38, ERK1-2 and IkbAlfa was evaluated by Western Blot. RT-PCR was used to measure IFNbeta, IRF-8, RANTES and IL-12p35 and IL-12p40 gene expression.

**Results:** BPZE1 was able to induce the production of IL-10, IL-23, and low level of IL-12p70 while RSV infection did not induce any of these cytokines. BPZE1 and RSV induced two different signal pathways: BPZE1 triggered the activation of p38 and ERK 1/2 MAPKs phosphorylation and expression of IL-6, IL12p40 and low level of IL-12p35 subunits; RSV triggered the activation of STAT1

phosphorylation and the expression of IFN $\beta$  and several IFN-regulated genes, including IRF-8, RANTES and high levels of IL-12p35 subunit. Remarkably, in BPZE1/RSV co-infection the intracellular pathways complement each other as demonstrated by the enhanced production of IL-12p70. In vitro polarization experiments showed that RSV-primed mDC drive the expansion of a mixed Th1/Th2. Strikingly, the co-infection greatly decreased Th2 and increased Th17 expansion.

**Conclusion:** BPZE1 is able to re-direct the RSV immune response by modification of the cytokine profile that has a profound effect on polarization of T helper cells. These results support a potential application of BPZE1 as adjuvant in a future RSV vaccine. Supported by EC grant agreement 201502 (ChildINNOVAC).

#### **P1048 Immunogenicity and safety of tick-borne encephalitis vaccination in healthy elderly individuals**

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**Introduction:** Tick born encephalitis (TBE) is a major viral cause for central nervous system disease in Europe. Serious disease with debilitating neurological sequelae is more frequently observed in older TBE patients compared to younger adults and children. Causal antiviral treatment is not available but prevention by active immunization is highly efficacious and widely recommended in Europe. However, evidence for TBE vaccine immunogenicity and protective efficacy in the elderly population is scarce. Since the risk for serious TBE is increased and immunogenicity of vaccines is generally reduced in the elderly, additional data on immunogenicity of TBE vaccine in a naïve elderly population is highly warranted.

**Objectives:** Detailed investigation of immunogenicity and safety of a licensed inactivated whole-virus TBE vaccine (FSME Immun CC<sup>®</sup>) after primary immunization of 137 healthy elderly subjects in a controlled clinical trial (NCT00461695).

**Patients and methods:** Of 183 screened patients 137 were included and immunised with a standard three dose regimen of TBE vaccine at week 0, 4 and 24. Major inclusion criteria were: age >70 years, healthy ( $\leq$ one co-morbidity;  $\leq$ one medication), negative TBE-serology. Immunogenicity was assessed longitudinally by neutralization and ELISA test, safety was evaluated clinically and by standardized questionnaires. Results were compared with previous studies in younger adults that had used the same assays.

**Results:** Four weeks after the 2nd and 3rd vaccination 98.5% and 99.3% of subjects were seropositive ( $\geq$ 10) in the neutralization test. Using a threshold of 126 VIEU/mL in the ELISA the seropositivity rates were 93.4% and 97.8%. Geometric mean titer (GMT) measured by neutralization test were 29.2 and 70.6 after the 2nd and 3rd vaccination, respectively. GMT were 799.4 VIEU/mL and 1933.8 VIEU/mL in the ELISA. In summary, the seropositivity rates for elderly subjects obtained in this study were similar to earlier studies in young adults and children using the same test assays. However, antibody titer were generally lower. Adverse events were mild, transient and comparable to earlier studies in adults.

**Conclusions:** These results suggest that primary TBE vaccination of healthy elderly leads to satisfactory immune responses well above levels considered protective. However, duration of protective immunity may be shorter than in younger individuals.

#### **P1049 Designing and studying of defensin peptides analogues as a mucosal adjuvant/microbicide: a study towards the development of peptide-based vaccine against AIDS**

T. Mohan\*, D. N. Rao (New Delhi, IN)

**Background:** The mechanisms of resistance to HIV infection in the human oral cavity are incompletely understood while salivary components have been implicated in protection.

**Methodology:** We have synthesized HIV and Defensin peptides and their corresponding analogues by making some modifications in the natural sequence. We have done Anti HIV, Anti-microbial and other characteristic study of defensins to prove them active. Then, immunized these formulations in Outbred and Two different Inbred mice (H2b, H2d) through IN route using Microsphere as delivery vehicle. We have studied Humoral Response of HIV peptides with and without Defensins by estimating antibody levels (IgG/IgA) in the serum as well as in lung, intestinal, vaginal and rectal washes till day 120. For cell mediated immune response, peptide specific T cell proliferation and cytokine/chemokine levels were studied in the cells isolated from the three different mucosal sites i.e. spleen, lamina propria and peyer's patches of the primed mice. We have also done Cytolytic activity analysis, by estimating IFN-gamma/Perforin secretion by CD4+/CD8+ also through FACS, which was checked by IFN-gamma/Perforin secretion.

**Result and discussion:** All the HIV peptide with defensins in different formulations has increased titre significantly both in sera (1 02 400–4 09 600) and washes (800–12 800) ( $p < 0.05$ ) as well as the proliferation response was also significantly higher (upto two fold ranging from 10 to 50 stimulation index) ( $p < 0.001$ ) as compared to HIV peptide alone. The cytokine measurement profile showed mixed Th1 and Th2 type of immune response. The FACS analysis data revealed that CD8+/CD4+ T cells showed significantly higher Cytolytic activity in the HIV with Defensin peptide formulations. Surprisingly, CD4+ T-cells were also showing Cytolytic property.

**Conclusion:** Thus, the results reported here demonstrate the effectiveness of synthetic defensin peptide analogues to induce strong and long lasting humoral and cellular immune response through intranasal route using PLG- microsphere as a delivery vehicle. Our findings may have implications in the development of new antiviral agent for AIDS therapy.

#### **P1050 Deficient long-term response to pandemic vaccine results in an insufficient antibody response to seasonal influenza vaccination in solid organ transplant recipients**

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**Introduction and objectives:** Long-term antibody response to the 2009-H1N1 vaccine in solid organ transplant recipients (SOTR) and its clinical repercussion on the efficacy of the following 2010–2011 influenza vaccine has been poorly studied. The aim of this study was to evaluate the long-term seroprotection of the 2009-H1N1 vaccine and its impact on the effectiveness of the following seasonal vaccine.

**Methods:** Multicenter prospective study in SOTR receiving one-dose of the non-adjuvanted 2010–2011 seasonal influenza vaccine. The immunological response at baseline and five weeks after vaccination by a microneutralization assay. Follow up: 90 days after vaccination, or 10 months if adverse effects were detected.

**Results:** One hundred SOTR were included (kidney 42.7%, liver 41.7%, heart 15.6%). Median time after the transplantation was 39.7 months (range from 2.3–277.8). Among the 73 patients that received the 2009-H1N1 vaccine, only 22 (30.1%) maintained antibody titers (GMT<sub>pre</sub> 11.1, 8.3–14.9) 1 year after vaccination. The overall rates of seroprotection and seroconversion were 79.2% and 77.1%, respectively for the 2009-H1N1 strain, 67.7% and 66.7% for the H3N2 strain and 72.9% and 67.7% for the B strain, with a median GMT<sub>post</sub> of 106.0 (73.8–152.4) for 2009-H1N1 strain, 98.6 (63.3–153.6) for the H3N2 strain and 119.0 (76.5–185.1) for the B strain, after vaccine. Patients with baseline titers had significantly higher seroprotection for all three influenza strains: 2009-H1N1 (100% vs. 73%, RR 1.37 CI95% 1.19–1.57,  $p = 0.006$ ), H3N2 (100% vs. 62.2%, RR 1.61 CI95% 1.36–1.90,  $p = 0.005$ ) and B (100% vs. 69%,  $p = 0.02$ ). GMT<sub>post</sub> was also higher if basal antibody titers were present: 2009-H1N1 (529.8 vs. 65.7,  $p < 0.001$ ), H3N2 (353.3 vs. 79.3,  $p < 0.001$ ) and B (239.7 vs. 107.7,  $p = 0.08$ ). Three patients (3.1%) were diagnosed with 2009-

H1N1 infection having mild disease and no one required hospital admission.

**Conclusions:** SOTR long-term response to the 2009-H1N1 influenza vaccine is insufficient. The response to 2010–2011 seasonal influenza vaccine is related to baseline titers, however, most of the patients did not exhibit detectable antibodies at the time of next-season vaccination. New strategies are necessary to improve seasonal vaccination efficacy in SOTR.

## Antibiotic use and antibiotic stewardship

**P1051** **The Antibiotic Resistance and Prescribing in European Children (ARPEC) project: the European neonatal and paediatric antimicrobial web-based point prevalence survey in 56 hospitals within Europe and globally in 2011**

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**Objectives:** The European neonatal and paediatric antimicrobial Point Prevalence Survey (PPS) is part of the ARPEC project (<http://www.arpecproject.eu/>). The aim of the study is to develop a standardized method for surveillance of antimicrobial use in hospitals within Europe and globally. The variation in antibacterial drug use will be used to identify targets for quality improvement and hospital Antimicrobial Stewardship Programmes.

**Methods:** Data collection was completed during 2 weeks between September 19th and 30th 2011. The pilot survey included all admitted children receiving an antimicrobial treatment on the day of survey. Mandatory data were age, gender, (birth) weight, underlying diagnosis, antimicrobial agent, dose and indication for treatment. Data were entered online using a web-based system for data-entry and reporting based on the WebPPS program developed by the University of Antwerp for the ESAC (European Surveillance of Antimicrobial Consumption) project.

**Results:** There were 5122 inpatients reported for the 56 participating hospitals originating from Europe, USA, the Middle East and Africa (22 countries). Overall, 37% of paediatric patients (N = 1436) and 35% of neonates (NICU, N = 299) were receiving at least one antibiotic. Mainly cephalosporins (30%), penicillins (22%), aminoglycoside antibacterials (17%) and other antibacterials (13%) were used. Third-generation cephalosporin use was highest in the Islamic Republic of Iran and Georgia (total proportional use >36%) followed by Latvia (32%) and Romania (30%) and the lowest use was reported for hospitals in Lithuania, Spain, Belgium and UK (<7%). The most common sites of infection for which antibacterials were prescribed were lower respiratory tract infections (15%), prophylaxis for medical problems (13%) and prophylaxis for surgical disease (12%) for paediatric patients, and sepsis (32%) and prophylaxis for newborn risk factors (12%) for neonates. Two quality indicators were identified: surgical prophylaxis for >24 hours (80%) and therapy with third-generation cephalosporins for community acquired lower respiratory tract infections (pneumonia) (28.3%).

**Conclusion:** The ARPEC-WebPPS method was successfully piloted in 56 hospitals worldwide. It offers a standardized tool to identify targets for quality improvement. The first global web-based ARPEC-PPS with quantifiable outcome measures will be organized in 2012. Interest in the project is high!

**P1052** **ASPEN© – Antimicrobial Stewardship PracticEs in the NHS (North London)**

N. Cooley\*, P. Panesar, M. Gilchrist, K. Hatton, T. Lyons, H. Wickens, W. Lawson (London, UK)

**Objectives:** Antimicrobial pharmacists in the UK have been embedded in clinical practice for over a decade. The role of the pharmacist has evolved and expanded over this time. We discuss the impact of such

posts across three acute London teaching Trusts (covering eight hospitals, incorporating ~3000 in-patient beds).

**Methods:** Antimicrobial stewardship activities were reviewed on medical wards at each of the Trusts over the last 4 years. Local data collection tools were used by antimicrobial pharmacists to ascertain practices. Where comparable methodology was applied across the sites, regional benchmarking was undertaken.

**Results:** Over the last 4 years, improvements have been observed in documentation of indication for antibiotic therapy (average 75% in 2008 to 95% in 2011) and recording of a stop/review dates (20% in 2008 to 80% in 2011) on drug charts. This has been achieved through the introduction of initiatives such as automatic stop dates, electronic prescribing systems, medication charts with specific areas for antibiotic prescriptions and education and training – both at induction and through continual feedback and via key performance indicators. The introduction of electronic stop dates, at one centre, has significantly reduced oral antibiotic usage by 9% (p = 0.07). Data has also shown an increase in the number of antimicrobial guidelines available to clinicians for treatment and prophylaxis (61% in 2008 to 85% in 2011), whilst improved electronic technology has provided a platform to broaden the access to guidelines e.g. pocket guide versions, local intranet and Smartphone applications. Documentation of allergy status on drug charts ranged from (88–98%), and compliance with guidelines has been maintained at a high rate (75–98%) despite the regular turnover of clinical staff. The establishment of joint antimicrobial pharmacist/microbiology ward rounds for the Acute Admissions ward and for patients diagnosed with *Clostridium difficile* infection has seen an increase in the numbers of patients reviewed, resulting in greater numbers of prescriptions being optimised.

**Conclusion:** Antimicrobial pharmacists have implemented novel approaches to support antimicrobial stewardship. This has resulted in clear improvements in antimicrobial use as well as changing healthcare professionals' attitudes towards antimicrobial prescribing.

**P1053** **Antimicrobial stewardship practices in North London hospitals**

N. Cooley\*, P. Panesar, M. Gilchrist on behalf of North London Antimicrobial Pharmacist Network

**Objectives:** The Antimicrobial Self-assessment tool (ASAT)1 was developed to aid identification of areas of strength and weakness within Trust stewardship practices. This tool was applied in 16 North London hospitals to benchmark regional practice and to identify differences and commonality between Trusts.

**Method:** Self assessment of practice was undertaken against seven aspects of stewardship. The mean scores for the assessed elements were: risk assessment (90.1%), operational delivery of strategy (86.7%), structure and lines of responsibilities (82.7%), antibiotic pharmacist post (82.3%), clinical governance (63.7%), education and training (50.2%), and information for public, patients and carers (43.7%).

**Results:** For the operational delivery of strategy element, teaching hospitals scored significantly higher than district general hospitals, 92.5% vs. 81% (p = 0.0003).

Greatest variability within the scores was identified for education and training and information for public and patients. These elements required quantifiable data to be able to score on the tool. Whilst these aspects are recognised as important and often performed, Trusts found it difficult to quantifiably demonstrate this practice.

**Conclusion:** The ASAT is a useful aid for organisations to identify current gaps in local antimicrobial stewardship activities. However, Trusts need robust mechanisms for demonstrating the quantifiable aspects. The tool may require refining to address this.

**Reference:** 1. Cooke J, Alexander K, Charani E et al. Antimicrobial stewardship: an evidence-based, antimicrobial self-assessment toolkit (ASAT) for acute hospitals. *J Antimicrob Chemother* 2010; 65(12): 2669–2673.

**P1054 Adherence to recommendations of an antimicrobial stewardship programme**

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**Background:** Several barriers have been identified to the development of an Antimicrobial Stewardship Program (ASP). Adherence to recommendations varies depending on who makes the intervention, the kind of suggestion and on the patient status. The objective of the present study is to identify the characteristics of the interventions with the lowest adherence.

**Methods:** Prospective study from June 2006 to December 2010 in a 850 beds community based Hospital in Balearic Islands in Spain. In the context of a program to improve antibiotic (AB) prescription in the Hospital, that also included educational activities and development and dissemination of guidelines, an AB prospective audit was performed. Interventions were made after chart review by a team of infectious diseases physicians by leaving a note with the justified suggestion. Recommendations could be to stop, to change or to maintain the antibiotic treatment. Forty-eight hours later the adherence to the recommendation was evaluated.

**Results:** During the study period 11 082 interventions were made with an adherence of 60%. A suggestion was given in 50% of the prescription reviewed, 13.2% to stop and 27.8% to modify. Adherence was analyzed by departments in the Hospital and by the reason to make the suggestion. The departments of Urology, General Surgery and Gastroenterology had the poorest adherence, around 40%, and the best adherence rates were obtained in Orthopedics, neurosurgery and Neurology, reaching 85% in orthopedics. The reasons to make interventions more associated to low adherence were to fit the prescription to the local guidelines and to stop due to lack of indication, 40%. However, interventions aimed to deescalate (60.8%) or to stop due to completed therapy (55.7%) or to set the ending date (90%) were more often followed.

Interestingly, adherence improved during the 3 years follow up, reaching 69% in the last year.

**Conclusion:** The map of adherence in the Hospital could be useful to identify those departments less engaged in antimicrobial stewardship programs and potential barriers to successfully implement the program as inadequate diffusion of the aim of the program, absence of consensus in the elaboration of protocols. Furthermore, some interventions as those devoted to improve adherence to local guidelines must be redesigned considering the low adherence obtained. New tools like the use of biomarkers could help to improve the adherence to those recommendations made to stop unnecessary antibiotic prescription.

**P1055 Trends in antibiotic use and antimicrobial resistance in French hospitals, 2008–2010. Data from the nationwide network “ATB-RAISIN”**

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**Objectives:** French programmes to control antimicrobial resistance (AMR) and antibiotic (AB) use put an emphasis on surveillance in hospitals. We performed three nationwide surveys since 2008 to monitor trends in AB use and AMR in hospitals and to provide a tool for benchmarking and to identify areas for improvement at national and at hospital level.

**Methods:** AB for systemic use (class J01 of WHO Anatomical Therapeutic Chemical classification, ATC-DDD system, rifampicin and oral imidazole derivatives) and AMR were surveyed according to the national surveillance network ATB-RAISIN. Data retrospectively collected each year, for the whole previous year, by voluntary hospitals were: hospital status; AB consumption for inpatients,

expressed in number of defined daily doses (DDD) per 1000 patients-days (PD); number of susceptible strains for specified AB/bacteria couples, number of PD.

**Results:** Eight hundred and sixty-one hospitals in 2008, 997 in 2009 and 1115 in 2010 participated in the survey, accounting for 52% of PD in France in 2010. In 662 hospitals participating each year, AB use (pooled mean) slightly increased by 3% each year: 366, 378 and 389 DDD/1000 PD from 2008 to 2010. Trends varied according to hospitals status: AB use increased by 28%, 17%, 9% and 7% in long-term care centres, rehabilitation centres, public hospitals and cancer centres. Major increase occurred in the use of piperacillin/tazobactam (+33%), carbapenems (+31%), ceftriaxone (+28%) and imidazole derivatives (+18%). Fluoroquinolone use remained stable over the study period; levofloxacin use increased by 12% in 2009 and remained stable in 2010. AMR increased in Enterobacteriaceae while meticillin-resistant *Staphylococcus aureus* incidence remained stable.

**Conclusion:** This national network, gathering standardized data from a large sample of hospitals, allows surveillance of trends in AB use and AMR. It also allows benchmarking between hospitals and providing help to outliers to analyse results and implement improvements. Despite the national action plan, AB use did not decrease in recent years in French hospitals when expressed in DDD/1000 PD. Moreover, increase in AMR of Enterobacteriaceae is worrisome. Considering these data, emphasis will be reinforced on rational use of carbapenems and ceftriaxone. Increase in carbapenem use was also reported in Sweden (+9%), in the Netherlands (+37% between 2007 and 2009) and in Denmark (+49%), illustrating the urgent need to better control the use of these last-line agents.

**P1056 Antibiotic stewardship programmes in French hospitals: time to change the national structure and process indicator, ICATB**

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**Objectives:** Since 2007, it is mandatory for French hospitals to report to health authorities a structure and process indicator on their antibiotic stewardship programme (ABS), ICATB, measuring implementation of recommended activities, for public disclosure. We conducted a study in 2009 to assess metrological performance of the indicator ICATB and to identify needs for its improvement.

**Methods:** A retrospective survey was performed in 440 Southwestern France hospitals. ICATB was retrieved from the national database provided by health authorities. Content validity, internal consistency and reproducibility of ICATB were assessed by multiple correspondence analysis, Cronbach's alpha and Kappa coefficients calculations. Information on ABS measures in 2009 was collected by auto-questionnaire sent to pharmacists, antibiotic (AB) advisors and/or infection control teams. Implementation of organisation measures, resources (human and information technology (IT) support), educational and restrictive activities was studied and compared with ICATB.

**Results:** Two hundred and forty-six hospitals participated (56%). ICATB showed low content validity and internal consistency but good reproducibility (assessed against replies to the auto-questionnaire). Most hospitals had implemented measures included in ICATB: ICATB median score was 16/20. However, some activities and resources, as detailed in the auto-questionnaire, were among the least frequent: training (32%) and IT support (in <50%). Practice audits had been carried out in 66% of hospitals but 33% had provided feedback to prescribers. Only 42% of AB advisor had passed one of the two recommended diplomas; the median weekly time spent was 4 hours. An antibiotic management team was in place in 41%.

**Conclusion:** Overall, the level of compliance with recommended ABS measures included in ICATB was high in 2009, thus limiting ICATB usefulness to trigger new improvements. In addition, its metrological characteristics could be improved. Replacement of some components, already widespread, by less frequent measures and addition of questions

on practical modalities of implementation could improve both its ability to foster progress and its consistency. We suggest to include measures that have proven their efficacy to improve AB use and able to discriminate between hospitals, such as AB advisor activity, feedback to prescribers, multidisciplinary teams. Items could be weighted so that known synergistic measures (bundle) could be given more value.

#### **P1057** Institutional programme for improvement of antimicrobial treatment (PRIOAM)

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**Objectives:** PRIOAM is an antimicrobial stewardship program with four basic objectives in 3 years: (i) To improve the antimicrobial prescription, (ii) To reduce the hospital mortality in severe infection, (iii) To reduce the morbidity of patients with severe infection (iv) To reduce the antimicrobial resistance and (v) To reduce the economic cost. We exposed the results of first 10 month of the first objective.

**Methods:** During the first 10 months has been reached to achieve the following tasks within the program. (i) The program was approved by the hospital's manager and publicized for all wards, (ii) We performed 17 local guidelines of infectious diseases in collaboration with the rest of units of the Hospital (iii) Clinical consultancies of antimicrobial prescription were performed by 13 clinician expert in infectious disease. The objective of the clinical consultancy does not the change of antimicrobial prescription if no the training of the doctor advice. Each clinical consultancy the expert checks specific items of the antimicrobial prescription with the doctor advice of a real patient. Each clinical consultancy was scored 0–10 and a prescription was considered inappropriate if at least one item in the check was wrong. To assess the different ratio of inappropriate prescription for months we performed a Chi square test and evaluated the change in the score with Kruskal–Wallis test. At the end of the clinical consultancy the doctor advised may completed a satisfaction survey.

**Results:** Since January to October was performed 975 clinical consultancies in 24 different clinical units. Of them 511 (52.4%) were empirical treatment, 240 (24.6%) were antimicrobial treatment with microbiological confirmation and 224 (23.0%) were surgical prophylaxis. In the January months the inappropriate treatment was 56.8% (79/139) and in October was 31.3% (36/152)  $p < 0.001$ . Surgical prophylaxis 63.2% (12/19) vs. 17.1% (7/41)  $p < 0.001$ ; empirical treatment 59% (49/83) vs. 28% (23/82)  $p < 0.001$  and 48.6% (18/37) vs. 20.7% (6/29)  $p = 0.019$  in treatment prescribed with microbiological confirmation. The satisfaction survey was completed by 321 (32.9%) of doctors advised and 97.8% (314/321) find it useful.

**Conclusions:** In this interim analysis we find that PRIOAM is useful for improve the antimicrobial prescriptions.

#### **P1058** The barriers and facilitators to optimal antimicrobial prescribing: a qualitative study

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**Objectives:** Successful interventions to optimise antimicrobial prescribing (AP) need to be underpinned by research investigating the barriers and facilitators to optimal APB. Published studies on interventions to optimise APB often omit or fail to report on the application of theory and primary research to inform intervention development and design. We report here on a qualitative study to identify (i) attitudes and perspectives of healthcare professionals (HP), including doctors, pharmacists and nurses on AP; (ii) key barriers and facilitators to APB; and (iii) key determinants of APB.

**Methods:** A random sample of 39 HP (19 nurses, 10 doctors and 10 pharmacists) working in three hospital sites of a large University Hospital Trust in the UK were interviewed. Data was extracted and

coded using a framework approach using inductive and deductive approaches. In depth analysis of the coded transcripts was conducted to identify emerging themes based on the initial objectives of the research.

**Results:** The analysis identified four key determinants of APB: (i) influence of peers and seniors; (ii) delineation of responsibility in AP; (iii) influence of local and organisational culture on APB; and (iv) experience and expertise as barriers and facilitators to optimal APB. APB was influenced by prescriber attitudes towards policy, evidence base, and more importantly by consultants and senior doctors. Junior staff expressed difficulty in questioning “out-of-policy” practices of their senior colleagues due to perceived social hierarchy or their own self-efficacy in being able to question APB. The barriers and facilitators to optimal APB identified by HPs differed by profession, specialty and their perceived position in organisational hierarchy. This study demonstrates a need to clarify HP involvement in antimicrobial stewardship e.g. empowering nurses, pharmacists and doctors to champion antimicrobial stewardship.

**Conclusion:** The influence of senior physicians as role models and the experience and expertise of individual HP's need to be acknowledged as key determinants of APB. Based on this research we recommend that APB interventions will benefit from targeting the prevailing cultural attitudes and behaviours, however we caution that social dynamics may differ by culture and specialty. Interventions targeting APB must be multimodal and include audience segmentation, and role definition for HCPs in antimicrobial stewardship.

#### **P1059** Development of a worldwide antimicrobial stewardship survey

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**Objectives:** Antimicrobial stewardship (AMS) has been surveyed at national and continental level, but never at a global level. At the 2011 ECCMID meeting, the ESCMID Study Group for Antimicrobial Policies supported a worldwide survey of antimicrobial stewardship.

**Methods:** A small multidisciplinary project group was established (England, France and Scotland). Volunteers were identified from each continent to be the development advisory group. A literature search was undertaken to identify published surveys and standards for antimicrobial stewardship. A draft survey was developed from these results using the good practice methodology for conduct and reporting survey research [1, 2] and CHERRIES criteria for improving internet surveys [3]. These suggested piloting the survey to ensure that questions could not be misinterpreted especially where English is not the predominant language, then pre-testing in a larger group. Pre-testing was planned in 11 countries in six continents (Asia: Hong Kong, China and India; Europe: UK, France, Switzerland, Austria or and Slovenia; Africa: South Africa; Oceania: Australia; South America: Argentina, and USA in North America. SurveyMonkey© software was used to develop the survey. This allowed question order to be randomised for each respondent.

**Results:** A survey has been developed to collect data on antimicrobial stewardship at a global level that collects data on AMS governance arrangements, staffing levels, strategies, audit and feedback and education within hospitals and community. The results will be presented at a future meeting.

**Conclusion:** It is possible to develop and test an internet based survey of antimicrobial stewardship that is applicable across all continents, but a thoroughly planned development phase is needed.

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3. Eysenbach G. Improving the quality of Web surveys: the Checklist for Reporting Results of Internet E-Surveys (CHERRIES). *J Med Internet Res* 2004; 6(3): e34.



**P1060** Lack of correlation between antibiotic use and mortality in Swedish intensive care units – report from Swedish registry of intensive care

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**Objective:** The high antibiotic use in intensive care is a driving force of antibiotic resistance. The aim of this study was to report and analyse antibiotic consumption and correlation with mortality among patients admitted to Swedish ICUs.

**Methods:** *Setting:* Starting 10 years ago an increasing number of ICU:s in Sweden reports each episode of care (EOC) after discharge systematically to the Swedish Intensive care Registry (SIR). Mortality is followed weekly for all patients in the registry. Data on antibiotic consumption was collected from the web based statistical system Concise (Apotekens Service AB), containing information on all sales of antibiotic drugs in Sweden.

*Participants:* 49 and 52 Swedish ICUs participated 2009 and 2010 respectively.

*Variables:* Antibiotic consumption expressed as defined daily doses (DDD) per 1000 occupied bed days (DDD1000), using the annually updated DDD calculated by the WHO Collaborating Centre for Drug Statistics Methodology (<http://www.whocc.no>). Standardized mortality rate (SMR) calculated as observed mortality within 30 days after admission/predicted mortality based on the Simplified Acute Physiology Score 3 (SAPS 3) model. Spearman rank correlation was used and statistical significance was assumed if  $p < 0.05$ .

**Results:** The median antibiotic consumption was 1354 and 1484 DDD1000 2009 and 2010 respectively. Antibiotic consumption varied widely (four fold) between units ranging from 680 to 2698 and 658 to 2515 DDD1000 2009 and 2010 respectively. Isoxazolylpenicillins (16%) were most frequently used, followed by cephalosporins (14%), carbapenems (14%), triazoles (9%) piperacillin-tazobactam (9%), fluoroquinolones (6%) and beta-lactamase sensitive penicillins (5%). Mean SMR (SD) was 0.63 (0.17),  $n = 75$ . No significant correlation between antibiotic consumption and standardised mortality rate was found 2009 and 2010.

**Conclusions:** The lack of correlation between antibiotic consumption and mortality suggest that it is possible to lower antibiotic consumption in high consumption units. The Swedish ICU Registry provides a quality control system which ensures that such reduction of antibiotic consumption does not compromise patient outcome.

**P1061** Improved antimicrobial susceptibility of uropathogenic *Escherichia coli* due to decline in ambulatory antibiotic consumption

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**Objectives:** The aim of this study was to assess the impact of ambulatory antibiotic consumption on resistance patterns of *Escherichia coli* strains isolated from patients with community-acquired urinary tract infections (CA-UTIs) in Zagreb region.

**Methods:** All urinary specimens with significant bacteriuria processed during two study periods (January–October 2005 and January–December 2010) were included in the survey. Urinary specimens were collected in the course of routine diagnostics procedures of Zagreb outpatient population. Resistance patterns of 3052 *E. coli* strains collected during the first study period were compared with those of 4817 *E. coli* strains from the second study period. Antimicrobial susceptibility of isolates to commonly prescribed antibiotics was tested by disk diffusion method according to CLSI criteria. Information on ambulatory consumption of antibiotics, expressed in defined daily doses per 1000 inhabitants per day (DID), was obtained from annually published report by The Croatian Academy of Medical Sciences.

**Results:** In the period from 2001 to 2009, ambulatory consumption of broad spectrum penicillins, first generation of cephalosporins and co-trimoxazole declined from 4.09 DID, 1.65 DID and 1.70 DID in 2001 to 3.60 DID, 1.21 DID and 0.98 DID in 2009, respectively. While consumption of fluoroquinolones (FQs) remained more or less unchanged, usage of nitrofurantoin showed since 2007 constant increase. Considering the resistance of *E. coli* between 2005 and 2010, significant decrease in resistance was observed for amoxicillin (from 46.1% to 42.9%;  $p = 0.006$ ), cephalixin (from 21.3% to 10.2%;  $p < 0.0001$ ) and co-trimoxazole (from 28.9% to 23.3%;  $p < 0.0001$ ). While resistance to nitrofurantoin remained very low in both study periods (2.4% in 2005, 2.1% in 2010), resistance to FQs significantly increased (8.6% in 2005, 12.0% in 2010;  $p < 0.0001$ ).

**Conclusion:** Due to the updates in Croatian guidelines on empirical treatment of CA-UTIs, changes in ambulatory use of antibiotics occurred. Withdrawal of amoxicillin, cephalixin and co-trimoxazole from the list of first line agents for empirical treatment of uncomplicated cystitis, elicited significant improvement in susceptibility of uropathogenic *E. coli* to these antibiotics. Since no obvious rise in FQ use occurred, the increasing trend in FQ resistance which might be caused by the spread of highly epidemic FQ-resistant uropathogenic *E. coli* clonal groups requires further investigation.

**P1062** An innovative study of intra-abdominal infections to test the appropriateness of the revised trust antibiotic prophylaxis in adult surgery guidance. Results from intra-abdominal infections study for monitoring antimicrobial resistance trend

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**Background:** Blackpool Teaching Hospitals (BTH) operate a successful healthcare associated infection (HAI) and antibiotic stewardship programme. Revised antibiotic prophylaxis in adult surgery (APAS) guidance including new antibiotic choices to reduce *C. difficile* infections (CDI) was implemented. BTH was one of the UK sites for the study monitoring antimicrobial resistance trend (SMART) in intra-abdominal infections (IAI). We present findings of an innovative project aimed to assess the appropriateness of the revised APAS antibiotic choices in abdominal surgery by comparing it to SMART IAI isolates/susceptibility results.

**Methods:** A review of database of 61 intra-abdominal infections (IAI) isolates (from pus/intra-abdominal swabs) submitted to SMART (2008–10). Comparing this to the revised APAS guidance for abdominal surgery **Results:** Revised APAS guidance for abdominal surgery is an aminoglycoside plus metronidazole at induction replacing previous guidance of cefuroxime plus metronidazole.

Results from SMART: *Escherichia coli* 60% (18/30), followed by *Klebsiella pneumoniae* 13% (4/30), *Enterobacter cloacae* 7% (2/30). Five percent *E. coli* isolated were ESBL positive but fully sensitive to gentamicin. MICs to following antibiotics were tested in the study: ertapenem (ETP), imipenem (IMP), cefepime (CPE), cefotaxime (CFT), cefoxitin (CFX), ceftazidime (CAZ), ceftriaxone (CAX), ampicillin/sulbactam (A/S), piperacillin/tazobactam (P/T), amikacin (AK), ciprofloxacin (CP), levofloxacin (LVX). Key susceptibility results included: *E. coli*: 100% to ETP, IMP and AK; >90% to cephalosporins; >80% to A/S, CP and LVX. *Klebsiella pneumoniae*: 100% susceptible to all antibiotics tested. ESBL+ *E. coli* organisms: 100% susceptible to ETP, IMP, A/S, P/T and AK.

From 2008 to 2010, a downward trend of reduced susceptibility was noticed – *E. coli* to cephalosporins, fluoroquinolones and piperacillin/tazobactam. *K. pneumoniae* remained 100% susceptible to all agents in this study over the years.

**Discussion:** The trust has successfully and significantly reduced rates of CDI, MRSA and ESBL +ve Gram negatives over the last 3 years. There is high emphasis on antibiotic stewardship, education/training, regular microbiology ward rounds and consultations, regular antibiotic compliance audits and feedback. This innovative project examined the

appropriateness of the revised APAS guidance. All isolates were susceptible to aminoglycosides while reduced susceptibility to cefuroxime was evident from this study. Details to be presented.

**P1063 Point prevalence study for local antibiotic prescription and usage trends used in a large non-academic teaching hospital in the Netherlands**

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**Introduction:** In order to optimize in-hospital antibiotic usage, it is essential to gain insight which antibiotics are used and how these choices are made in the local situation. Recently, the European Surveillance of Antibiotic Consumption (ESAC) has developed a Point Prevalence Study for antibiotic surveillance. This is a limited time consuming method to gain insight in local prescribing policies. We adapted this method to see whether, how and when infection specialists (microbiologists and ID physicians) are involved in the decision to start a certain antibiotic.

**Methods:** The Canisius–Wilhelmina Hospital is a large 653-bed non-academic teaching hospital in the Netherlands with all the major hospital specialties present. Via the electronic prescription system of the hospital pharmacy, all patients that used one or more antibiotics at 1 day in the fall of 2007 and spring of 2011 were identified. Patients with surgical prophylaxis were excluded. With a standardised questionnaire, the treating physicians were interviewed to gain insight how the choice for a certain antibiotic prescription was made.

**Results:** Of all admitted patients, 27% (2007) and 22% (2011) were using antibiotics. Types of antibiotics prescribed and indications were similar between 2007 and 2011. An infection specialist was involved in 39% (2007) and 28% (2011) of prescriptions. Reasons for consultation were a positive culture result in 23%, a direct question about empirical therapy in 23% and a multidisciplinary meeting in 44% of cases. If an infection specialist was consulted, antibiotic therapy was often aimed at a certain pathogen, in contrast to patients that received prolonged (>72 hours) empirical therapy, for which in a minority of patients consultation was sought (50% vs. 8.8% of patients respectively  $p < 0.001$ ).

**Conclusions:** A point prevalence study is a fast way to gain insight into local antibiotic prescription and usage trends in a hospital. The PPS can be used to detect reasons for infection consultation. In our hospital we found that multidisciplinary meetings are very important for advice on antibiotic decision making. However, we had poor control on patients that are using prolonged empirical therapy, which is an important group for streamlining antibiotics.

**P1064 Adherence to recommendations by infectious disease consultants and its influence on outcomes of intravenous antibiotic-treated hospitalised patients**

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**Background:** There is a well-established role of infectious diseases (ID) specialists in antimicrobial stewardship. A prospective, randomized, 1-year study was performed to analyse the effects of ID recommendations and adherence by treating physicians on clinical outcome in hospitalized antibiotic-treated patients in a tertiary university hospital in Spain.

**Methods:** Patients receiving intravenous antimicrobial therapy prescribed by the primary service for 3 days were identified and randomised to intervention or non-intervention. Interventions consisted of treatment recommendations by IDs. Appropriateness of empirical treatments (prescribed by treating physicians) was classified as adequate, inadequate or unnecessary. In the intervention group, the

degree of adherence to intervention was classified as complete, partial or non-adherence.

**Results:** A total of 1173 antibiotic-treated patients were included in the study, 602 patients in the non-intervention and 571 patients in the intervention group (199 [34.9%] showing complete adherence, 141 [24.7%] partial adherence and 231 [40.5%] non-adherence to recommendations). In the multivariate analysis for adherence (R2 Cox = 0.065,  $p = 0.009$ ), non-adherence was associated with prolonged antibiotic prophylaxis ( $p = 0.004$ ; OR = 0.37, 95%CI = 0.19–0.72). In the multivariate analysis for clinical outcome (R2 Cox = 0.126,  $p < 0.001$ ), Charlson index ( $p < 0.001$ ; OR = 1.19, 95%CI = 1.10–1.28), malnutrition ( $p = 0.006$ ; OR = 2.00, 95%CI = 1.22–3.26), nosocomial infection ( $p < 0.001$ ; OR = 4.12, 95%CI = 2.27–7.48) and length of hospitalization ( $p < 0.001$ ; OR = 1.01, 95%CI = 1.01–1.02) were associated with clinical failure, while complete adherence ( $p = 0.001$ ; OR = 0.35, 95%CI = 0.19–0.64) and adequate initial treatment ( $p = 0.010$ ; OR = 0.39, 95%CI = 0.19–0.80) were associated with clinical success.

**Conclusions:** Intervention and adherence to recommendations were associated with favorable clinical outcome, which in turn was associated with shortened length of hospitalization. This may have important health–economic benefits and stimulates further investigation.

**P1065 Comparison of antimicrobial consumption measured by defined daily dose and days of therapy in VINCat hospitals**

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**Background:** Several units of measurement have been performed to estimate antimicrobial consumption. Studies focused on relationship between defined daily dose (DDD) and days of therapy (DOT) are scarce.

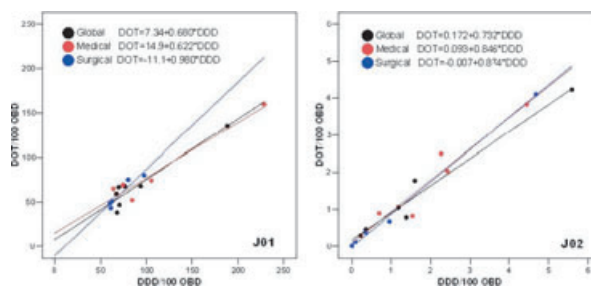
**Objectives:** The aim of this study is to assess the relationship between DDD and DOT measures in antimicrobial consumptions in several VINCat hospitals in Catalonia (Spain).

**Methods:** The average of antimicrobial consumption of a representative sample of 7 VINCat hospitals in 2010 was calculated in DDD/100 occupied bed-days (OBD) and DOT/100 OBD.

Global, medical and surgical antimicrobial consumption was analyzed. Antibacterial (J01) and antimycotic (J02) consumption were calculated separately.

Data of antimicrobial consumption are presented with means and standard deviation. Relationship and differences between two measures was assessed using simple linear regression and paired Student's *T* test. Agreement was also calculated with intraclass correlation coefficient (ICC). *p* Values <0.05 were considered statistically significant.

**Results:** The mean of antibacterial consumption in global area was 90.4 DDD/100 OBD ( $\pm 44.1$  of standard deviation) and 68.8 DOT/100 OBD ( $\pm 31.6$ ), with differences statistically significant ( $p = 0.016$ ). The ICC was 0.787 (95%CI: –0.029; 0.964). In surgical wards, the antibacterial consumption was 72.1  $\pm$  16.2 (DDD/100 OBD) and 59.5  $\pm$  16.7 (DOT/100 OBD);  $p = 0.006$ , ICC = 0.743 (95%CI:



-0.063; 0.971). In medical wards, the differences were not significant ( $p = 0.092$ ).

The antimycotic consumption in global area was  $1.72 \pm 1.97$  (measured in DDD/100 OBD) and  $1.43 \pm 1.46$  (in DOT/100 OBD). The differences between the two measurements were not significant ( $p = 0.283$ ) and the ICC was 0.938 (95%CI: 0.677; 0.991).

Graphic 1 shows the relationship between DDD/100 OBD and DOT/100 OBD, including estimated formula from simple linear regression.

**Conclusions:** Differences between DDD and DOT were observed in antibacterial (J01) group, mainly in surgical wards. In general, antimicrobial consumption measured with DOT seems to be lower than in DDD. Simple linear regression shows greater differences when consumption is higher.

Wide confidence intervals in estimations have been observed. Further studies based on bigger samples should be performed to confirm our results.

#### **P1066** Analysis of antibiotic usage in Ukraine

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**Objectives:** To estimate dynamics of antibiotic consumption in Ukraine expressed in DDD per 1000 inhabitants per day (DDD/1000/day).

**Methods:** Data about antibiotic selling in Ukraine during 2007–2010 years from data base SMD company was analyzed. Information about DDD was taken from site of WHO. Dynamics of total antibiotic consumption and different groups was evaluated.

**Results:** In Ukraine antibiotic consumption had tendency to decreasing: in 2007 it was 9.30 DDD/1000/day, in 2008 – 9.20, in 2009 – 9.16 and in 2010 it was decreased till 8.64 (-8.5%). Penicillines was the most frequently prescribed antibiotics. Level of their use was almost in two times higher than antibiotics of the other groups and varied in range 2.82–2.95 DDD/1000/day. Top in this group was amoxicillin but with little diminishing use in dynamics. Consumption of amoxicillin/clavulanate during 4 years was increased on 76.9%. Ampicillin use was decreased on 40%, ampicillin/oxacillin – on 46.2%. Second place of antibiotic use occupied fluoroquinolones with tendency to diminishing from 1.77 DDD/1000/day in 2007 till 1.5 in 2010. More than 86% of prescriptions were ofloxacin, norfloxacin, ciprofloxacin. But part of their use was gradually decreasing on 76.5% in 2010 in comparing with 2007. During this period use of levofloxacin increased in five times. Macrolides consumption had tendency to diminishing from 1.65 DDD/1000/day in 2007 till 1.30 DDD/1000/day in 2010 (-12.9%). Leading position among macrolides took up azithromycin with growth +113.3% and clarithromycin. Use of midecamycin was decreased in 2.5 times. Consumption of cephalosporines raised on 19.4%. The most frequently used drug from this group was ceftriaxone with growth from 0.45 DDD/1000/day in 2007 till 0.59 in 2009 and little decrease to 0.54 in 2010. Consumption of cefuroxime rose in 2.5 times and cefazolin use was diminished more than 50.0%. Use of tetracyclines decreased on 28.0% co-trimoxazole on 42.9% and chloramphenicol on 22.8%. It reflected positive changes in antibiotic prescriptions.

**Conclusion:** Level of antibiotic consumption in Ukraine is low. We found positive tendency at the penicillines, macrolides, cephalosporines use that may restrain developing antibiotic resistance in our country. Level of fluoroquinolones use requires conducting actions for diminishing consequence of non-rational antibiotic therapy.

#### **P1067** Experience of a large Belgian centre in multidrug-resistant *Pseudomonas aeruginosa* bloodstream infections

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**Objectives:** Emergence of MDR, including strains producing metallo- $\beta$ -lactamase, has been increasingly reported in *P. aeruginosa*. As these

strains are frequently resistant to all antibiotics except colistin, treatment of infections by these strains remains an unevaluated therapeutic challenge. We aimed to review our clinical experience in treating patients (pts) who developed bacteraemia due to MDR *P. aeruginosa*.

**Methods:** Retrospective, observational study on all pts who developed bacteraemia due to MDR *P. aeruginosa* from 2004 to 2011, in our institution. An MDR *P. aeruginosa* was defined as a strain resistant to carbapenems, 3rd and 4th generation cephalosporins and aminoglycosides or quinolones. The presence of MBL was confirmed by PCR for blaIMP and blaVIM genes detection.

**Results:** Thirty pts (19M, 11F), median age 55 years (range 24–87) were included. All had received broad spectrum antibiotics during the preceding month, and 29/30 had at least one co-morbidity factor. Infection developed a median of 17 days after admission (0–139). VIM producing *P. aeruginosa* was identified in 18 episodes. Previous colonization by MDR-*P. aeruginosa* was detected in five pts. Primary sources of infection were: pulmonary ( $n = 13$ ), intra-abdominal ( $n = 8$ ), upper urinary tract ( $n = 2$ ), catheter ( $n = 2$ ), others ( $n = 2$ ) and unknown ( $n = 3$ ). Twenty-seven pts needed intensive care unit management. Susceptibility testing showed that all strains were susceptible to colistin, and that aztreonam, gentamicin and piperacillin-tazobactam were the next most effective drugs, but all with high MIC's. Empirical antimicrobial treatment consisted in a combination of a median of two antimicrobial drugs (range 1–4). Appropriate treatment (at least some in-vitro activity) was administered in a combination of 4 ( $n = 2$  pts), 3 ( $n = 11$  pts), 2 ( $n = 7$  pts) or 1 ( $n = 1$  pt) drugs. The most frequently used combination was aztreonam, colistin and either piperacillin-tazobactam, ciprofloxacin or gentamicin/amikacin (10 pts), given at very high dosages. Source control was performed in six pts. Eighteen pts died (60%) after a median delay of 4 days (0–16). Death was infection-related in 15/18 pts.

**Conclusion:** The mortality rate of our patients was very high, probably because patients had many co-morbidities, and because of the delay in administering appropriate antimicrobial treatment, due to the paucity of active drugs.

#### **P1068** Latvian point prevalence study for healthcare associated infections and antimicrobial use in acute care hospital by using ECDC PPS protocol

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**Objective:** Point prevalence studies on antibiotic use were performed in Latvian hospitals as a quality improvement tool since 2002. Lack of standardised methods and strict definitions of infections was the most important shortcoming of these studies. Our goal was to examine the prevalence of antibiotic use and healthcare-associated infections (HAI) in acute care hospitals with the new ECDC protocol designed for all EU countries.

**Methods:** Study was done in accordance with ECDC Point prevalence survey of HAI and antimicrobial use in European acute care hospitals (ECDC PPS) protocol version 4.2. All acute care hospitals were invited to participate in the first ECDC PPS organised in Latvia. Eighteen hospitals and hospital unions were invited and 14 voluntary agreed to participate. Two of them were specialized hospitals. Hospitals that didn't participate in the study were small size local hospitals. Investigators were trained in the special course before the start of the study. Training included detailed explanation of all variables collected during the study and practical exercise with case studies. Data were entered in HelicsWin database and analysed with EpiData 3.1.software.

**Results:** There were 3367 patients eligible for study and in average 37.8% of patients received antibiotics on the study date. The three most prevalent antibiotics were ceftriaxone, ciprofloxacin, and amoxicillin with enzyme inhibitor. There was substantial variability in antibiotic use across hospitals from the highest 59.3% to the lowest 26.2%. 9.8% of all antibiotics were prescribed for surgical prophylaxis. Antibiotics were predominantly used intravenously. The most common indications

for antibiotic treatment were community acquired pneumonia (CAP) and urinary tract infection (UTI). In 3.6% of patients investigators reported HAI, but not in all cases it corresponded to study HAI definitions due to lack of detailed investigations and laboratory data. The most common HAI recorded were pneumonia and surgical site infection.

**Conclusions:** We think that ECDC PPS protocol was successfully implemented in Latvia. High proportion of participating hospitals provided a good overview on situation in our country and the protocol could be used to assess interventions. At the same time it should be noted that strict HAI definitions constituted a problem for investigators since different diagnostic algorithms and financial possibilities exist in our hospitals.

#### **P1069** Antibiotic sales in rural and urban pharmacies in Northern Viet Nam: an observational study

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**Background:** In Vietnam, antibiotics can only be sold with a prescription. But previous studies have shown that there is a high inappropriate use of antibiotics in the community in Vietnam. The majority of antibiotics are sold without a prescription, often for acute upper respiratory infections that do not need antibiotic treatment. The problem of irrational use of antibiotics should be minimized as it drives resistance development. Better understanding of practices and economic incentives of antibiotic dispensing is needed to design effective interventions to reduce inappropriate antibiotic use.

**Objectives:** This study aims to assess the current practices and economic profitability of antibiotic sales for rural and urban pharmacies in northern Vietnam.

**Method:** This cross-sectional study used both quantitative and qualitative techniques. All drug sales were observed and recorded for 3 days at thirty private pharmacies (15 urban, 15 rural). Pharmacy staffs were interviewed by a semi-structured questionnaire and this was followed by in-depth interviews of drugstore owners and drug sellers.

**Results:** In total 2953 drug sale transactions (2083 urban and 870 rural) were observed. Twenty-four percent (499/2083) of the urban transactions contained antibiotics and 29.5% (257/870) of the rural transactions. Antibiotics contributed 13.4% in urban and 18.7% in rural to the total sales of pharmacies. Most antibiotics were sold without a prescription: 88% in urban and 91% in rural pharmacies. The most frequent reported reason for buying antibiotics in urban area was cough (31.6%) and fever in the rural area (21.7%). The three most common sold antibiotics were ampicillin/amoxicillin (29.1%), cephalexin (12.2%) and azithromycin (7.3%). Consumer often demanded antibiotics without a prescription: 49.7% in urban and 28.2% in rural, respectively. The qualitative data revealed that the knowledge of antibiotics and antibiotic resistance of drug sellers and customer's awareness are low, especially in rural area.

**Conclusion:** Suggested areas of improvement are enforcement of regulations and increase knowledge of drug sellers as well as customer's awareness to reduce pressure for drug sellers to dispense antibiotics inappropriately.

#### **P1070** Antimicrobial pharmacists in the UK – a role model for Europe

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**Objectives:** To describe the evolving role of UK hospital pharmacists in antimicrobial stewardship, to allow pharmacists and hospital managers from other European countries to benchmark their pharmacy services.

**Methods:** Review of the UK published literature and informal discussion with practising hospital antimicrobial pharmacists.

**Results:** UK hospital pharmacists have established specialist roles and become integrated into multi-disciplinary clinical teams. The majority of UK hospitals now have specialist antimicrobial pharmacists (APs). Networks and training: Over 450 hospital pharmacists with an interest in infection subscribe to an infection management network e-mail discussion forum hosted by the UK Clinical Pharmacy Association and there are over 250 APs. Regional networks of APs exist throughout the UK, providing opportunities for shared learning and experience. Formal links have also been forged with several UK medical infection societies. A 2-year post-graduate Infection Management MSc course for antimicrobial pharmacists and short course are offered at Imperial College, London. Guidelines and education: A primary role of APs is facilitating the development, dissemination and implementation of treatment guidelines. APs lead projects to deliver innovative solutions to implementation including: intranet websites; pocket-sized booklets; and Smartphone applications.

APs lead on auditing adherence to antimicrobial prescribing policy with regular feedback to prescribers. APs from over 100 hospitals contributed to the first national point prevalence study on antimicrobial use as part of the 2011 European survey of Healthcare Associated Infections. APs provide expert advice to pharmacy colleagues and hospital doctors on the clinical pharmacology of antimicrobials. Many APs refer patients to and attend regular antimicrobial stewardship ward rounds with their microbiology or Infectious Diseases medical colleagues. Surveillance: and governance Antimicrobial consumption data are derived from hospital pharmacy dispensing computer systems in the UK. APs convert dispensing data to the standard currency of WHO defined daily doses for benchmarking and quality assurance reports for hospitals management boards. Such data are collated nationally for hospitals in Scotland, Wales and Northern Ireland.

**Conclusions:** UK hospital pharmacists have successfully established a specialist role in antimicrobial stewardship, bringing unique skills and adding value to the multi-disciplinary team.

#### **P1071** Off-label use of anti-infective drugs in an Italian paediatric cohort

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**Objectives:** The European Medicines Agency (EMA) estimated that 50–90% of drugs used for treating children were studied exclusively in adults. There are few clinical trials about safety of drug prescriptions in children. The aim of this study was to evaluate off-label (OL) anti-infective drugs prescriptions in hospitalized children.

**Methods:** We retrospectively reviewed 712 anti-infective systemic prescriptions in children hospitalized at Regina Margherita Children's Hospital (Turin, Italy), who underwent an infectious disease (ID) consult, from December 2010 to February 2011. Anti-infective drugs were assessed according to the following variables of prescription: indication, age, dose, method of administration and duration. We divided patients in two groups in relation to their acute or chronic disease. Data were assessed in each group according to drug-prescribing physician, pediatrician or ID consultant. The use of these anti-infective drugs was compared to the Italian Medicines Agency (AIFA) recommendations and to current available guidelines (see Table 1).

**Results:** One hundred and fifty patients received anti-infective drugs (mean age 5.7 years, 54% males). The most frequent conditions in

Variable of prescription	Prescription OL for AIFA	Prescription OL for AIFA and not conformed to current guidelines*
indication	11.5%	10%
age	12.9%	6%
dose	39.2%	36%
method of administration	24.3%	35%
duration	12.1%	13%

\* Sanford Guide to Antimicrobial Therapy (2010); Sanford Guide to HIV/AIDS Therapy (2011); National Institute for Health and Clinical Excellence's British Guidelines for urinary tract infections (2007), tuberculosis (2011) and meningitis (2010); Recommendations of the Italian Association of Pediatric Hematology and Oncology for anti-infective therapy and prophylaxis in oncohematological patients (2010)

acute group were respiratory (32%) and infectious diseases (28%), whereas in chronic group were oncological (31%) and cardiological (24%) ones. Ninety percent of children received at least an OL drug, without significant difference between acute and chronic group (91% vs. 90%). 53.8% of total prescriptions were OL: antibiotics, antivirals and antifungals were OL in 62%, 50% and 20% of cases, respectively. Including drugs with more than one OL variables, the total OL prescriptions were 502 (see Table 1). Seventy-two percent of OL drugs were prescribed by ID consultant (71% in acute and 73% in chronic patients). Twenty-three percent of OL prescriptions for AIFA agreed with current guidelines, whereas 41% of anti-infective prescriptions were OL for AIFA and not agreed with these guidelines (see Table 1). Sixty-seven percent of ID consultant's prescriptions agreed with analyzed guidelines.

**Conclusions:** Our study confirmed that there is a discrepancy between official licenses and use of drugs in paediatric clinical practice. The EMEA, with the institution of the European Regulation, the Task Force in Europe for Drug Development for the Young and the Paediatric Investigation Plans, is working to improve safety and propriety of children treatment. Innovative study design for paediatric clinical trials are emerging. A greater number of guidelines is needed.

#### **P1072** Creation of the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) project

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**Objectives:** The ESVAC project was launched in 2009 following a request from the European Commission to the European Medicines Agency (EMA) to establish a surveillance program for the collection of data on the sales of veterinary antimicrobial agents in the EU. The purpose is to obtain valid and standardised data for risk profiling, setting risk management priorities and to assess the impact of recommendations on responsible use of antimicrobials for animals.

**Methods:** In 2009, a pilot project was set up, including an advisory group consisting of representatives from already existing national surveillance programs. Representatives from ECDC, EFSA and ESAC (European Surveillance of Antimicrobial Consumption in man) were also involved in order to harmonise surveillance of consumption and antimicrobial resistance across the human and veterinary sectors. The pilot project defined the ESVAC protocol and form for data collection and submission, following recommendations from international organizations such as WHO and OIE.

To document the situation of antimicrobial consumption in animals, including agents critically important for humans, the project collected aggregated, retrospective data from nine countries.

In 2011, the project sent its first call for data collection to the EU countries.

**Results:** The ESVAC protocol and data collection form have been developed (<http://www.ema.europa.eu>). The annual sales data will be collected as the number of packages for each antimicrobial product, including the product's details and linked to the ATCvet classification. A technical unit for estimating the animal population potential exposed to treatment with antimicrobials has been created: The Population Correction Unit (PCU) taking into account weight at treatment and import/export of animals. Eighteen European Countries have already delivered data for 2010 using the ESVAC protocol. In parallel, ESVAC analysed 5 years of aggregated data from nine countries.

**Conclusions:** ESVAC developed a standardised protocol for collecting sales data for veterinary antimicrobial agents and an indicator for measuring the animal population potentially exposed to antimicrobial treatment. In 2011, nine of 18 countries delivering data started collecting such data for the first time. During 2011 two workstreams on collection of harmonised data per animal species in order to obtain use per species and on the development of standardised units of measurement have been initiated.

#### **P1073** Using milk from cows on antimicrobial therapy as feed for calves – a potential risk for dissemination of resistant bacteria?

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**Objectives:** The aim of this ongoing cross-sectional study is to describe how and to what extent milk from cows on antimicrobial therapy is fed to calves in Swedish dairy herds and whether this approach can be linked to the high prevalence of resistance among fecal *Escherichia coli* from calves.

**Methods:** In a web-based survey, a representative sample of 457 farmers responded to questions covering the use of colostrum and milk from cows subjected to antimicrobial therapy. In 300 of these herds, fecal swabs from three calves in the age of 1–4 weeks will be collected for antimicrobial susceptibility testing. For each sample, the minimum inhibitory concentration for 14 antimicrobial substances and the within-sample prevalence of *E. coli* resistant to cefotaxime, streptomycin and quinolones will be determined. Additional data regarding use of antimicrobials in the herd will be collected from databases as well as from the farmers.

**Results:** Eleven percent of the farmers always discarded colostrum from cows treated during the dry period, while 45% of the farmers always fed such colostrum to calves. In the rest of the herds such colostrum was used only under special conditions, e.g. if colostrum from untreated cows was unavailable. When antimicrobial therapy was used during lactation, 56% of the farmers always, or with some restrictions, gave milk from these cows to calves during treatment as well as withdrawal period. Twenty-four percent of the farmers gave such milk during the withdrawal period only and the remaining 20% of the herds never fed such milk to calves. These results together with antimicrobial susceptibility results and data on antimicrobial usage will be analyzed to find significant associations or trends. Preliminary results from these analyses will be presented.

**Conclusions:** This study indicates that the use of milk from cows treated with antimicrobials is widespread on Swedish dairy farms, but at this point in time no conclusion can be drawn whether this has any effect on the prevalence of antimicrobial resistance among fecal bacteria from calves.

#### **P1074** ESBL-producing enterobacteria in retail chicken meat, Berlin, Germany

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**Objective:** To examine the role of retail chicken meat as a reservoir of ESBL-producing Gram-negative bacteria.

**Methods:** Between 16 and 26 August, 2011, 199 fresh raw chicken meat samples purchased from Berlin stores of six supermarket chains, two whole food shops and a butcher were cultured for ESBL-producing Gram-negative bacteria on selective chromogenic plates after broth enrichment. ESBL-positive isolates were affirmed by DDST using 3rd generation cephalosporins with/without clavulanic acid. Species identification and antimicrobial susceptibility testing was performed as well as PCR for ESBL genes, plasmid-mediated AmpC genes and PMQR genes. Strain typing by PFGE will be performed.

**Results:** Of the 199 retail chicken meat samples tested, 76 (38%) turned out to be positive for ESBL-producing Gram-negative bacteria (*Escherichia coli* n = 71, *Serratia fonticola* n = 3, *Escherichia fergusonii* n = 1 and *Enterobacter cloacae* n = 1). Using PCR and sequencing, ESBL-types CTX-M-1 (n = 36), SHV-12 (n = 32), TEM-52 (n = 6), CTX-M-14 (n = 1) and CTX-M-15 (n = 1) were identified. *E. coli* isolates from 43 samples additionally contained TEM-1 beta-lactamase and in two *E. coli* isolates the SHV-1 enzyme was identified. Two further *E. coli* strains isolated from two meat samples exhibited no effect in DDST with clavulanic acid. Molecular analyses revealed AmpC-production (CMY-2) in both strains. Antimicrobial

susceptibility testing showed increased MICs for ciprofloxacin (MIC 0.25–16 mg/L) in *E. coli* from 37 samples (19%) and resistance to SXT in *E. coli* from 30 samples (15%). PMQR genes were not detected indicating mutations in chromosomal-encoded genes (parC, gyrA) might be the cause for increased ciprofloxacin MICs.

There were no significant differences with respect to origin and point of purchase. All chicken meat samples were exclusively produced in Germany.

**Conclusion:** A substantial proportion of retail chicken meat samples purchased from Berlin groceries yielded ESBL-producing Gram-negative bacteria. Therefore, retail chicken meat may act as reservoir for the transmission of ESBL-positive bacterial strains or genes to humans via the food chain. Further molecular analysis has to be done to prove this hypothesis.

#### **P1075** Increased raw poultry meat colonisation by extended-spectrum beta-lactamase-producing *Escherichia coli* in southern Spain

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**Objectives:** The present study was conducted in order to assess trends in the prevalence and diversity of extended spectrum beta lactamase producing *E. coli* (ESBLEC) colonizing retail poultry meat samples in our area. Results were compared to those obtained in a previous study in 2007 where a high percentage of retail meat (39.8%) colonized by ESBLEC was found. Spread of the O25b:H4-ST131 clonal group was also investigated.

**Methods:** In 2010, 15 of either chicken and turkey breasts samples were purchased in local supermarkets in Seville (Southern Spain) along a period of 12 weeks. Samples were processed by stomaching, incubated overnight in pre-enrichment broth and plated in MacConkey agar with cefotaxime and ceftazidime. Suspected *E. coli* colonies were selected from each sample based on morphological criteria and further identification was performed by standard biochemical tests. ESBL screening was performed by double disk synergy test according to CLSI. Characterization of ESBL and phylogenetic grouping of isolates were carried out by PCR and sequencing. Antibiotic susceptibility was determined by agar diffusion. Genetic relatedness was assessed by PFGE for phylogroup A1 isolates. Results were compared with those obtained in a previous study performed in 2007. For categorical variables comparison, chi-squared or Fisher's tests were performed.

**Results:** ESBLEC was detected in 14 (93.3%) of 15 chicken samples and in 14 (93.3%) of turkey meat samples. The prevalence of poultry meat ESBLEC colonization increased from 62.5% (2007) to 93.3% (2010) in our area ( $p = 0.005$ ). Sixty ESBLEC isolates were recovered from meat samples. Phylogroups B1 and A1 encompassed more than 60% of the isolates. Sequence type ST131 or B2 phylogroup isolates were not detected. Clonal relationship was found between 2 CTX-M-1-producing isolates included in A1 phylogenetic group. SHV-12 was the most frequently detected ESBL harboured by *E. coli* (65%), although its prevalence has significantly decreased since 2007 at expenses of CTX-M ESBLS. Resistance to quinolones continues to be significantly high, with 85.35% and 32.79% of isolates showing resistance to nalidixic acid and ciprofloxacin, respectively.

**Conclusion:** An increasing trend of ESBLEC colonization in raw poultry meat, particularly by CTX-M-producing isolates, has been observed in our area in a short period of time.

#### **P1076** Comparison of ESBL contamination in organic and conventional retail chicken meat

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**Objectives:** Contamination of retail chicken meat by Extended Spectrum Beta-Lactamase (ESBL) producing bacteria likely contributes to the increasing incidence of infections with these bacteria in humans. This study aimed to compare the prevalence and load of ESBL positive isolates between organic and conventional retail chicken meat samples, and to compare the distribution of ESBL genes, strain genotypes and co-resistance.

**Methods:** In 2010, 98 raw chicken breasts ( $n = 60$  conventional;  $n = 38$  organic) were collected from 12 local stores in the Netherlands. ESBL producing micro-organisms on the meat samples were detected by homogenizing 25 grams per meat sample with peptone water. The homogenate was directly inoculated on an ESBL selective agar for quantitative cultures, and after a pre-enrichment step for qualitative cultures. Species determination was performed usingaldi-tof. Phenotypic ESBL production was confirmed using ESBL Etests. ESBL genes were detected by PCR and sequencing. Minimum inhibitory concentrations (MICs) of antibiotics were determined by broth micro-dilution. Strain typing was performed using multi locus sequence typing.

**Results:** Prevalence of ESBL producing micro-organisms was 100% on conventional and 84% on organic samples ( $p < 0.001$ ). Median loads of ESBL producing micro-organisms were 80 CFU (range <20–1360) in conventional, and <20 (range 0–260) CFU/25 gram in organic samples ( $p = 0.001$ ). The distribution of ESBL genes in conventional samples and organic samples was 42% vs. 56%, respectively (N.S.), for CTX-M-1, 20% vs. 42% (N.S.) for TEM-52, and 23% vs. 3% ( $p < 0.001$ ) for SHV-12. CTX-M-2 (7%), SHV-2 (5%) and TEM-20 (3%) were exclusively found in conventional samples. Co-resistance rates of ESBL positive isolates were not different between conventional and organic samples (co-trimoxazole 56%, ciprofloxacin 14%, and tobramycin 2%), except for tetracycline, 73% and 46%, respectively,  $p < 0.001$ . Six of 14 conventional meat samples harbored four MLST types also reported in humans and five of 10 organic samples harbored three MLST types also reported in humans (2 ST10, 2 ST23, ST354).

**Conclusion:** The majority of organic chicken meat samples were also contaminated with ESBL producing *E. coli*, and the ESBL genes and strain types were largely the same as in conventional meat samples.

#### **P1077** Contamination of retail samples of roasted pork (Sui Mei) in Hong Kong

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**Objectives:** *Staphylococcus aureus* is a major cause of food poisoning and in Hong Kong the majority of such outbreaks have been ascribed to intake of roasted pork although there appear to have been no studies to determine rates of contamination of this popular local food. This study aimed to determine rates of *S. aureus* contamination. The presence of enterotoxin and leukocidin genes and resistance to methicillin was determined.

**Methods:** Samples were purchased from 50 retail outlets preparing roasted pork on the premises. Five millilitres of a 25 g homogenate were enriched in 6% NaCl Tryptone Soy Broth (TSB) for 24 hours and then streaked onto ChromeID SA agar. Five millilitres of the TSB were further enriched in TSB with 5 mg/L ceftiozime and 75 mg/L aztreonam for 24 hours before plating onto ChromeID MRSA agar. *S. aureus* isolates were confirmed by latex agglutination and susceptibility to cefoxitin determined by disc diffusion. Isolates appearing resistant were confirmed as methicillin-resistant *S. aureus* (MRSA) by presence of *mecA* and SCCmec type was determined. All isolates were characterized for presence of enterotoxins SEA-SEE and Panton-Valentin Leukocidin (PVL).

**Results:** Twenty-four (48%) samples yielded *S. aureus*, two samples having two strains. Of these three were resistant to cefoxitin, but only two (4%) were *mecA* positive (one SCCmec IVb, one V). The remaining strain was shown to be a beta-lactamase hyper producer (MIC penicillin 10 g/L). Enterotoxin genes were present in five methicillin-sensitive isolates and PVL in one.

**Conclusions:** Almost half of the samples were positive for *S. aureus* and 10% of samples yielded enterotoxin-positive strains, confirming that this food could be an important vehicle for staphylococcal food poisoning since it is usually kept at room temperature before sale and not reheated before consumption. The presence of community associated MRSA in two samples may be of concern as it demonstrates its ability to be transferred by contaminated foodstuffs. High rates of MRSA have been reported in pork carcasses in Hong Kong and further study of the source of contamination on roasted meat are needed.

**P1078** Did SARS have a long-lasting effect on levels of nasal colonisation and hand contamination of food handlers in Hong Kong?

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**Objectives:** Hand and nasal carriage of *Staphylococcus aureus* in food handlers is recognized as a major source of food poisoning. During the SARS epidemic in 2003, strict enforcement of hand hygiene, use of face masks and gloves was implemented in efforts to reduce viral transmission. Immediately before SARS onset, we commenced a longitudinal study of *S. aureus* colonization of food handlers. A marked drop in both hand and nasal colonization was observed in the follow-up performed 2 months post-SARS, which we attributed to compliance with improved hygiene measures. Long term effects of the interventions have not been investigated. This study investigated colonization in food handlers 8 years on.

**Methods:** One hundred and fifty workers at four catering establishments provided nose and hand samples. Nasal swabs were enriched in Brain Heart Infusion broth supplemented with 5% NaCl at 37°C overnight and then subcultured onto ChromID *S. aureus*. Hand contamination was estimated by fingerprint impressions of the dominant hand on Mannitol Salt agar. All isolates were confirmed by Staphaurex and tested for susceptibility to a range of antibiotics.

**Results:** Pre-SARS nasal carriage was 35% and hand contamination 41%, reducing in the immediate post-SARS period to 22.3% and 11.5% respectively ( $p = 0.001$ ). In the current follow-up the nasal carriage rate remained stable (22.8%) and hand contamination dropped to 6.5%. Resistance to fusidic acid rose significantly over the period from 25% to 59%. Reductions in tetracycline and erythromycin resistance rates were noted though not reaching significance. Resistance to other antibiotics appeared unchanged (Table 1). Methicillin resistance rates remain low. Strains isolated were SCCmec type V.

**Conclusions:** The remarkable decrease in colonization rates following SARS may be attributed to reduced opportunities for spread to transient carriers due to use of protective measures accompanied by improved hand hygiene. Since SARS, food handlers' routine use of gloves and masks if having respiratory symptoms has resulted in sustained low

colonization rates. A significant increase in fusidic acid resistance may reflect increased use due to concern about community-associated MRSA infection. Decreases in erythromycin and tetracycline resistance may reflect reduced local use of these agents. Resistance to other agents had not increased and may be related to sustained efforts to reduce antibiotic prescribing in the community.

## Bacterial fitness and antibiotic resistance of Gram-negative bacteria

**P1079** In silico search for QnrVC-like family members

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**Objectives:** The present work was designed to analyse the phylogenetic relationships of QnrVC-like sequences with respect to the established plasmid-encoded Qnr-families (QnrA, QnrB, QnrC, QnrD and QnrS), as well as to search GenBank for possible QnrVC-related sequences.

**Methods:** Representative sequences of the established plasmid encoded Qnr-families such as those present in <http://www.lahey.org/qnrStudies> were included in the phylogenetic analysis. Additionally, a similarity search in GenBank was performed using the DNA and protein sequences of QnrVC1 (protein GenBank access No: ACC54440; DNA GenBank access No: EU436855) as a template. Following established criteria sequences were considered as possibly related if their similarity was higher than 70%.

**Results:** The results show the presence of 10 different Qnr sequences, either plasmid or chromosomal-encoded with amino acid similarities higher than 70% with respect to QnrVC1. Thus, two chromosomal-encoded sequences of *Vibrio cholerae*, two of *Photobacterium profundum* and one of *Vibrio orientalis* and *Allivibrio salmonicida* respectively, as well as three plasmid-encoded sequences, one recovered from *Aeromonas caviae* (protein GenBank access: ADI55014), other from *Vibrio fluvialis* (protein GenBank access: AEM62764) were detected, and, surprisingly, QnrC was also included. Finally, a non-indicate full closely related ORF was found in a class 1 integron environment (DNA GenBank accession number: GU944730) from *Acinetobacter baumannii*, but no information related to its chromosomal or plasmid location was found.

**Conclusion:** Eleven sequences that following the current established normative may be considered as family-related has been found in GenBank. From these, at least two sequences has been found as plasmid encoded, then QnrVC is need to be considered as a new transferable Qnr family. The close similarity (higher than 70%) between the QnrC and QnrVC families may suggest the need for nomenclature unification following the current established normative.

**P1080** Comparison of the virulence genetic pool of *Pseudomonas aeruginosa* clinical and environmental isolates with public health relevance

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**Objectives:** Virulence factors (VF) are intrinsic characteristics of bacteria related to their infection ability. Several VF have been described in *Pseudomonas aeruginosa* (PA). Although thoroughly known in clinical setting, PA is also an aquatic bacterium. Thermae is a health care unit where physicians treat several health conditions using natural mineral water (NMW) that cannot be disinfected prior to use. If PA is present in thermae NMW, users contact with contaminated water and a health problem may occur. The aim of this study was to understand the prevalence of VF in NMW PA and clinical isolates, in order to better understand the health risk associated to PA contamination of thermae NMW.

**Methods:** Seventy-seven PA clinical isolates (PACI) and 77 PA thermae isolates (PATI) were screened for 20 VF encoding genes, by Polymerase Chain Reaction (PCR): motility (flag, orfF, pilA, pilB),

Table. Antibiotic resistance rates of *S. aureus* isolates over an eight year period

Antibiotics	Sampling period		p-value (chi-square test)
	2002/03 (%)	2011 (%)	
Fusidic acid	25	59	<0.001
Ciprofloxacin	2	6	0.25
Co-trimoxazole	1	0	0.56
Gentamicin	3	3	0.98
Clindamycin	29	32	0.71
Erythromycin	25	18	0.51
Tetracycline	21	12	0.33
Methicillin	1	1	NA

phenazines (phzI, phzII, phzH, phzM, phzS), proteases (apr, lasA, lasB), type III effector system (exoS, exoY) and genomic islands PAG11 (orf3, orf12), PAG12 (C22, C105) and PAG13 (SG8, SG100). PCR products sequencing and Blast analysis was performed. Statistical analysis was executed on IBM® SPSS® version 19.0, to compare the distribution of VF genes individually (Fisher's exact test) and by classes (Mann-Whitney test).

**Results:** One thousand five hundred and forty PCR screens were performed in each population. Six hundred and twenty-seven and 653 positive results were obtained in PACI and PATI, respectively. Statistically significant differences between populations were observed in the distribution of flag, phzH, phzM, phzII, apr, lasA, lasB, exoY, orf3, orf18, C105, SG8 and SG100 ( $p < 0.05$ ). Fisher's exact test showed that phzM, apr and lasA were more prevalent in PACI and other genes were more prevalent in PATI. Mann-Whitney test showed that PAG12 genes were more prevalent in PACI; phenazine and type III effector system genes were more prevalent in PATI and no significant differences in the distribution of motility, protease, PAG11 and PAG13 gene classes were observed ( $p > 0.05$ ).

**Conclusion:** PACI and PATI presented similar amounts of VF genes, but significant differences in their distribution. This shows that PA isolates, regardless of source, have basic pathogenic mechanisms necessary for infection. The different VF genetic pool observed may suggest that the type of VF present in populations are relevant for their infection ability, since PA infections associated to thermae practice are not known and clinical isolates are effectively causing illness.

#### P1081 Impact of antibiotic resistance and beta-lactamase carriage on virulence of *Klebsiella* spp.

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**Objectives:** In severe infections, Extended Spectrum beta-lactamases (ESBLs) and expression of various virulence factors may work in harmony, resulting in the treatment failure of Multidrug Resistant (MDR) *Klebsiella* spp. It remains somewhat unclear how beta-lactamase carriage affects virulence. We assessed the effects of  $\beta$ -lactamases and ESBL carriage on the virulence of *Klebsiella* spp. clinical isolates.

**Methods:** This study included 93 representative clinical isolates of *Klebsiella pneumoniae* ( $n = 86$ ) and *Klebsiella oxytoca* ( $n = 7$ ) that were collected between 1980 and 2011. The isolates were characterized in groups according to the  $\beta$ -lactamases produced, namely TEM, SHV, CTX-M and KPC. The virulence of each isolate was assessed by PCR amplification for six virulence genes: k2A (K2 serotype), fimH (fimbrial adhesins type 1), mrkD2 and mrkD3 (fimbrial adhesins type 3), khe (haemolysin) and iucC (aerobactin). A  $p$  value of  $\leq 0.05$  was used to indicate statistical significance. Average number of virulence factor genes per isolate was calculated.

**Results:** The isolates TEM-type  $\beta$ -lactamase producers (1980) showed an average number of virulence genes per isolate of 2.0 although the CTX-M-15 ESBL producers (2004–2009) showed 3.0. These results were higher when the *Klebsiella* spp. isolates are associated with carbapenemase KPC-3 genes (2009–2011), especially KPC-3 coupled with TEM-type, SHV-type and CTX-M-15 that showed an average number of 4.8. Among the KPC-3 producers no significant differences in virulence factor production were found. In the TEM-type isolates ( $n = 5$ ) was identified only khe (4/5, 80%), mrkD2 (3/5, 60%) and mrkD3 (3/5, 60%); The CTX-M-15 isolates ( $n = 40$ ) showed fimH (36/40, 90%), khe (22/40, 55%), mrkD2 (36/40, 90%), mrkD3 (24/40, 60%) and iucC (1/40, 2.5%). Finally the KPC-3/TEM/SHV/CTX-M-15 isolates ( $n = 5$ ) with K2 (3/5, 60%), fimH (5/5, 100%), khe (4/5, 80%), mrkD2 and mrkD3 (both with 5/5, 100%) and iucC (2/5, 40%). The presence of K2 serotype and iucC aerobactin in KPC-3 producers *Klebsiella pneumoniae* isolates was more significant ( $p \leq 0.05$ ) than in the other isolates.

**Conclusions:** The presence of ESBL enzymes suggested an association to virulence among the producing isolates. Carriage of

KPC-3 carbapenemase enzymes significantly impacts on the virulence of *K. pneumoniae* isolates producing these enzymes.

#### P1082 Colistin susceptible *Acinetobacter baumannii* that turns resistant after colistin treatment: effect on virulence and bacterial fitness

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**Objectives:** Recently, we have observed the development of stable colistin (COL) resistance in *Acinetobacter baumannii* after COL treatment of a nosocomial infection by a COL susceptible *A. baumannii* strain (*J Infect Dis* 2011; 204: 1147–1148). The aim of this study was to evaluate the effect of COL resistance acquisition on the virulence and fitness of *A. baumannii* clinical strains, and the mechanism of resistance.

**Methods:** *Strains:* *A. baumannii* CS01 (MIC  $\leq 0.03$  mg/L) and its COL resistant mutant (CR17, MIC  $\geq 16$  mg/L) isolated after colistin treatment were used. For in vitro growth, bacterial duplication time, and competition index (CI) experiments, growth curves in Mueller-Hinton broth were performed for both strains separately and in combination, and bacterial concentrations at 2, 4, 8, 24, 48, and 72 hours were determined. Virulence was assessed in a murine peritoneal sepsis model by inoculating groups of five C57BL/6 mice with 0.5 mL of an 8 log CFU/mL inoculum, and diminishing the inoculum by a factor of 10 until 0% mortality was reached (mortality and time to death were measured). For in vivo growth and CI experiments, groups of 19 mice were infected with both strains separately, and with a 50% mixture of both. In each group, three animals were sacrificed at 2, 4, and 8 hours, and 10 animals at 24 hours (were the CI was calculated), and the bacterial concentration in the spleen was determined. In order to characterize pmrA and pmrB mutations, genomic DNA from both strains was extracted, amplified with specific primers, and sequenced.

**Results:** Duplication time was 43 min for CS01 and 40.7 min for CR17. There were no in vitro growth significant differences between strains when grown separately, but CR17 growth was lower than CS01 when grown in competition (24 hours CI 0.097, 72 hours CI 0.008). CS01 was more virulent than CR17 in terms of mortality (see Table 1) and time to death. During in vivo growth, CS01 reached a maximal concentration in the spleen of 10 log CFU/g, whereas CR19 reached a maximal concentration of 9.17 log CFU/g. Growing in competition, CS01 decreased to 9.31 log CFU/g (0.69 log decrease), while CR17 decreased to 6.97 log CFU/g (2.2 log decrease). The in vivo CI was 0.016. In pmrA a mutation was identified: met12lys; no mutations were found in pmrB.

Mortality	8 log CFU/mL	7 log CFU/mL	6 log CFU/mL	5 log CFU/mL	4 log CFU/mL	3 log CFU/mL	2 log CFU/mL
CS01	100 %	100 %	100 %	100 %	100 %	20 %	0 %
CR17	100 %	100 %	100 %	100 %	10 %	0 %	0 %

**Conclusion:** The acquisition of COL resistance in *A. baumannii* clinical isolates after COL treatment, related to a PmrA mutation (met12lys), leads to an in vivo fitness loss and a decreased virulence.

#### P1083 Contribution of a different pattern of stimulation of the innate immune system in virulence of *Klebsiella pneumoniae*-producing carbapenemase

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**Objectives:** It is postulated that mortality of nosocomial infections by multidrug-resistant (MDR) *Klebsiella pneumoniae* is related to the lack of active antimicrobials. It was investigated if part of the effect of MDR may be related with the stimulation pattern of host immune responses.

**Methods:** Twenty blood isolates were studied: five isolates susceptible to antimicrobials, five ESBL-producing, five VIM-producing and five



KPC. Genetic diversity was defined by PFGE. Peripheral blood mononuclear cells of healthy volunteers were in vitro stimulated at a density of  $5 \times 10^6$  CFU/mL by live and heat-killed (HK) isolates for the production of pro-inflammatory cytokines in the presence of monosodium urate (MSU) that is a NLRP3-inflammasome agonist. Cytokines were measured by an enzyme immunoassay. Survival of 30 C57B6 male mice was recorded after intraperitoneal challenge with three susceptible and three KPC-producing isolates in five mice each.

**Results:** Respective mean release of TNF $\alpha$  after 1.5 hours stimulation with  $5 \times 10^3$  CFU/mL of live susceptible, ESBL-producing, VIM-producing and KPC isolates was 1492.1, 1144.3, 1459.5 and 1381.0 pg/mL; it became 4104.8, 4619.3, 4157.0 and 4791.8 pg/mL after 2.5 hours. Respective mean release of IL-1 $\beta$  after 1.5 hours stimulation with  $5 \times 10^3$  CFU/mL of live susceptible, ESBL-producing, VIM-producing and KPC isolates was 38.9, 35.3, 53.4 and 63.5 pg/mL; it became 499.9, 572.7, 517.8 and, 684.2 pg/mL after 2.5 hours ( $p = 0.017$  KPC vs. susceptible). Surprisingly, mean release of IL-1 $\beta$  after stimulation of PBMCs with HK-susceptible and HK-KPC isolates was 383.2 and 311.9 pg/mL respectively; it was changed to 1902.8 and 2150.2 pg/mL after addition of MSU denoting significant synergy at the NLRP3-stimulation level. Median survival of mice challenged by the susceptible isolates was 166 hours as opposed to 122 hours after challenge by KPC isolates (log-rank: 4.908,  $p = 0.027$ ).

**Conclusions:** Considerable differences are encountered in the stimulation of human PBMCs by susceptible *K. pneumoniae* and KPC probably related with the potential for NLRP3 stimulation. These differences may have an impact on therapeutics.

#### **PI084** Inverse relationship between pathogenicity and NDM-1 carriage in *Escherichia coli*

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**Objectives:** Microbial virulence potential and antibiotic resistance are major contributors promoting survival and colonisation of an infected host. It has been hypothesised that the evolution of these two factors may be intrinsically linked. Herein we investigate the virulence potential of Multi-drug-resistant (MDR) NDM-1-carrying *E. coli* (EC), and whether this may be directly or indirectly related to the expression and transfer of blaNDM-1 plasmids.

**Methods:** Isolates in this study were EC of four categories: (i) parental NDM-1 positive isolates ( $n = 13$ ), (ii) 12 year old ESBL-positive EC from India ( $n = 12$ ), (iii) Cardiff susceptible EC from UTI specimens ( $n = 20$ ), (iv) Transconjugants of 1) were obtained by transferring blaNDM-1 plasmids into EC J53, with confirmation by NDM-1 PCR (PrimerF-GAA GCT GAG CAC CGC ATT, PrimerR-TGC GGG CCG TAT GAG TGA TT). Virulence was assessed in *Galleria mellonella* larvae using inocula of  $1 \times 10^7$ ,  $10^6$ ,  $10^5$ ,  $10^4$  and  $10^3$  CFU/larva for each test isolate administered in  $10 \mu\text{L}$  aliquots through the rear-left proleg of the larvae hemocoel. Larvae were incubated at  $37^\circ\text{C}$  and survival rates were scored at 24 hour intervals until 72 hours, death accepted once there is no longer a response to touch.

**Results:** Statistical analysis of mean survival rates showed all test categories were significantly different, apart from NDM-1 and ESBL EC. The NDM-1 EC were least virulent, with larvae survival rates comparatively high and LD90s seen for only five isolates, at  $10^7$  and  $10^6$  CFU/larva. In comparison, 80% of susceptible EC had LD90s as low as  $10^4$  CFU/larva, with ESBL isolates slightly less virulent with LD90s of  $10^5$  CFU/larva. Of 13 blaNDM-1 positive EC, 12 gave positive transconjugants. Of four NDM-1 with reasonable virulence, the corresponding transconjugants gave similar virulence with LD90s much lower than the mainly avirulent J53 recipient. All other transconjugants had low virulence reflecting that of J53.

**Conclusions:** We have highlighted the increased virulence of J53 by introduction of NDM-1 plasmids, suggesting the recruitment of mobile virulence-determinants onto these MDR-gene constructs. Contradictory

however, overall our results show a definite trend where virulence is inversely proportional to resistance phenotype, highlighted by the survival rate hierarchy seen; Susceptible EC > ESBL EC > NDM-1 EC. This suggests divergent evolution, favouring either a strongly resistant, or highly-pathogenic existence, but not both.

## Emergence of bacterial resistance in humans, animals and the environment

### **PI085** First report of an OXA-23 carbapenemase-producing *Acinetobacter baumannii* strain in Spain

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**Background:** Resistance of *A. baumannii* isolates to carbapenems is on the rise, and it is mainly associated with a variety of combined mechanisms, among which, the production of carbapenem-hydrolyzing class D beta-lactamases (CHDLs) particularly OXA-23, OXA-24, and OXA-58 have been identified worldwide.

**Objective:** The aim of this study was to analyze the genetics of the blaOXA23 gene in an *A. baumannii* clinical isolate recovered in Spain.

**Methods:** We studied an *A. baumannii* clinical isolate recovered from the sputum of a 70-year-old male with chronic obstructive pulmonary disease, at the San Dureta hospital in Mallorca-Spain. The isolate was identified by MicroScan and confirmed by MALDI-TOF-MS. Antibiotic susceptibility was performed by MicroScan and interpreted according to CLSI guidelines. Multiplex PCR was performed to identify clonal lineages according to Turton et al. (CMI, 2007, 13: 807). Genes encoding CHDLs (OXA-23, OXA24, OXA-51 and OXA-58) and ISAb elements were detected by PCR and sequencing. Southern blot was performed by digestion with the S1 nuclease and hybridization with the blaOXA-23 probe. Genetic surrounding of the blaOXA-23 gene was obtained by inverse PCR mapping.

**Results:** The strain belonging to pan-European clone II showed resistance to amikacin (>256 mg/mL), ceftazidime (>128 g/mL), tetracycline, ciprofloxacin, gentamicin and netilmicin (>64 mg/mL), piperacillin (>512 mg/mL), imipenem and meropenem (16 mg/mL) and susceptibility to tigecycline and colistin (0.5 mg/mL). Multiplex PCR for CHDLs was only positive for blaOXA-51 and blaOXA-23. Southern blot clearly revealed that blaOXA-23 was located in the chromosome. Genetic surroundings showed two copies of ISAb1 in opposite directions of the blaOXA-23 gene, suggesting a composite transposon structure. An additional ATPase gene identical to that identified in Tn2006 was located downstream from the gene.

**Conclusion:** The presence of two ISAb1 sequences flanking the blaOXA-23 gene and constituting a Tn2006-like compound transposon might suggest their involvement in both blaOXA-23 expression and mobilization. While OXA-24 producing *A. baumannii* isolates are commonly found in Spain and Portugal, this is the first time that an OXA-23 carbapenemase producing *A. baumannii* isolate has been reported in Spain.

### **PI086** A case of New Delhi metallo-beta-lactamase 1 in the Netherlands with secondary transmission

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**Objectives:** Acquired carbapenemases such as New Delhi metallo-beta-lactamase 1 (NDM-1) are emerging resistance determinants in Gram-negative bacteria, leaving few or no therapeutic options. Here we describe a NDM-1 case in the Netherlands, imported from the Balkan area, and a secondary case in the same hospital.

**Materials and methods:** The study was performed as part of a retrospective analysis on a collection of 485 Enterobacteriaceae isolates, from patients in the east of the Netherlands. Antimicrobial susceptibility testing and species identification were performed by the VITEC 2. Carbapenems MIC values were determined by the E-test. Phenotypic ESBL confirmation was performed by a combination disc diffusion. For phenotypic confirmation of carbapenemase production, the modified Hodge test (MHT) and two inhibitor-based tests: ertapenem-boronic acid and imipenem-EDTA, were used. A microarray was used for genotypic characterisation of ESBL's and carbapenemases. Characterisation of NDM-gene and plasmid analysis, were done using PCR, sequencing and cloning. Genetic relatedness is tested by Amplified Fragment Length Polymorphism (AFLP). For genotyping the Multilocus sequence typing (MLST) was used.

**Results:** Two ESBL producing *K. pneumoniae* strains from two patients were selected for further analysis by their elevated MIC's to meropenem. One patient was transferred from a hospital in Belgrado, Bosnia, to hospital in the east of the Netherlands on the 27th of August 2008 and directly placed in a separate room in isolation because of MRSA carriage. The second patient, with no travel history outside the Netherlands, was admitted to the same hospital between the 10th of October and the 7th of November 2008. The results of antimicrobial susceptibility testing and phenotypic confirmations are shown in Table 1. Molecular gene analysis revealed NDM-1, CTX-M-15, SHV-12, TEM-1, and OXA-1. Molecular plasmid analysis revealed the presence of a 70 kb, Inc II plasmid containing the bla NDM-1, and a 140 kb plasmid in both isolates. AFLP showed that both strains were identical. MLST analysis showed that both isolates belong to ST15.

patient	strain	MIC (µg/ml)										phenotypic confirmation				
		amikacin	aztreonam	cefepime	ceftriaxone	colistin	ertapenem	imipenem	meropenem	netilmicin	teicoplanin	vancomycin	MHT	ERT/EDTA	CD	ERT/EDTA
index case	<i>K. pneumoniae</i> strain	≥ 64	≥ 32	≥ 16	≥ 16	≥ 4	0	0	0	0.25	1.5	yes	yes	yes	yes	yes
secondary case	<i>K. pneumoniae</i> strain	≥ 64	≥ 32	≥ 16	≥ 16	≥ 4	0	0	0	0.19	1.5	yes	yes	yes	yes	yes

MHT: modified Hodge test; CD: combined disc

**Conclusions:** This first encountered NDM-1 producing *K. pneumoniae* in the Netherlands underlines further that Balkan states constitute a secondary reservoir for NDM-1 producers. While cross transmission in Europe of NDM-1 producers has sporadically been reported, the index case was associated with a secondary case despite proactive measures aimed to control spread of resistant bacteria.

#### P1087 Retrospective summary report of integron study in a local hospital in southern China

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**Objectives:** To retrospectively summarize the occurrence and prevalence of resistance integron elements within clinical bacteria from the First Affiliated Hospital of Jinan University (FAHJU) in Guangzhou, China.

**Methodology:** During 1998 to 2006, integron screening had been conducted on a total of 583 clinical isolates, and data of this summary study were partially obtained from some of our PhD dissertations and local reports.

**Results:** Class 1 integrons were found in 73.6% (243/330) of the gram-negative strains, ranging from 85% to 90% for *Escherichia coli* (89.3%, 109/122), *Klebsiella pneumoniae* (87.5%, 28/32), *Acinetobacter* spp. (91.3%, 21/23), *Enterobacter cloacae* (86.7%, 13/15) and other organisms (90%, 18/20), except *Pseudomonas aeruginosa* (45.8%, 54/118). In gram-positive bacteria, the detection rate was 49.0% (124/253), with 42.5% (76/179) in *Staphylococcus aureus*, 56.6% (30/53) for coagulase-negative staphylococci, 84.6% (11/13) for *Enterococcus faecalis*, 100% (2/2) for *Enterococcus faecium* and 83.3% (5/6) for *Streptococcus* spp., summing up the total class 1 integron prevalence as 63.0% (367/583). The most frequently detected resistance genes were

*aadA* (88.3%, 324/367) and *dfrA* (74.9%, 275/367) family, with the identification rate of cassette arrays found to be 54.5% (200/367) for *dfrA12-orfF-aadA2*, 18.8% (69/367) for *dfrA17-aadA5* and 16.1% (59/367) for *aadA2*, respectively. Class 2 integron had been occasionally observed, taking up 5.7% (33/583) of all isolates, including 23 *P. aeruginosa*, 6 *E. coli*, 2 *E. faecalis*, 1 *Proteus vulgaris* and 1 *Proteus mirabilis* strains, and cassettes arrays were detected to be *dfrA1-sat1-aadA1*. None of the tested strains was positive for class 3 integron. Nevertheless, one *S. aureus* strain isolated from sputum in 2003 was found to be positive for *intI3*, while the variable region and both flanks remained unknown.

**Conclusions:** The commonly carriage of integron elements may confer multi-resistance for clinical organisms and further contribute to the unleashing waves of "Super Bugs".

#### P1088 Macrolide and tetracycline resistance in *Moraxella catarrhalis* isolates from 2009 to 2011 is higher in the Asia Pacific Region than in other regions of the world

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**Objective:** To evaluate the activity of macrolides and other agents against *Moraxella catarrhalis* by geographic region for isolates from 2009 to 2011. *M. catarrhalis* are generally susceptible to most agents except penicillins due to the production of beta-lactamases. Resistance (R) to tetracycline (TET), cephalosporins, and macrolides is generally <1% in most regions of the world.

**Methods:** Susceptibility (S) testing for *M. catarrhalis* was performed by Clinical and Laboratory Standards Institute (CLSI) broth microdilution methodology on isolates from 2009 to 2011 from medical centers in the SENTRY Antimicrobial Surveillance Program platform in the European Union (EU), United States (USA), Latin America (LA), and Asia-Pacific region (APAC). S interpretations were performed using CLSI guidelines (Clarithromycin [CLR], S ≤ 2 mg/L; TET, S ≤ 2 mg/L; trimethoprim/sulfamethoxazole [TMP/SXT], S ≤ 0.5 mg/L).

**Results:** In the EU, there were more than 400 isolates and none were R to CLR or TET. In the USA there was 1 (0.1%) isolate R to CLR and 1 (0.1%) R to TET. In LA, no isolate was CLR- or TET- (0/70) R. However in the APAC region 13 of 172 (7.6%) isolates were CLR-R and eight of 251 (3.2%) were TET-R. Twelve of 13 of the CLR-R and seven of eight of the TET-R isolates were from China. 5.8% of the isolates in APAC had telithromycin MIC values ≥ 2 mg/L (isolates were from China and Korea). TMP/SXT-R ranged from 5.1% in the US and EU to 7.0% and 7.1% in APAC and LA, respectively. Beta-lactamase production ranged from 97.1% (EU) to 100.0% (LA). 10.4% (APAC) to 15.7% (LA) of the isolates had cefepime MIC values at 2 mg/L. In APAC 2.8% of MIC values for ciprofloxacin were ≥ 0.5 mg/L.

**Conclusions:** Macrolide and tetracycline R in *M. catarrhalis* for isolates from the period 2009–2011 was ≤ 0.2% in NA and EU. In the APAC region CLR-R and TET-R were significantly elevated (3.2–7.6% R). The higher rate in the APAC region was primarily due to isolates from multiple sites in China. TMP/SXT-R occurred in all regions ranging from 5.1% to 7.1%.

#### P1089 Report of linezolid resistance from the Zyvox® Annual Appraisal of Potency and Spectrum programme (Europe, Latin America, Asia Pacific)

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**Objectives:** To monitor the in vitro activity and to detect resistance (R) to linezolid (LZD) in various geographic areas of the world, excluding the United States (USA), the Zyvox® Annual Appraisal of Potency and Spectrum Program (ZAAPS) surveillance program was established in 2002. LZD, the first oxazolidinone agent clinically applied, is an important therapeutic option for infections caused by antimicrobial-R Gram-positive (GP) pathogens. Although rare, LZD-R has been observed among coagulase-negative staphylococci (CoNS) in more

frequency than enterococci (ENT). R rates remain extremely low for indicated *S. aureus* (SA) and streptococci.

**Methods:** Five thousand seven hundred and sixty-nine isolates were collected from 57 sites in 34 countries in 2011. Isolates were received from the following organism groups (n): SA (2831), CoNS (656), ENT (747), *Streptococcus pneumoniae* (SPN; 878), viridians group streptococci (VGS; 244) and beta-haemolytic streptococci (BHS; 413). At least 200 isolates from each country (except China [n = 800]; the United Kingdom and Japan [n = 400]) were requested to be sent to a reference laboratory. CLSI broth microdilution susceptibility (S) testing was performed using TREK Diagnostic (Cleveland, OH, USA) panels. LZD-R isolates were confirmed with frozen broth microdilution, Etest (BioMerieux, Marcy l'Etoile, France) and disk diffusion methods. PCR and sequencing were performed to detect mutations in 23S rRNA, L3, L4, and L22 proteins, and acquired genes (cfr).

**Results:** Overall LZD-S in the ZAAPS study was >99.8% with only nine isolates identified as non-susceptible (NS). LZD-S by organism group: SA 100.0%; CoNS 98.9%; ENT 99.7%; all streptococci had LZD MIC values of  $\leq 2$  mg/L (S). The cfr gene was identified in three CoNS strains with LZD MIC values of 4 mg/L (Mexico and France), also in a *S. epidermidis* from Spain with a LZD MIC of 8 mg/L and a *S. epidermidis* from France had both a G2576T mutation and cfr gene with a LZD MIC of >128 mg/L (three isolates with cfr in 2010 ZAAPS study). MRSA rate was 33.4% overall (27.7% Europe [EU]; 42.4% Latin America [LA]; 42.2% Asia Pacific [AP]). VRE rates were 9.2% in EU, 8.9% in LA and 1.1% in AP. SPN had overall penicillin and erythromycin R rates of 24.7% (MIC  $\geq 2$  mg/L) and 45.0%, respectively.

Table: Linezolid-R isolates found in the 2011 ZAAPS Program.

Species	City/Country	LZD MIC (mg/L)	R-mechanism
<i>E. faecium</i>	Leipzig/Germany	8	G2576T
<i>E. faecium</i>	Dublin/Ireland	8	G2576T
<i>S. capitis</i>	Athens/Greece	16	G2576T
<i>S. epidermidis</i>	Sao Paulo/Brazil	8	G2576T
<i>S. epidermidis</i>	Sao Paulo/Brazil	8	G2576T
<i>S. epidermidis</i>	Sao Paulo/Brazil	16	G2576T
<i>S. epidermidis</i>	Madrid/Spain	8	cfr
<i>S. epidermidis</i>	Genova/Italy	16	G2576T
<i>S. epidermidis</i>	Lille Cedex/France	>128	G2576T and cfr

a. 3 CoNS had a cfr associated with LZD MIC at 4mg/L.

**Conclusions:** LZD-R remains low at <1% among contemporary pathogens from surveyed organism groups. Continued worldwide monitoring of in vitro LZD activity appears warranted as the species containing the cfr gene continue to evolve.

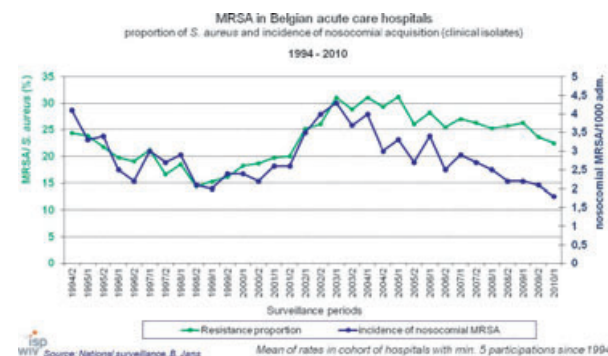
#### P1090 Fifty per cent reduction of MRSA incidence in Belgian acute care hospitals: yes we can!

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**Background:** Already in 1945, Sir Alexander Fleming warned scientists about the risk for antimicrobial resistance. Methicillin resistant *Staphylococcus aureus* (MRSA), first reported half a century ago, terrorises acute care hospitals in most European countries and abroad. In the early nineties, a Belgian policy for MRSA management in acute care hospitals was worked out including guidelines, microbiological and epidemiological surveillance, and improvement of identification techniques in the lab.

**Methodology:** Since 1994, the Scientific Institute of Public Health and the National MRSA Reference laboratory set up an epidemiological surveillance in acute care hospitals, collecting semi-annual MRSA data, aggregated at hospital level. Hospitals participated on voluntary basis until 2006, when participation became mandatory. Semestrial resistance rates (MRSA/*S. aureus*) and incidence of nosocomial MRSA (>48 hours after admission) were calculated on data excluding duplicates (patients counted only once). Semi-annual national and local feedback reports were sent to all hospitals. Surveillance data collected during 32 observation periods (1994–2010) are discussed here.

**Results:** Between 1994 and 1998, the resistance rate decreased from 24.4% to 14.4% and the incidence of nosocomial MRSA from 4.1 to 1.1 cases/1000 admissions. Afterwards, the resistance proportion and nosocomial incidence increased dramatically and doubled in a 5-year time span, reaching respectively 31.1% and 4.3 cases/1000 admissions. The year 2003 seemed to be a turning point. The resistance rate remained stable until 2005 and then slowly decreased (–8.7%) attaining 22.5% in 2010. In the same time span the incidence of nosocomial MRSA dropped from 4.3 to 1.8 cases/1000 admissions. Since 2006 all acute care hospitals perform MRSA screening of patients at risk or transferred from other healthcare facilities.



**Discussion:** Belgian hospitals successfully incurred the dramatic evolution. Since 2003, nationwide actions for the control of MRSA in Belgian healthcare facilities focus on five action lines: revision of guidelines for MRSA management in acute care hospitals, establishment of an antibiotic policy committee in each hospital, investigation of MRSA carriage in nursing homes (NH) resulting in specific NH MRSA guidelines, promotion of screening at admission and during hospital stay and the implementation of a successful Belgian hand hygiene program with four consecutive campaigns in healthcare settings.

#### P1091 In vitro doxycycline selection of *Bacillus thuringiensis*, *Bacillus cereus* and *Bacillus anthracis* Sterne with a one-step method

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*Bacillus anthracis* is a potential bio-threat agent. According to the recommendations of European Medicine Agency, doxycycline is an alternative therapy after ciprofloxacin. The ability of this pathogenic agent to develop quinolones and doxycycline resistance is a major concern.

**Objectives:** The first aim of this study was to determine the ability of *Bacillus cereus* related species, *B. thuringiensis*, *B. cereus* and *B. anthracis* to express resistance mechanisms to cyclines with a one step method using antibiotic selection pressure. The second aim was to test tige cycline in doxycycline resistant derivative strains.

**Methods:** *B. cereus* ATCC 7704, *B. thuringiensis* ATCC 36404, *B. anthracis* Sterne, and two clinical isolates of *B. cereus* and *B. thuringiensis* were used in this study. *B. anthracis* Sterne was handled in biosafety level 3 laboratory. Current cyclines resistance genes, tetL, tetK, tetM, tetO and tetS with specific PCR were screened before selection. Inoculation process was performed in triplicate with Mueller-Hinton agar containing increased doxycycline concentrations, using Whatman paper strips with different doxycycline concentrations (0.125–256 mg/L). After inoculation with a strong inoculum ( $10^8$ – $10^{10}$  CFU/mL), mutants were screened and mutational rate was estimated. MICs with and without efflux pump inhibitors were determined with agar dilution method.

**Results:** Screening of tet genes was negative for all wild-type strains. In vitro selection of *B. anthracis* Sterne was not possible with the

method used. We obtained doxycycline resistant mutants of *B. cereus* and *B. thuringiensis*. The two respective obtained mutants were resistant to doxycycline, tetracycline and minocycline but only *B. cereus* derivative resistant strain exhibited resistance to tigecycline. Efflux inhibitors were only effective in *B. thuringiensis*.

**Conclusions:** Opposite to *B. thuringiensis* and *B. cereus*, the selection of stable *B. anthracis* Sterne mutants resistant to doxycycline after in vitro antibiotic selection was not possible with our technique, confirming previous data with Szybalski method. These data have a clinical significance, since a course of doxycycline during 60 days is one of the recommended therapy/prophylaxis for inhalation anthrax related to intentional release. Mechanisms involved in the differences in mutational rate among *Bacillus cereus* related species need to be further investigated.

**P1092** Antimicrobial susceptibility and species identification of *Corynebacterium* spp. strains collected in Europe and USA medical centres, 2006–2010

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**Objectives:** To evaluate the antimicrobial susceptibility of *Corynebacterium* spp. clinical strains collected from European and USA medical centers. The accuracy of species identifications performed at local labs was also assessed.

**Methods:** One hundred and twenty-one *Corynebacterium* strains were collected from serious infections between 2006 and 2010 as part of the SENTRY Antimicrobial Surveillance Program from 15 and 18 medical centers in nine European countries and USA, respectively. The strains were susceptibility (S) tested by the CLSI broth microdilution method in cation-adjusted Mueller–Hinton broth against numerous antimicrobial agents. MIC results were interpreted according to CLSI M45 breakpoint criteria. There are yet no criteria from EUCAST. Species identifications were performed by MALDI-TOF methodology using the Bruker Biotyper system.

**Results:** Isolates were from bacteremia (53.6%), skin and skin structure (17.3%), pneumonia (12.7%), among other infection sites. Vancomycin (100.0% S) and daptomycin (99.1% S) showed potent activity against *Corynebacterium* with MIC<sub>50/90</sub> of 0.25/0.5 and ≤0.06/0.25 mg/L, respectively. *C. jeikeium* and *C. urealyticum* species groups demonstrated lower S rates, 0.0% for penicillin and 36.8–40.0% for gentamicin, compared to other *Corynebacterium* species groups (see Table 1). Sixty-one of 121 strains (50.4%) were identified to species level by the clinical laboratories and species identification was confirmed by the MALDI-TOF analysis on 47 (77.0%) of those strains. The remaining 14 strains had incorrect species (12) or genus (two strains, *Brevibacterium* and *Microbacterium*). Among the 60 strains submitted as unspiculated *Corynebacterium*, 51 (85.0%) strains were confirmed as *Corynebacterium* and identified to species level, while the remaining nine strains were identified to a different genus (*Brevibacterium* [3], *Microbacterium* [2], *Dermabacter* [1], *Rhodococcus* [1], *Staphylococcus* [1], *Truicella* [1]) by the MALDI-TOF analysis.

Organism/species (no. tested)	MIC <sub>50</sub> (mg/L) / % susceptible				
	DAP	ERY	GENT	PEN	VANC
All <i>Corynebacterium</i> spp. (110)	≤0.06/99.1	>2/24.6	≤2/80.9	1/51.8	0.25/100.0
<i>C. amycolatum</i> (33)	≤0.06/100.0	>2/27.3	≤2/84.9	1/63.3	0.5/100.0
<i>C. jeikeium</i> (19)	0.12/100.0	2/10.5	>8/36.8	>4/0.0	0.5/100.0
<i>C. striatum</i> (37)	≤0.06/99.3	>2/24.3	≤2/97.3	1/56.8	0.25/100.0
<i>C. urealyticum</i> (5)	≤0.06/100.0	>2/40.0	>8/40.0	>4/0.0	0.5/100.0
Other <i>Corynebacterium</i> spp. (16)	≤0.06/100.0	2/31.3	≤2/100.0	0.25/93.8	0.25/100.0

DAP = daptomycin, ERY = erythromycin, GENT = gentamicin, PEN = penicillin, VAN = vancomycin

**Conclusions:** Antimicrobial resistance differences among *Corynebacterium* species were noted with higher rates among the *C. jeikeium* and *C. urealyticum* species groups. Identification to the species level in clinical laboratories for *Corynebacterium* remains a challenge; however, the MALDI-TOF method appears to generate more complete and accurate identifications to be compared to antimicrobial-S profiles.

**P1093** Surveillance of non-O157 STEC isolates in the Netherlands, 2007–2011

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**Introduction:** In the Netherlands, an intensified surveillance of STEC O157 has existed since 1999. In 2007, the scope of this surveillance was widened to include non-O157 STEC isolates.

**Methods:** From 2007, laboratories were encouraged to use a real time PCR targeting shigatoxin (stx) genes 1 and 2 and submit up to five cultured isolates (sorbitol positive or negative) from a PCR positive faecal sample in an effort to obtain the isolate responsible for the positive signal. These isolates were tested for the presence of shigatoxin and other virulence genes and when found positive, were serotyped. Over the years, more laboratories included a PCR in their routine diagnostic workup. To limit the workload and to increase the recovery rate, in 2011 laboratories were asked only to submit isolates from faeces samples with a high DNA load (Ct ≤ 35).

**Results:** During 4 years (2007–2010) of STEC surveillance, this strategy resulted in submission of 3536 isolates from 703 different patients. Only for 193 patients (27.5%), an isolate could be found harbouring either the stx-1 or stx-2 gene. The preliminary results from 2011 indicate a significantly higher success rate (47.5%) for finding a shigatoxin gene positive isolate. In only 12 of these patients (6.2%), the isolate found was O157, but O157 is also submitted based on sorbitol negativity on SMAC, so this does not reflect the prevalence of O157 in the general population. Most frequently isolated serotypes are O63 (11.4%), O26 (10.9%) and O91 (7.3%). Remarkably, shigatoxin subtype 2f, which was added as a target for PCR in 2008, was found in 21.2% of all positive isolates.

**Discussion:** The increased success rate of isolating an STEC isolate in 2011 is most likely caused by a change in the submission criteria. Even though the number of O157 isolates found using this strategy does not represent prevalence in the general population, it is clear that diagnostic methods should also include non-O157 STEC. However, the clinical and public health relevance is not well-established for many of the non-O157 STEC serogroups. A new insight that this surveillance strategy has provided is the high occurrence of serotype O63, which carry subtype stx2f, and other STEC harbouring stx2f in the Netherlands. This shigatoxin subtype has also been described to be emerging in Germany but only occasionally in other countries.

**P1094** Occurrence of extended-spectrum beta-lactamases in *Shigella sonnei* and *S. flexneri* isolated after 2007 in Korea

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**Objectives:** A nationwide survey was carried out in the Republic of Korea to know the prevalent type of extended-spectrum beta-lactamases (ESBLs) those produced by *S. sonnei* and *S. flexneri*

**Methods:** From 2007 to 2010, 507 clinical strains were isolated and screened for resistance to extended-spectrum cephalosporins based on the synergistic effect between clavulanate and selected beta-lactams (ceftazidime and cefotaxime) by the Clinical and Laboratory Standards Institute (CLSI) criteria. Classification of beta-lactamases was performed using multiplex PCR. Sequence analysis of the beta-lactamases including ESBL encoding genes was carried out.

**Results:** Twenty-six *S. sonnei* and two *S. flexneri* isolates were found to be ESBL positive by their phenotype. Twenty-four strains including two *S. flexneri* isolates, found to possess CTX-M ESBLs. Among these, 12 *S. sonnei* strains also contained TEM beta-lactamases. All 26 isolates found to possess CMY beta-lactamases by multiplex PCR. Nucleotide sequence analysis revealed that they harbored blaCTX-M-15 (13 *S. sonnei* and 2 *S. flexneri* strains), blaCTX-M-14 (10 *S. sonnei* strains), blaCTX-M-27 (10 *S. sonnei* strains), and blaTEM-1 (12 *S. sonnei* strains). In one strain, SS084469, we found two new TEM beta-

lactamses, did not reported so far. One contained a substitution (Phe55Leu), and the other had two substitution (Ser253Pro and His255Arg) in the blaTEM.

**Conclusion:** From these results, We found that, after 2007, the common type of ESBL in *S. sonnei* in Korea, changed from TEM type to CTX-M type. The predominance of CTX-M types in *S. sonnei* appears to be similar to that seen in other Enterobacteriaceae. This is the first report of the finding of two new TEM type beta-lactamases.

**P1095 Systemic and extraintestinal salmonellosis in Bulgaria: review of clinical cases, resistance to antimicrobial agents and PFGE typing of human non-typhoid *Salmonella* isolates**

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The self-limiting gastroenteritis is the most common form of human salmonellosis, but sometimes systemic generalization occurs and diverse extraintestinal foci appear as a consequence of the infection's spread. Multidrug-resistant isolates enhance the risk of a fatal outcome, because of the restricted choices for antimicrobial treatment.

**Objectives:** To review the clinical cases of systemic or extraintestinal salmonellosis diagnosed between 2005 and 2010 in Bulgaria, to screen for antimicrobial resistance and to analyze PFGE profiles of non-typhoid salmonellae.

**Methods:** Conventional methods for identification and serotyping of salmonellae. Resistance to 12 antimicrobial agents was studied with disc diffusion method. Screening for ESBLs was performed using the double-disc synergy method. PFGE typing was applied for comparative investigation of *S. corvallis* isolates.

**Results:** Between 2005 and 2010 2227 human non-typhoid *Salmonella* isolates have been investigated at the Reference Laboratory of Enteric Pathogens, NCIPD. Thirty-three strains (1.48%) from nine national regions were isolated from patients with systemic and extraintestinal forms of salmonellosis. The serotype distribution was: Enteritidis (n = 21), Choleraesuis (diphasic n = 3, monophasic n = 3), Typhimurium (n = 2), Corvallis (n = 2), Montevideo (n = 1) and Javiana (n = 1). Eight patients have developed severe forms of systemic infections: sepsis (n = 2), septic shock (n = 1 with fatal outcome), meningitis (n = 3), acute renal failure (n = 2). Diverse complications caused by non-typhoid salmonellae were diagnosed in surgical and haematological wards due to underlying diseases or to compromised immunological status. Twenty-two percent of isolates were resistant to ampicillin and gentamicin, 17, 64% – to tetracycline, 14, 28% – to nalidixic acid and 10% – to chloramphenicol. All but one *S. montevideo* were susceptible to ciprofloxacin. One *S. corvallis* recovered from a patient with chronic chemolytic anaemia produced an ESBL of CTX-M type and its PFGE profile demonstrated <96% similarity to fecal and wound *S. corvallis* isolates with susceptible phenotypes.

**Conclusions:** *S. enteritidis* was the most common cause of systemic and extraintestinal forms of human salmonellosis in Bulgaria. Resistance to ampicillin and gentamicin were the predominant profiles, one *S. corvallis* produced an ESBL and one *S. montevideo* was resistant to ciprofloxacin. The ESBL-producing *S. corvallis* clustered separately from the susceptible *S. corvallis* isolates.

**P1096 Comparative analysis of shiga toxin-producing *Escherichia coli* isolates from humans and animals in Peru**

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**Objectives:** The objectives of this study were to compare the distribution of virulence genes, phylogeny, antimicrobial susceptibility and the mechanisms of antimicrobial resistance in Shiga toxin-producing *Escherichia coli* (STEC) strains isolated from animals (cattle) and humans in Peru.

**Methods:** We analysed 29 STEC strains isolated from stool samples of children (<36 months of age) with and without diarrhoea from four previous cohort studies, and 12 STEC strains isolated from farm animals (cattle) all recovered in Lima, Peru. STEC was identified by a multiplex real-time PCR for stx1 and/or stx2 genes. The strains were analysed to identify the presence of intimin (eae), alpha-hemolysin (hlyA) and enteroaggregative *E. coli* enterotoxin 1 (EAST1) genes. The phylogenetic group was determined by Clermont's method. The susceptibility to twelve antimicrobial agents was tested by disk diffusion, and mechanisms of resistance to ampicillin, to trimethoprim-sulfamethoxazole (Sxt) and to tetracycline were searched by PCR.

**Results:** Among all STEC isolates, stx1 was the most common toxin-gene (37/41, 90%), followed by stx2 (3/41, 7%) and stx1/stx2 (1/41, 2%). Also, eae gen (31/41, 76%) was frequently identified. Meanwhile hlyA was identified more frequently in animal than human strains (100% vs. 66%, p < 0.05); also EAST1 tended to be more frequent (42% vs. 21%). Strains mainly belong to B1-group (66% vs. 75%) and A-group (17 vs. 17%). Animal isolates tended to be more multiresistant than human isolates (33% vs. 27%). STEC isolates from animals were more resistant than those of humans isolates to Amp (58% vs. 17%, p < 0.05), to Sxt (34% vs. 17%, p < 0.01) and to amoxicillin-clavulanic acid (9% vs. 0%, p < 0.05). Animal isolates also tended to be more resistant than human isolates to Tet (42% vs. 17%), nalidixic acid (33% vs. 13%), nitrofurantoin (25% vs. 0%), and gentamicin (17% vs. 13%). Among all isolates, antibiotic resistance was mainly related to the presence of tem-like beta-lactamases (9/11, 82%) for Amp, while dfrA1, dfrA5 and dfrA7 genes account for the 50% of the Sxt resistance. Finally tetA (3/5, 60%) and tetB genes (2/4, 50%) were related to the Tet resistance in animal and human strains respectively.

**Conclusion:** Similarity of virulence profiles between STEC strains from human and animals and the higher resistance levels in animal strains suggest both a role of food chain in transmission to humans and the high use of antimicrobials in veterinary practice in the area.

**P1097 Prevalence and characterisation of extended-spectrum beta-lactamases in human and bovine isolates of *Escherichia coli* from Oyo state, Nigeria**

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**Objectives:** The advent of extended spectrum beta-lactamases' (ESBL) producers has posed a great threat to the beta-lactam antibiotics. The aim of this study was to determine the prevalence of ESBL producing human and bovine *Escherichia coli* isolates collected in Oyo state, Nigeria, characterize their resistance determinants, and assess their transfer.

**Methods:** Human isolates (n = 57) from two tertiary and secondary hospitals and bovine isolates (n = 57) were collected during August 2010–2011. Antimicrobial susceptibility was done by the Kirby-Bauer method. ESBLs were screened by the double disc synergy test. Positive ESBL strains were identified by PCR, and analyzed by sequencing. Plasmids were identified by PCR based replicon typing. The genetic relatedness of the isolates was determined by a rep-PCR genomic fingerprinting method (BOX-PCR). Conjugation was used to assess the transfer of the ESBL phenotype (cefotaxime resistance), using *E. coli* J503 (azide resistant) as a recipient cell.

**Results:** Eight (14%) human isolates were found to produce ESBLs. They were all resistant to cefotaxime, while only two showed reduced susceptibility to ceftazidime. No ESBL producer was identified among bovine strains. A high prevalence of resistance to amoxicillin (88%), trimethoprim (83%) and nalidixic acid (47%) among the human strains was remarkable compared with the bovine strains. All ESBL positive strains had AmpC, blaTEM, blaCTX-M genes. CTX-M-1 group enzymes were identified. ESBL positive strains were also resistant to gentamycin (87.5%), nalidixic acid (62.5%) and ciprofloxacin (50%). BOX-PCR identified five patterns among the ESBL positive isolates. All ESBL positive isolates had multiple plasmids belonging to Inc

FrepB, FIA, FIB, H12 and K plasmid groups. ESBL resistance was demonstrated to be conjugative in two strains.

**Conclusion:** Our findings showed a moderate prevalence of ESBL in clinical human strains, and not a major prevalent clone. However, ESBL were associated with conjugative plasmids. In contrast with other reported Nigerian studies, the healthy animals did not show considerable resistance, which may be associated with the geographic location. Nevertheless, this study highlights the need to adopt measures to monitor the dissemination of ESBLs in Nigeria. The detailed and reliable knowledge on the resistance of human and bovine pathogens will be required for a controlled and safe use of antibiotics in human and veterinary medicine.

**P1098** Analysis of trends in *P. aeruginosa* antibiotic resistance of animal and human isolates: a retrospective observational study in central Italy

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**Objectives:** *Pseudomonas aeruginosa* (PA) is a clinically significant pathogen responsible of nosocomial and community infections both in human and veterinary medicine. The high level of acquired resistance as well as the horizontal and clonal spread of resistant PA strains has become a serious problem, representing a limit for adequate antibiotic therapy and resulting in a serious threat to Public Health. A retrospective observational study was carried out to assess the antibiotic resistance trends in PA strains of animal and human origin from 2006 to 2011.

**Methods:** A total of 384 isolates of PA were obtained from different clinical specimens from animals (n = 298) and humans (n = 86) living in central Italy. The isolates were identified by PCR and were tested against 12 different antibiotics usually active against PA by disk diffusion method (Table 1). The significance of differences in resistance was evaluated using chi squared test (STATA software version 9.1), and p value <0.05 was considered statistically significant.

**Results:** The microorganisms were cultured from ear (43%), tonsillar swabs and bronchial alveolar washes (19%), naso-pharyngeal swabs (17%), wound swabs (13%), high vaginal swabs and urine samples (8%).

During 6-year period, the development of PA resistance to antibiotics is given in Table 1. There was evidence of increased resistance to gentamicin (p = 0.0431), fluoroquinolones (p = 0.0167), cephalosporins, and carbapenems in human isolates. At the same time, the resistance of PA to amikacin decreased from 6.4% in 2006 to 3.2% in 2011. Similar trends were observed for PA of animal origin: a significant increased resistance was observed for tetracyclines (p = 0.0483), and penicillins (p = 0.0325). Different trends were obtained for amikacin, cefquinome and carbapenems for which PA animal strains demonstrated a decreased, but not significant, resistance (p > 0.05).

Table 1. Resistance (%) of *Pseudomonas aeruginosa* to antipseudomonal antibiotics in veterinary and human isolates.

Antibiotics	Disk potency (µg)	2006		2007		2008		2009		2010		2011	
		Animal n=38	Human n=8	Animal n=41	Human n=12	Animal n=46	Human n=13	Animal n=49	Human n=11	Animal n=23	Human n=7	Animal n=18	Human n=8
Tetracycline	30	22.3*	12.6	21.6	12.7	29.4	10.8	33.8	8.8	41.5	11.9	42.0*	17.8
Chloramphenicol	30	14.3	6.4	14.2	4.9	13.5	4.2	11.6	3.9	10.2	3.8	9.6	3.2
Amikacin	10	23.2	14.3*	23.6	20.1	24.6	33.1	24.1	25.5	29.3	31.2	29.0	32.3*
Tobramycin	10	12.0	3.7	13.6	2.9	13.9	4.0	15.8	7.9	15.9	6.7	16.4	7.2
Trimethoprim-Co-Amoxicillin 2:1	30	17.9*	12.6	23.4	18.4	27.9	18.1	33.2	13.6	35.7	14.7	39.6*	14.1
Cephalosporin	30	42.2	34.7	43.2	29.7	46.7	33.8	49.6	43.1	48.0	37.5	48.9	43.4
Cefepime	30	14.3	-	14.1	-	14.6	-	13.9	-	12.7	-	12.3	-
Carbapenem	10	3.2	24.0	3.7	20.8	3.3	19.5	4.0	16.7	3.3	26.4	3.1	27.3
Imipenem	10	2.0	26.2	2.4	22.8	2.1	37.6	2.2	32.4	2.0	35.0	2.0	35.8
Colistin	5	15.8	18.5*	14.0	19.6	15.7	25.9	16.0	32.3	16.9	35.6	17.7	39.4*
Neofloxacin	10	10.4	7.9*	12.5	7.7	12.2	8.5	13.7	11.8	13.9	13.7	14.1	19.9*
Sulfamonomethoxazole	1.25/23.75	31.5	43.3	30.0	32.9	30.6	42.5	31.4	54.1	31.9	53.5	32.5	53.4

\* Significant differences P<0.05

**Conclusion:** This study confirms that resistance profile of PA is changing in central Italy, acquiring gradually less antibiotic susceptibility to molecules used both in veterinary and human

medicine. Considering the potential role of PA as zoonotic microorganism, due care must be taken in the antibiotic treatment to prevent the increase of the spread of multidrug resistant zoonotic PA. This study confirms that the widespread antibiotic misuse could be the main risk factor for the occurrence of multiresistant PA strains.

**P1099** Antimicrobial susceptibility monitoring of non-typhoidal *Salmonella* from healthy pigs and poultry in Belgium, 2008–2011

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**Objectives:** For severe salmonellosis in man, fluoroquinolones (FQs) and 3rd generation cephalosporins (CPs) are the drugs of choice. From 2008 to 2011, a susceptibility survey with focus on FQs and CPs was conducted among Belgian *Salmonella* (Sal) strains from healthy pigs and chickens, the major sources of human food-borne Sal infections. Both epidemiological cut-off values (ECVs) and clinical breakpoints (CBPs) were applied to categorize antimicrobial susceptibility.

**Methods:** Non-repetitive faecal sampling was conducted at various farms and abattoirs across Flanders. Susceptibility to ciprofloxacin (CIP), cefotaxime (CTX), and seven older molecules was assessed by agar dilution (CLSI; M31-A3). Clinical resistance (CR) was based on CLSI breakpoints (M100-S21); decreased susceptibility (DS; % isolates with MICs > ECV and <CBPs) was determined for four antibiotics based on ECVs as defined by EUCAST and EFSA (2007).

**Results:** In all, 348 and 419 strains from pigs and chickens comprising various distinct serovars were recovered. Following serovars were mainly identified: Typhimurium (124), Typhimurium var. Copenhagen (88) and Derby (35) from pigs and Enteritidis (79), Paratyphi (60) and Typhimurium (32) from chickens. CR to CIP was absent in isolates from both host species, but mean DS of the pig and poultry isolates was 3.2% and 22.0%. *S. Hadar*, *S. Paratyphi* B, *S. Blockley* and *S. Virchow* were the most frequent serovars with DS to CIP. CTX resistance was 1.4% and 12.2%, DS to CTX was 0.9% and 1.9% in pig and poultry isolates. In pigs, CR or DS to CTX was mainly observed in *S. Typhimurium*; in chickens in nine different serovars, most frequently in *S. Paratyphi* and *S. Virchow*. Virtually all CTX-resistant strains were phenotypically determined as ESBL producers (47). For the older drugs CR percentages for respective pig and chicken isolates were: ampicillin (A) 63, 34; chloramphenicol 19, 3; nalidixic acid 2, 22; streptomycin 53, 24; tetracycline 58, 15; and trimethoprim/sulfadiazine 39, 27. Gentamicin (G) CR was low (0–1%) in isolates from either animal species. DS for A and G was negligible (0–1%).

**Conclusions:** Clinical resistance among Sal from pigs and chickens was absent for CIP and low for CTX, two essential drugs for treating salmonellosis in humans. Decreased susceptibility to CIP and CTX varied from 1% to 22%. For most older drugs, notably higher rates of CR were detected, with marked differences between the two animal species.

**P1100** Antimicrobial susceptibility of zoonotic *Salmonella enterica* from cattle, pigs and poultry isolated in eight countries over a 4-year period (2002–2006) (EASSA programme)

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**Background:** The European Antimicrobial Susceptibility Surveillance in Animals (EASSA) is the first ongoing program monitoring antimicrobial susceptibility relevant for human medicine of zoonotic and commensal bacteria from healthy food-producing animals at slaughter across Europe. Susceptibility results of *S. enterica* (Se) isolated from 2002 to 2006 are presented here.

**Methods:** Colon or caecal content from healthy beef cattle, fattening pigs and broiler chickens was randomly sampled in eight EU countries (five countries/host; four slaughterhouses/country; one sample per herd/

flock). *Salmonella* isolation, identification and serotyping were performed by standard methods. MICs of 11 antimicrobials were determined by agar dilution (CLSI, M31-A3) in a central laboratory. Results were interpreted using clinical breakpoints (CLSI M100-S20, except colistin: EUCAST) and Epidemiological Cut-off Values (ECVs) as defined by EFSA (2007). Decreased Susceptibility (DS), i.e. MIC values exceeding the wild-type MIC distribution (>ECV) but not the clinical breakpoint, was determined for four antimicrobials.

**Results:** In total, 659 *Se* strains (48 different serotypes) were identified: 57 from cattle, 420 from pigs and 182 from chickens. Following serotypes were predominantly recovered: Typhimurium (18), Dublin (12), Enteritidis (9) and Montevideo (7) from cattle; Typhimurium (136), Derby (111) and Rissen (51) from pigs and Enteritidis (61), Indiana (43) and Typhimurium (17) from chickens. Overall, mean resistance percentage for each respective animal host was: ampicillin 19.3, 33.1, 12.6; chloramphenicol 17.5, 25.2, 4.4; colistin 10.5, 3.1, 27.5; gentamicin 0.0, 3.6, 1.6; nalidixic acid 3.5, 3.6, 36.3; sulfisoxazole 21.8, 53.0, 12.2; tetracycline 22.8, 73.8, 13.2 and trimethoprim/sulfamethoxazole 5.3, 23.6, 4.9. Clinical resistance against cefepime, cefotaxime and ciprofloxacin was absent. DS was particularly apparent for ciprofloxacin: 3.5% for cattle, 2.9% for pigs and 35.2% for chickens, whereas the corresponding figures for cefotaxime were 1.8, 1.9 and 1.1%. For ampicillin and gentamicin, DS was negligible (0.5% or less).

**Conclusion:** This pan-European survey demonstrates high variability in antimicrobial susceptibility among zoonotic *Salmonella enterica* isolates from healthy food producing animals at slaughter. For older molecules, prevalence of clinical resistance was notably high, but most isolates displayed high susceptibility to newer compounds, critically important in human medicine.

#### P1101 Serogroup screening and antimicrobial resistance in *Salmonella* isolated from broiler chickens

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The development of antimicrobial resistance has been a public health issue worldwide. The aim of this study was therefore to determine the antibiotic resistance pattern of *Salmonella* species isolated from broiler chickens in Iran. Thirty-seven isolates of *Salmonella* recovered from broiler chicken carcasses were analyzed for resistance to 14 antimicrobial agents. *Salmonella* isolates were serogrouped by standard agglutination test using O and H antisera before antibiotic susceptibility testing. Serogroup screening showed four different serogroups (D1, B, C1, C2) among 37 *Salmonella* isolates. *Salmonella* serogroup D1 with frequency 48.64% was the most prevalent serogroup. Antimicrobial susceptibility testing was performed by the standard disc diffusion method according national committee for clinical laboratory standards. Antibiotic sensitivity tests showed that Amikacin, Cephalotin, Tylosin, and Chloramphenicol, were the most effective antibiotics (100% sensitivity). Antibiotics for which *Salmonella* isolates exhibited resistance were: Neomycin (10.81%), Amoxicillin (18.91%), Streptomycin (29.72%), Chlortetracyclin (32.43%), Trimethoprim (13.51%), Nalidixic acid (18.91%), Tetracyclin (29.72%), Kanamycin (16.21%), Ampicillin (13.51%). Also 51% of resistant isolates were belonged to serogroup D1 and serogroups B, C1, and C2 included 37%, 9%, and 3% of resistant isolates respectively. In all 28% of *Salmonella* isolates were resistant to more than four antibiotics (MDR). Findings of present study indicated that the development of antimicrobial resistance in *Salmonella* is an increasing problem in Iran and resistant strains are widespread.

#### P1102 Antimicrobial susceptibility monitoring of respiratory tract pathogens isolated from diseased cattle and pigs across Europe

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**Objectives:** VetPath is an ongoing pan-European resistance monitoring program for veterinary pathogens isolated from diseased antimicrobial-naïve cattle, pigs and poultry. Here, antimicrobial susceptibilities of isolates from cattle and pig respiratory tract infections are presented.

**Methods:** Lung samples or deep nasopharyngeal swabs from cattle or nasal swabs from pigs were collected from animals with acute clinical signs, not recently treated with antibiotics, in 11 EU countries, predominantly in 2002–2006. *Pasteurella multocida* (Pm) and *Mannheimia haemolytica* (Mh) from cattle samples and Pm, *Actinobacillus pleuropneumoniae* (Ap) and *Streptococcus suis* (Ss) from pigs samples were isolated by standard methods (one isolate per farm per outbreak). Ss was also isolated from meningitis cases. MICs of 17 antibiotics were determined in a central laboratory by broth microdilution as per CLSI recommendations. Results were interpreted using CLSI resistance breakpoints (M31-A3, 2008) where available.

**Results:** In all, 1388 isolates were recovered. In cattle, 343 Pm and 230 Mh were isolated, the majority of these were susceptible to antibiotics for which resistance breakpoints are available (Table 1). For amoxicillin, 4 Pm and 8 Mh isolates showed MIC  $\geq$  64 mg/L while the highest MIC observed for cephalixin was 8 and 0.12 mg/L for cefquinome. MIC distributions of marbofloxacin and enrofloxacin were similar. With a susceptibility breakpoint of  $\leq$ 0.25 mg/L, 94.8% Mh and 96.5% Pm were susceptible to danofloxacin. Tylosin and lincomycin showed similar MIC patterns with MIC90 of  $\geq$ 32 mg/L for Mh and 32–64 mg/L for Pm. A total of 326 Pm, 262 Ap and 227 Ss were recovered from pig samples. For antibiotics having CLSI breakpoints, % resistance is shown (Table 1); for the others, similar MIC ranges as those obtained for cattle isolates were observed. For Pm and Ap, MIC90 amounted to 0.03 mg/L for cefquinome and 2 mg/L for cephalixin; amoxicillin MIC90 was 0.25 mg/L. In contrast, for Ss MIC90s were 0.03 mg/L for amoxicillin and cefquinome; cephalixin MIC90 was 0.25 mg/L. For Pm and Ap, tylosin and lincomycin showed similar MIC patterns with MIC90s of 32 mg/L and  $\geq$ 32 mg/L, respectively.

Antimicrobial (breakpoint)	Cattle		Pigs	
	Pm (n=343)	Mh (n=230)	Ap (n=262)	Ss (n=227)
Amoxicillin (R <sub>20</sub> mg/L)	0	0	0	0
Cefixur (R <sub>8</sub> mg/L)	0	0	0.3	0
Enrofloxacin (R <sub>2</sub> mg/L, R <sub>4</sub> mg/L, S <sub>8</sub> )	0.6	0.4	0	0.4
Florfenicol (R <sub>2</sub> mg/L)	0.3	0	0	0.4
Spectinomycin (R <sub>12</sub> mg/L)	3.2	0	nc	nc
Tetracycline (R <sub>8</sub> mg/L, cattle, R <sub>2</sub> mg/L, pigs)	2.9	16.1	19.8	11.1
Tiamulin (R <sub>2</sub> mg/L)	nc	nc	nc	0
Trimoxin (R <sub>2</sub> mg/L)	nc	1.7	0.8	0
TS (co-trimoxazole) (R <sub>4</sub> mg/L)	2.3	6.1	2.5	3.8

nc: not calculated; CTC was used 2003-2004

**Conclusion:** The results show an absence or low antimicrobial resistance, except to tetracycline, among the major respiratory tract pathogens isolated from diseased but non-treated cattle and pigs across the EU.

#### P1103 Antimicrobial susceptibility of mastitis pathogens isolated from diseased dairy cows across Europe: VetPath monitoring results

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**Objectives:** VetPath is an ongoing pan-European resistance monitoring program for veterinary pathogens isolated from diseased antimicrobial-naïve cattle, pigs and poultry. Antimicrobial susceptibilities of mastitis pathogens isolated from lactating cattle are presented here.

**Methods:** Milk was sampled from dairy cows with acute mastitis, not recently exposed to antimicrobial treatment, in eight EU countries, mainly during 2002–2006. *Escherichia coli* (Ec), *Staphylococcus aureus* (Sa) and *Streptococcus uberis* (Su), were isolated and biochemically identified (one isolate per farm). MIC values of 9 (Ec) and 12 (Sa, Su) antibiotics were determined in a central laboratory by

broth microdilution as per CLSI guidelines. Susceptibility was expressed as MIC<sub>50</sub> and MIC<sub>90</sub>, and % resistance was determined for antibiotics with defined CLSI breakpoints (M31-A3, 2008).

**Results:** In total, 1066 isolates were recovered, 363 Ec, 356 Sa and 347 Su. For Ec, resistance to beta-lactam antibiotics was virtually absent; tetracycline resistance amounted to 16%. For the cephalosporins tested without CLSI breakpoints, MIC<sub>90</sub> amounted to 0.06 mg/L for cefquinome and 8 and 64 mg/L for cephalexin and cephapirin. MIC distributions of marbofloxacin and enrofloxacin were similar, with MIC<sub>50</sub> and MIC<sub>90</sub> of 0.03 and 0.03–0.06 mg/L. For neomycin, five Ec isolates showed MICs > 128 mg/L, while MIC<sub>50</sub> was 1 mg/L. For Sa, resistance to amoxicillin/clavulanic acid was absent, whilst penicillin resistance was 38%, but MIC<sub>90</sub> for cloxacillin was as low as 0.5 mg/L. MIC<sub>90</sub> of cefquinome and cephapirin were 0.25 and 0.5; for cephalexin 4 mg/L. Erythromycin and tetracycline resistance were low (1.4% and 7.3%, respectively). MIC<sub>50/90s</sub> of enrofloxacin and marbofloxacin varied from 0.12 to 0.5 mg/L. Neomycin MICs ranged from 0.25 to 2 mg/L, except for two isolates. In contrast, for Su beta-lactam resistance was absent, whereas erythromycin and tetracycline resistance were 19% and 25%. For the beta-lactam antibiotics without CLSI breakpoints, MIC<sub>90</sub> of cefquinome, cephalexin and cephapirin were 0.25–0.5 mg/L; cloxacillin MIC<sub>90</sub> was 4 mg/L. Fluoroquinolone MIC<sub>50/90s</sub> were 0.5–2 mg/L, whereas neomycin MIC<sub>50</sub> and MIC<sub>90</sub> were 64 and ≥128 mg/L.

**Conclusions:** Overall, the prevalence of antibiotic resistance is low among the three major mastitis pathogens isolated from diseased but non-treated dairy cows across the EU, however penicillin resistance of Sa is fairly high. Generally, the tested antibiotics exhibited high efficacy.

#### **P1104** *Campylobacter* spp. in raw materials of animal origin and their resistance to antibiotics in the Czech Republic

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**Objectives:** *Campylobacter jejuni* and *Campylobacter coli* are the most common bacterial pathogens causing alimentary tract infections in Europe. An important source of infection is poultry; a potential source is also raw cow's milk. The presentation is concerned with the prevalence of thermotolerant *Campylobacter* spp. in broilers and raw cow's milk in the Czech Republic. Resistance of isolates to selected antibiotics is studied.

**Methods:** In 2006–2007, cloacal swabs were collected from broilers in slaughterhouses to determine the presence of *Campylobacter* spp. In 2008, caeca and skin of broilers at slaughterhouses were examined. In 2009, fresh and frozen poultry skin was tested in screened supermarkets. In 2010, poultry caecum tests were continued at slaughterhouses and milk filters at dairy farms were examined. In 2011, more tests of poultry caeca were carried out. In all isolates, resistance to antibiotics was quantitatively determined by the microdilution method.

**Results:** The prevalence of *Campylobacter* spp. in cloacal swabs (years 2006 and 2007) was about 50%. The rates were higher in broiler caeca – 61%, 72% and 65% in 2008, 2010 and 2011 (as of 30 September), respectively. Skin tests revealed rates of 70% in broilers at slaughterhouses, 75% in fresh and 37% in frozen poultry in supermarkets. In dairy farm milk filters, the prevalence of *Campylobacter* spp. was 3%. *C. jejuni* was detected significantly more frequently than *C. coli*. Poultry isolates were highly resistant to quinolones, as opposed to *Campylobacter* spp. isolated from milk.

**Conclusion:** The prevalence of thermotolerant *Campylobacter* spp. in poultry is high in the Czech Republic. Broiler skin contamination is higher than the primary prevalence in the intestine, suggesting secondary contamination. Poultry isolates are characterized by higher resistance to quinolone antibiotics. The resistance is relatively low in milk isolates.

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#### **P1105** A comparison of primary and secondary clarithromycin and levofloxacin resistance of *Helicobacter pylori* in southern Poland between 2006–2008 and 2009–2011

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**Objectives:** An increasing resistance of *Helicobacter pylori* (*H. pylori*) strains to the currently used antimicrobial agents is the serious therapeutic problem. Therefore, the aim of this study was to compare the primary and secondary resistance to clarithromycin and levofloxacin of *H. pylori* strains isolated between years 2006–2008 and 2009–2011.

**Methods:** One hundred and sixty dyspeptic patients before or after failed therapy who underwent gastroscopy in the Center of Medical Care Falck in Krakow were enrolled to the study. During the gastroscopy two biopsy specimens were taken from each patient. Minimal Inhibitory Concentration (MIC) values of clarithromycin and levofloxacin were determined by E-test method. The breakpoints used to classify strains as resistant according to the MIC value was 1 mg/L for clarithromycin and levofloxacin. The statistical analysis was conducted with the use of chi-squared test at the 0.05 significance level ( $p \leq 0.05$ ).

**Results:** One hundred and fifteen *H. pylori* strains (90 primary, 25 secondary) were isolated between 2006 and 2008, and 45 strains (37 primary, eight secondary) between 2009 and 2011.

2006–2008: In total, 34% of *H. pylori* strains were resistance to clarithromycin and the percentage of the secondary strains was significantly greater than the primary strains (80% vs. 21%,  $p < 0.001$ ). Five percent of strains were resistant to levofloxacin, and the difference between primary and secondary was statistically significant (2% vs. 16%,  $p = 0.006$ ).

2009–2011: In total, 24% of strains were resistant to clarithromycin and the percentage of the secondary strains was also significantly greater than the primary strains (38% vs. 22%,  $p = 0.01$ ). Sixteen percent of strains were resistant to levofloxacin, and the difference between primary and secondary was statistically significant (11% vs. 38%,  $p = 0.04$ ).

The noteworthy result is the decrease of *H. pylori* resistance to clarithromycin in 2009–2011 compared to the years 2006–2008 which is not significant (24% vs. 34%,  $p = 0.06$ ), whereas an increase of resistance to levofloxacin is significant (16% vs. 5%,  $p = 0.007$ ).

**Conclusion:** Higher rate of *H. pylori* resistant strains to levofloxacin may be justified by an increasing usage of levofloxacin instead of clarithromycin in the treatment of *H. pylori* infection. The resistance to fluoroquinolones is rapidly acquired, therefore levofloxacin, which is recommended for a rescue therapy, should not be used routinely. Scientific work partially funded by Polish Government as a research grant in the years 2011–2013 (NN404547640).

#### **P1106** *Helicobacter pylori* primary and secondary resistances to antimicrobials in a Portuguese adult population

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**Objectives:** *Helicobacter pylori* (Hp) resistance to antimicrobials is increasing and information about susceptibility patterns in each country could be very relevant. The prevalence of primary and secondary resistance of Hp to antimicrobials was determined in order to improve the outcome of these patients and for studying the evolution of resistance patterns.

**Methods:** Evaluation of the prevalence of primary and secondary resistance in Hp isolates to antimicrobials on a population ( $n = 88$ ) of dyspeptic adult patients with positive 13<sup>C</sup> urea breath test (UBT) from May 2009 to May 2011. Patients were divided in two groups: A ( $n = 51$ ) – no previous Hp treatment (primary resistance); B ( $n = 37$ ) – previous failed Hp treatment (secondary resistance). All patients were submitted to upper digestive endoscopy with biopsies for histological and microbiologic characterization. Susceptibility to metronidazole



(MET), clarithromycin (CLA), levofloxacin (LEV), amoxicillin (AMX) and tetracycline (TET) was determined by E-test. Patients were treated with: group A-pantoprazole + AMX + CLA during 14 days; group B-pantoprazole + AMX + LEV for 10 days. Hp eradication rate was assessed with UBT after 8–12 weeks. Statistical analysis was performed with SPSS v17.0.

**Results:** Eradication was successful in 64.8% (A-74.5%; B-51.4%; p = 0.041). All Hp isolates were susceptible to AMX and TET. 46.6% of isolates were resistant to CLA and 40.9% to MET, however there was a statistically significant difference between group A and B (CLA: A-15.7%; B-89.2%; p < 0.0001; MET: A-27.5%; B-59.5%; p = 0.004). Relatively to LEV, 30.7% of the isolates were resistant, and difference between the two groups was observed but there was no statistical significance (A-25.5%; B-37.8%; p = 0.247).

**Conclusions:** Rates of Hp eradication associated with first and second line empirical treatments were lower than the usually accepted. The results also showed a high prevalence of Hp primary and secondary resistance to CLA, MET and LEV regimes. Hp secondary resistance rates to CLA and MET exceed 50%, dissuading the use of these antimicrobials agents in second line empirical anti-Hp protocols. This study suggest the development, in our country, of strategies of antibiotic therapy based on susceptibility tests, in order to improve Hp eradication rates.

**P1107** Prevalence of third-generation cephalosporin-resistant *Escherichia coli* and their resistance mechanisms in dogs in Finland

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**Objectives:** Third generation cephalosporin resistance among *Escherichia coli* is usually due to production of extended-spectrum betalactamases mediated by ESBL or AmpC genes. In humans, ESBL-producing *E. coli* strains are increasing rapidly. In veterinary medicine such isolates have been rare so far in Finland. The purpose of this work was to study the prevalence of third generation cephalosporin resistance among *E. coli* and to characterize their resistance genes in Finnish dogs.

**Methods:** Fecal specimens were taken from 471 dogs. Screened dogs were the patients of the Small Animal Veterinary Teaching Hospital (30%) which were presented for other reason than diarrhea, or were other healthy dogs (70%). The fecal specimens were cultured on selective plates (Brilliance, Oxoid). Identification of the isolates was done with standard techniques and susceptibility testing with the disk diffusion method. Isolates with decreased susceptibility to 3rd generation cephalosporins were further tested with the double disk method and AmpC detection disks (MAST, UK). The major ESBL genes (CTX-M, TEM, SHV) and AmpC genes (CIT, FOX, DHA, ACC) were detected by multiplex PCR.

**Results:** Fecal specimens were obtained from 471 dogs. The mean age of the dogs was 4.5 (SD ± 3.5 years) and 53% of them were females. Twenty-five dogs (5%; 95% CI 3–7%) carried a 3rd generation cephalosporin resistant Enterobacteriaceae isolate, of which 23 were *E. coli* and 2 *E. cloacae*. Of *E. coli* – isolates, eight carried CTX + TEM genes and four had CTX gene, eight had CIT + TEM and one had CIT gene alone.

**Conclusion:** Rather high proportion (5%) of the companion dogs carried either ESBL- or AmpC-producing *E. coli*. Further studies are needed to find a possible source, dog feed being one example. The next step is to type animal *E. coli* isolates by MLST in order to compare these with *E. coli* isolates of human origin. Due to this rather high prevalence of ESBL- and AmpC-producing strains, it is recommended that 3rd generation cephalosporin resistance is routinely screened in clinical specimens by veterinary laboratories.

**P1108** Comparison of veterinary medicines and natural feed constituents on curing efficacy and conjugation frequency of CTX-M plasmids in *Escherichia coli*

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**Objectives:** In recent years, *Escherichia coli* containing large promiscuous plasmids carrying the blaCTX-M gene, that confers resistance to Extended-Spectrum Beta Lactam (ESBL) antibiotics, have become increasingly common in humans and farm animals. These CTX-M *E. coli* can cause human infections and there is a concern that in some cases people can become infected via animal products. The aim of this study was to determine if passage of *E. coli* in the presence of veterinary medicines and natural feed constituents could promote plasmid loss and therefore reduce the prevalence and potential dissemination of CTX-M genes.

**Methods:** The plasmid curing activity of veterinary medicines and natural feed constituents was determined in two field isolates (C159/11, LR8 3A) and a mutant (DH5alpha::pCTaph) by up to 21 passages at 1/2 and 1/4 MIC of these agents in Luria-Bertani broth. Ethidium bromide and LB broth were used as positive and negative controls respectively. Potentially cured strains were selected by replica plating to selective agar plates. Those colonies which grew in the absence of selective agent were selected for further plasmid analysis by PCR of specific genes and size profiling. The ability of test compounds to inhibit plasmid conjugation from test strains to a *Salmonella Typhimurium* (26R) recipient was evaluated in conjugation studies by prior growth with sub-lethal concentrations of test agents.

**Results:** The mutant strain lost its plasmid more readily than the two field strains tested but all three strains did show evidence of plasmid loss in the presence of test compounds (Table 1). PCR analysis showed loss of genes from around the plasmid as well as the loss of the blaCTX-M or Kanamycin gene. Further analysis by plasmid profiling, however, showed significant genomic rearrangement in a number of ‘‘cured’’ strains but in the majority of cases; no plasmid loss. Plasmid conjugation was not detectable following exposure to sub-lethal concentrations of enrofloxacin, whilst the pre-biotic Bimuno and streptomycin also significantly (p < 0.05) reduced conjugation rates for both isolates.

Strain	Conc. used (MIC)	Named curing rates (% cured) after 7, 10, 17 or 21 passages with drug and antibiotic																			
		Ethidium bromide			Acetic acid usage			Fluorocytin			Enrofloxacin			Streptomycin			Colistin				
Summary		7	10	17	21	7	10	17	21	7	10	17	21	7	10	17	21	7	10	17	21
C159	%	0	0	0	2.2	0	0	0	0	4.3	0	0	0	1.7	3.1	0	0	0	0	0	0
LR8	%	0	0	0	0	0	0	0	0	13.5	2.6	0	0	1.3	1.0	0	0	4.1	0	0	0
DH5	%	0	0	0	0	0	0	0	0	0	2.1	0	0	0	0	0	0	0	0	0	0
TEM	%	0	2.4	2.6	0	0	0	0	0	0	1.9	0	0	0	1.4	0	0	0.6	0	0	0
SHV	%	1.6	2.0	3.0	0	0	0	0	0	0	0	0	0	0.8	0	0	0	1.7	0	0	0
CT-Mph	%	0.7	3.9	3.0	0	0	0	0	0	7.8	10.0	0	0	69.2	30.0	0	0	18.2	0	0	0

a. Bacteria contain forward controls and is included as a replacement to feed to animals & the ‘‘probiotic’’ consists of equal media replacement of a *Lactobacillus plantarum* strain & 30000 yeast extract and yeast cells were not added (they at concentrations listed, or % and %MIC refers to how much concentration for these substances only)

**Conclusions:** The results suggest that some veterinary antibiotics and or natural feed or dietary constituents may promote loss of plasmids from bacteria and/or reduce the transfer of plasmids. These findings warrant further investigations in the battle against plasmid-mediated antibiotic resistance.

**P1109** Comparison of a rapid multiple combination bactericidal testing method vs. conventional time-kill studies for patients infected with extreme drug-resistant *Acinetobacter baumannii* with decreasing susceptibilities to polymyxin B

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**Objective:** Extreme-drug resistant (XDR) bacterial infections are increasingly prevalent worldwide as a public health problem.

Combination (combi) therapy may be the only viable option until new antibiotics (abx) are available. Conventional time-kill studies (TKS) are the gold standard for evaluating abx combi. But, they are time-consuming and labourious, suffering from long turnaround time to obtain the results and evaluating very few abx combi. We aim to develop a high-throughput method using multiple combination bactericidal testing (MCBT) to identify optimal abx combi against XDR *Acinetobacter baumannii* (AB) and compare them with previously published results by TKS.

**Methods:** Thirty-five non-clonal XDR AB harbouring blaOXA-23/51 were collected from five Singapore hospitals over a 2006–2007. All XDR AB isolates were resistant to all abx except polymyxin B (P) (MIC 0.5–64 mg/L). MCBT was performed in 96-well microtiter trays using maximum achievable clinical, unbound concentrations (mg/L) of rifampicin (R) (2), tigecycline (T) (2) and P (2) alone and in combi against the test isolates. A final inoculum of  $5 \times 10^5$  CFU/mL was used and the trays were incubated at 37°C for 24 hours. Quantitative counts were obtained at 24 hours in duplicate. Three abx combi (P + R, P + T and T + R) were evaluated. A MCBT was scored as positive if it predicted bactericidal activity ( $\geq 3$  log CFU/mL decrease from baseline and compared to its most active abx alone), synergism ( $\geq 2$  log decrease from baseline and compared to its most active abx alone) or indifferent activity ( $\leq 2$  log decrease from baseline) that were similar to TKS results. An agreement for an isolate is determined from complete agreement amongst the three abx combi evaluated. Evaluation of agreement within abx combi regardless of isolates was also performed.

**Results:** In TKS, P + R, P + T and T + R were bactericidal against 15/35, 8/35 and 8/35 isolates respectively; synergistic against 1/35, 2/35 and 0/35 isolates respectively and indifferent against 19/35, 25/35 and 27/35 isolates respectively. 32/35 isolates were scored positive and in complete agreement for all three abx combi evaluated using synergy definitions when compared to TKS. Overall, a 91.4% agreement was achieved. There was 94.3% (33/35) agreement for P + R, P + T and T + R within abx combi regardless of isolates.

**Conclusion:** Our MCBT can be used to objectively assess the killing activity of two abx in combination against XDR AB. It may guide clinicians in selecting the optimal abx combi and warrants further investigations.

**P1110** **In vitro activity of the novel ceragenin compound, CSA-131 alone vs. a diverse collection of Gram-negative pathogens and in combination with vancomycin vs. multidrug-resistant *Acinetobacter baumannii***

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**Objectives:** Ceragenins are cholic acid derivatives that exhibit broad range antimicrobial activity. They act as antimicrobial peptide (AMP) mimics, permeabilizing the outer membrane of Gram-negative bacteria. These agents have a number of advantages over AMPs; most importantly they are resistant to cleavage by proteolytic enzymes. It is hoped that ceragenins will find clinical utility in the treatment of infections caused by MDR bacterial pathogens. We investigated the in vitro activity of the novel ceragenin compound, CSA-131 against both clinical isolates with defined resistance mechanisms and type strains of important Gram-negative species. In addition, the potential of a CSA-131/vancomycin combination vs. MDR *A. baumannii* (MDRAB) was studied using representative isolates of successful UK clones.

**Methods:** Isolates were identified by MALDI-TOF mass spectrometry and *A. baumannii* clinical isolates were assigned to specific clones by PFGE. MICs were determined by broth microdilution in IsoSensitest broth. Synergy of the CSA-131/vancomycin combination was assessed in standard checkerboard assays performed in microtitre plates. Endpoints were visualised with the aid of the redox dye-containing reagent, alamarBlue. Fractional inhibitory concentrations indices (FICI) were calculated.

**Results:** MICs of CSA-131 for all *A. baumannii* (including colistin-resistant), *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia* and *Escherichia coli* (including ST131) isolates tested were in the range

of 8–16 mg/L and for all *Enterobacter* sp. and *Klebsiella pneumoniae* isolates the MICs were in the range 16–32 mg/L. The compound was active against ESBL and carbapenemase-producing strains. CSA-131 was less active vs. *Proteus mirabilis* and *Serratia marcescens* (MICs > 64 mg/L). Though marked synergy (FICI  $\leq$  0.05) with vancomycin was not observed, in the presence of 2 mg/L CSA-131 the MICs of the former agent were reduced for all *A. baumannii* isolates (at least 32-fold).

**Conclusions:** CSA-131 displayed useful in vitro activity vs. type strains and problematic clinical isolates of several frequently encountered Gram-negative species. The majority of the isolates studied were MDR; many displaying resistance mechanisms of increasing importance including production of the New Delhi metallo-beta-lactamase (NDM). We also demonstrated the potential of a ceragenin/glycopeptide combination for the treatment of infections caused by MDRAB including colistin-resistant isolates.

**P1111** **Monitoring the activity of antimicrobials against multidrug-resistant *Clostridium* spp.**

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**Background:** The incidence of multi-drug resistant (MDR) *Clostridium* spp. is unclear. Surveillance studies aimed at determining their incidence and susceptibility to antibiotics are necessary in order to better understand the incidence of such etiologic agents. This study evaluated the activity of tigecycline and comparators against MDR *Clostridium* spp. isolated from infections during 2009–October 2011.

**Methods:** A total of 1450 isolates were collected from Europe, of which 462 were multi-drug resistant (MDR; resistant to two antibiotic classes). Isolates were tested for susceptibility following the CLSI guidelines for anaerobes and interpreted using EUCAST guidelines or FDA guidelines for tigecycline.

**Results:** The susceptibility of all and MDR isolates are shown in the Table below:

%S, %I, %R, percent isolates susceptible, intermediate or resistant; \*, susceptibility determined using FDA breakpoints due to absence of EUCAST breakpoints.

Antibiotic	All (n = 1,450)				MDR (n = 462)				
	MIC <sub>90</sub>	%S	%I	%R	Antibiotic	MIC <sub>90</sub>	%S	%I	%R
Clindamycin	>8	65	-	35	Clindamycin	>8	9	-	91
Meropenem	2	92	4	4	Meropenem	>8	80	8	12
Metronidazole	2	99.6	-	0.4	Metronidazole	2	99.6	-	0.4
Penicillin	4	38	10	52	Penicillin	>32	0	0	100
Pip-Tazo	16	89	9	2	Pip-Tazo	16	71	22	7
Tigecycline*	0.5	99.9	0.1	0	Tigecycline*	0.12	100	0	0

**Conclusions:** Of the 1450 isolates collected, 462 (32%) were resistant to two antibiotic classes. The most active antibiotic was tigecycline with MIC<sub>90</sub> of 0.12–0.5 mg/L, followed by metronidazole (MIC<sub>90</sub> 2 mg/L). Susceptibilities were highest for metronidazole and tigecycline (>99% susceptible). Against MDR isolates, only these two agents exhibited susceptibilities >90%.

**P1112** **Chlorhexidine-resistance in clinical isolates of coagulase-negative staphylococci, a descriptive study**

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**Objectives:** Coagulase-negative staphylococci (CoNS) comprise the major part of the normal flora of the human skin. Despite regarded as nosocomial pathogens, especially in infections associated with implanted foreign body materials. Pre-operative antiseptic preparation

is an important strategy for reducing the risk of complications such as surgical site infection (SSI). The most widely used compounds today are alcohols and quaternary ammonium compounds (QACs), predominantly chlorhexidine.

The aim was to investigate, by using both phenotypic and genotypic methods, if decreased susceptibility to chlorhexidine among CoNS isolates was present in our setting.

**Methods:** Coagulase-negative staphylococci (n = 150) obtained from four different studies were investigated; *S. epidermidis* isolates (n = 61) obtained from prosthetic joint infections (PJI), commensal *S. epidermidis* (n = 24), *S. epidermidis* from two trials investigating post-operative infections after cardiothoracic surgery (n = 31), and finally CoNS isolated from the skin of the thorax after routine disinfection prior cardiothoracic surgery (n = 34). Determination of MIC against chlorhexidine was performed on Müller Hinton agar plates supplemented with serial dilutions of chlorhexidine. PCR was used for the detection of five different QAC resistance genes, qacA/B, SMR, qacH, qacJ, and qacG.

**Results:** Decreased susceptibility to chlorhexidine was found among 54% of the PJI isolates, 64% of the cardiothoracic isolates, in 17% of the commensals, and 12% of the isolates obtained from the skin of cardiothoracic patients, respectively.

The qacA/B gene was present in 64 of 150 isolates (43%), SMR in 8/150 (5%) and qacH was found in one isolate (0.7%). The qacA/B gene was found among 52% of the PJI isolates, 61% of the cardiothoracic isolates, in 25% of the commensals, and 21% of the isolates obtained from the skin of cardiothoracic patients, respectively.

**Conclusion:** Decreased susceptibility to chlorhexidine as well as QAC resistance genes is present among *S. epidermidis* causing deep SSIs.

#### **P1113** High antibiotic resistance patterns of *Escherichia coli* in hospital wastewater in Nicaragua

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**Objectives:** The emergence of antibiotic resistant bacteria presents a major threat to public health because it reduces the effectiveness of antibiotic treatment, leading to increased morbidity, mortality and health care expenditure. Resistant bacteria found in the aquatic environment may disseminate among the population and the genes conferring resistance may be introduced in the natural bacterial ecosystems. The aim of this study was to determine the antibiotic resistance patterns of *Escherichia coli* isolates from different aquatic environmental sources in León, Nicaragua.

**Methods:** Antibiotic resistance patterns were studied among 493 *E. coli* isolates from different aquatic sources collected through October 2008 to May 2009 in León, Nicaragua. The following antibiotics were tested: ampicillin, amoxicillin-clavulanic acid, cefotaxime, ceftazidime, ceftriaxone, ciprofloxacin, chloramphenicol, gentamicin, nalidixic acid and trimethoprim-sulfamethoxazole by the agar dilution method. Phenotypic detection of extended-spectrum beta-lactamase (ESBL) was analysed using the Etest® system. All ESBL positive *E. coli* strains were screened for the resistance genes by PCR. To identify the beta-lactamase genes, sequencing was performed.

**Results:** High levels of antibiotic resistance were found in *E. coli* isolates in all hospital sewage water samples and in eight of 87 well water samples. Among the resistant isolates from the hospital sewage, ampicillin, chloramphenicol, ciprofloxacin, nalidixic acid, trimethoprim-sulfamethoxazole showed the most common multi-resistance profile. *E. coli* producing ESBL and harbouring the genes for CTX-M enzymes were detected in one of the hospital sewage samples and in 26% of the resistant isolates from the well water samples. CTX-M-9 group was more prevalent in *E. coli* isolates from the hospital sewage samples and CTX-M-1 group in the well water samples.

**Conclusion:** The presence of antibiotic resistant *E. coli* strains was frequent in the environmental water samples. Isolates carrying the

CTX-M group of enzymes have become one of the main public health concerns due to their ability to be involved in nosocomial and community acquired infections. In the present study, it was found that CTX-M-15 and CTX-M-9 were the specific beta-lactamases present in the *E. coli* isolates. Our results suggest that multi-resistant ESBL-producing *E. coli* are widely spread in hospital sewage water and in some well water samples in Nicaragua.

#### **P1114** Recovery of extended spectrum beta-lactamase (ESBL) producing bacteria from the hospital environment

*C. Judge, L. Burke, S. Galvin, H. Humphreys, D. Fitzgerald Hughes\* (Dublin, IE)*

**Objectives:** Inanimate surfaces adjacent to hospitalized patients are a potential reservoir for the transmission of bacteria. The contribution of the hospital environment to the transmission of antimicrobial-resistant Gram-positive bacteria in hospitals is widely documented. Due to their relatively short-term survival on surfaces, spread of antimicrobial resistant Gram-negative bacteria such as extended spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae by this route is less common and therefore is often overlooked. We investigated inanimate surfaces adjacent to ESBL-positive patients for the presence of ESBL-producers.

**Methods:** Recovery of ESBL-producing Enterobacteriaceae from six surfaces adjacent to three patients with confirmed ESBL infections was investigated using both pre-moistened environmental swabs and chromogenic selective contact plates. Recovered isolates were characterised using phenotypic combination disc tests, multiplex PCR for the detection of blaTEM, blaSHV and blaCTX-M genes and pulsed field gel electrophoresis (PFGE).

**Results:** ESBL-producing *Klebsiella pneumoniae* which harboured bla-SHV, blaTEM and blaCTX-M-1 genes were recovered from multiple surfaces adjacent to one of three patients. Isolates recovered from the patient's environment were closely-related (90–100%) by PFGE analysis to the clinical isolate that caused the urinary tract infection in the patient.

**Conclusion:** Using the recovery methods optimized in this study, we have shown that viable ESBL-producing Enterobacteriaceae may be present on inanimate surfaces close to patients and that environmental isolates are genetically closely related to the infecting isolates. Therefore, in relation to ESBL-producing Gram-negative bacteria, the hospital environment is a potential reservoir for further cross-contamination and infection.

## Environmental control and healthcare-associated infections

#### **P1115** In-use evaluation of an automated ultraviolet lamp (Germ Genie®) for bio-decontamination of hospital computer keyboards

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**Objectives:** As high-touch surfaces in hospitals, computer keyboards can be a source for nosocomial infection. The electrical nature and irregularity of keyboards makes them difficult to clean by traditional methods. This study evaluated an automated ultraviolet lamp (Germ Genie®) for bio-decontamination of such keyboards. A second objective was to get feedback from hospital staff on its usability in a busy working environment.

**Methods:** Prior to the hospital field trial, computer keyboards across a number of settings including the hospital were sampled using sterile, cotton-tipped swabs. Additionally, a laboratory evaluation of efficacy using deliberately inoculated keyboards was also undertaken. For the hospital setting evaluation the Germ Genie® ultraviolet lamp was placed at two keyboards on each of four wards and there were a further two control keyboards on each ward for simultaneous sampling.

Samples were taken at least six times per keyboard over a 2-week period. The bacteria on sample keyboards were enumerated using direct plating and dilution methods. Bacterial identification was done by standard clinical microbiological methods including use of API strips. Semi-structured interviews about the device were conducted with 15 members of staff.

**Results:** Baseline sampling of keyboards both within and outside the hospital environment indicated significant contamination with fifty-eight skin-type and environmental morphotypes including *Acinetobacter* sp., *P. aeruginosa* and *S. aureus*. Results in the laboratory setting with inoculated keyboards showed that ten 1-minute cycles of the lamp were sufficient to kill 99–100% of artificially inoculated *E. coli*, *B. subtilis* and *S. aureus*. In the hospital, in all cases where Germ Genie® was correctly positioned (40 samples), no bacteria were recovered by any of the enumeration methods. Where the Germ Genie® was not installed (67 samples), significant levels of bacteria were found. Staff interviews indicated the view that the Germ Genie® was a valuable addition to efforts to combat hospital infection.

**Conclusions:** Our results show that hospital keyboards are colonised by a range of bacteria, including potential pathogens, and that installation of the tested automated UV lamp significantly reduces bacterial numbers to near-zero by the sampling methods employed. The UV lamp did not adversely affect workflow and there were favourable views from healthcare workers about its potential use for prevention of nosocomial infection.

**P1116** Effect of a decontamination device producing hydroxyl radicals on the survival of airborne opportunistic pathogens

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**Objectives:** There is increasing evidence that decontamination of the clinical environment can help reduce nosocomial infections. Systems that can disinfect air and surfaces whilst patients and staff are still occupying a room are being sought. The Inov8 Air Disinfection (AD) device releases hydroxyl radicals into room air continuously. These highly reactive oxygen species are thought to have multiple effects on microbes, reducing their viability in air and on surfaces. Few investigations of the AD device have been published so far, and results from hospital rooms have been conflicting. We thus evaluated the device against two nosocomial pathogens under defined conditions, in an aerobiological chamber set to mimic airflow rates typical of those encountered in wards.

**Methods:** *Staph aureus* NCTC 10399 and *Acinetobacter baumannii* NCTC 12156 were nebulised into the centre of a 72 m<sup>3</sup> chamber (6 m L × 4 m W × 3 m H). Air was sampled at regular intervals with a 6-stage Andersen sampler, and surviving bacteria calculated as CFU/m<sup>3</sup> of air. Steady-state experiments were carried out with the ventilation set at ~3 air changes/hour, ambient temp (20°C) and RH (~50%). Within each run, levels of bacteria in the air during a control period (AD off) were compared to levels when the device was on. Runs were done in triplicate.

**Results:** When placed in Position A (centre of room next to point of aerosol dispersal), the AD device achieved a mean percentage reduction of 37.3% (SD 22.5%) in *S. aureus* viable counts, with the AD ‘‘on’’ and ‘‘off’’ counts being significantly different ( $p < 0.001$ ) in two of the three runs. With *A. baumannii*, the mean reduction was 36.3% (SD 29.2%) with only one of the three runs displaying a statistically significant ( $p < 0.001$ ) difference when the device was on.

When in Position B (back of room, 2.7 m from point of dispersal), viable counts of airborne bacteria generally increased. A mean % increase of +10.8% (SD 8.9%) was observed with *S. aureus*, and +3.9% (SD 8.2%) for *A. baumannii*. With the device at Position B, none of the results for the AD ‘‘on’’ vs. ‘‘off’’ periods were significantly different ( $p > 0.05$ ).

**Conclusion:** Some modest reductions in airborne counts of the two pathogens were obtained when the AD device was close to the source of

bacterial dispersal. When the device was further away (2.7 m), there was no significant effect on viability. The AD device appears to have a limited range, which might explain the variations in results reported by others.

**P1117** Sporicides: the importance of in-use volume for effective surface disinfection

S. Ali\*, G. Moore, A. Wilson (London, UK)

**Objective:** We previously demonstrated the spread and persistence of *Clostridium difficile* spores during and after cleaning with sporicidal disinfectants. The aim of this study was to determine whether the in-use volume of two commercially available sporicides is sufficient for adequate disinfection of surfaces contaminated with *C. difficile*.

**Methods:** Microfibre cloth swatches (15 × 15 cm) immersed in either chlorine dioxide (140 ppm) or hypochlorite (1000 ppm) solution retained ~15 mL of sporicide. Subsequent wiping of a polypropylene surface deposited 20 µL of sporicide onto each consecutively wiped test area (25 cm<sup>2</sup>).

Sporicidal activity in suspension: microtitre wells containing 20 µL of test sporicide were inoculated with 10 µL of *C. difficile* spore suspension (10<sup>4</sup> CFU). Sporicidal activity was stopped after 0, 1, 3, 5, 10, 15, 30, 45, and 60 min by adding 20 µL of neutralising solution to each well (n = 5). Well contents were plated onto Brazier’s agar.

In-use activity: test areas (25 cm<sup>2</sup>) were inoculated with 10 µL of *C. difficile* spore suspension (10<sup>4</sup> CFU). Twenty micro litres of test sporicide was added and spread over the surface. After the appropriate contact time, test areas (n = 10) were sampled using cotton-tipped swabs. Each swab was transferred to 10 mL neutralizing solution prior to plating.

**Results:** When incorporated within the microtitre assay, 20 µL of chlorine dioxide or hypochlorite solution reduced the number of *C. difficile* spores to below the detection limit (20 CFU) within 5 min achieving a 3.69 and 4.72 log reduction respectively. In contrast, when 20 µL of sporicide was applied to a surface, a 5 min contact time resulted in spore numbers being reduced by between 0.42 (hypochlorite) and 1.15 (chlorine dioxide) log values. Increasing the contact time to 60 min achieved a further 1.00 (chlorine dioxide) and 1.20 (hypochlorite) log reduction. However, neither sporicide achieved a 3 log reduction and surfaces remained contaminated 60 min after cleaning.

**Conclusions:** When using microfibre cloths, the volume of sporicide (20 µL) transferred to a surface during cleaning, although effective when tested in suspension, was insufficient to decontaminate a surface. These findings confirm that suspension tests do not accurately reflect in-use activity and imply that sporicides, even when applied to a surface at the correct in-use concentration, may fail to reduce the number of *C. difficile* spores to a level that minimises patients’ risk of acquiring an infection.

**P1118** A pilot before and after intervention study on the effect of a liquid oxygen releaser sporicidal surface disinfectant compared with the usual disinfection procedure on the risk of nosocomial CDI in a university hospital, Austria, 2007–2011

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**Background:** *Clostridium difficile*, an important nosocomial pathogen is the leading cause of hospital-acquired diarrhea associated with high risk of fatal outcome.

A conventional, sporicidal surface disinfection applied in response to the occurrence of CDI was compared with daily application of a new sporicidal agent at two wards of a University hospital with respect to the risk reduction of CDI.

**Methods:** The routine CDI-surveillance data indicated a high endemic incidence of community-acquired (CA)-CDI and health-care associated (HA)-CDI (n = 118) at two wards (A, B) of an internal medicine department. CDI patients were classified into CA-CDI and HA-CDI according to ECDC definitions. Two intervention studies with a before-after design were performed at the wards A and B: ward A including four to six-bed-rooms and ward B single or two-bed-rooms. At ward A the "pre-intervention phase", in which the hospital policy based usual disinfection procedure (sporocidal surface disinfection in response to occurrence of nosocomial CDI only) was applied, took place from November 2007 until April 2009; the intervention phase, in which a new liquid oxygen releaser disinfectant with high sporocidal activity was daily used regardless of CDI occurrence, took place from May 2009 until July 2010. At ward B the pre-intervention phase lasted from August 2009 until June 2010 and the intervention phase from July 2010 until May 2011.

**Results:** A total of 67 cases of CDI (including 26 CA- and 41 HA-CDI cases) occurred at ward A and 51 cases (including 34 CA- and 17 HA-CDI cases) at ward B.

At ward A, the incidence rate difference of nosocomial CDI cases (0.4/1000 hospital days) observed was insignificant. At ward B, the incidence rate of nosocomial CDI at the end of the pre-intervention phase was 1.84/1000 hospital days compared with an incidence rate of 0.77/1000 at the end of the intervention phase, resulting in a rate difference of 1.07/1000 hospital days at borderline significance (p = 0.10) (see Table 1).

		Nosocomial CDI cases		*Per 1000 Person Time Units			**Two-Tailed	
		Beddays	Rate*	Rate Ratio	Rate Difference	P value		
Ward B	Intervention	5	0.42	0.42 (0.15-1.19)	-1.07 (-2.31, 0.17)	0.10		
	N	12	1.84					
Ward A	Intervention	16	1.45	0.78 (0.41-1.46)	0.40 (-1.42, 0.61)	0.45		
	N	25	1.85					

**Conclusion:** We concluded that the daily use of a liquid oxygen releaser disinfectant with high sporocidal activity at a ward with single or two bed-rooms was superior in preventing occurrence of nosocomial CDI to the sporocidal surface disinfection, applied as needed.

#### P1119 Equal efficacy of glucoprotamin vs. an aldehyde product for environmental disinfection in haematologic transplant unit: a prospective cross-over trial

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**Objectives:** Traditionally, aldehydes as broad spectrum microbicides have been used for surface disinfection in many settings. Glucoprotamin, an aldehyde-free disinfectant had been introduced as an active ingredient of disinfectants. In-practice data for surface disinfection with glucoprotamin are lacking and we therefore initiated a cross-over study to evaluate the effectiveness of glucoprotamin in comparison to the current standard of an aldehyde containing compound in a high risk hospital environment.

**Methods:** The study was a prospective, cross-over study with random allocation of the sequence of the disinfectant from October 2010 to December 2010. It was run at the hematological transplant unit of the University Hospital Basel, Switzerland. Access to this unit is limited, and protective care is applied for every patient. The study unit was split into two equal parts, the first part of the study lasted 4 weeks, after this time a cross over of the used disinfectants followed after a neutral cleaning of the ward. The used disinfectants were Deconex® 50 FF (Bode, Germany) and Incidin® Plus (Ecolab, Germany). Swabs of defined size were taken from five dedicated places in each room and cultured on blood agar and selective media for *Clostridium*, Gram-positives and *Staphylococcus aureus*.

**Results:** A total of 1528 samples (negative and positive) were available for analysis from surfaces disinfected with Deconex® 50 FF and 1540 (negative and positive) samples from surfaces disinfected with Incidin®

Plus. Ten percent of the samples with Deconex® 50 FF showed positive results (detection of pathogens) and 12% of the samples with Incidin® Plus. High counts of Enterococci were detected even shortly after disinfection with both disinfectants.

Tab: Species of pathogens detected in swabs

CFU/10C cm <sup>2</sup>	Deconex® 50 FF	Incidin® Plus	p-value
Overall bacterial counts	160	205	0.686
Mean (range)	0-8840	0-30028	
Enterococci	0	0	1.0
Mean (range)	0-2000	0-8000	
Other pathogens	0	0	1.0
Mean (range)	0-100	0-2000	

**Conclusions:** No significant difference was observed after applying the aldehyde, and aldehyde-free compound. *C. difficile* was not detected despite lack of activity against spores in the aldehyde-free compound. Enterococci – despite being highly susceptible to both compounds – did rapidly recolonize surfaces after successful disinfection. In conclusion, the aldehyde-free and the aldehyde-containing compound appear to be equally effective for disinfection of the hospital environment.

#### P1120 Microbial air contamination in operating theatres during hip and knee arthroplasties: first results from the ISChIA project

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**Objectives:** The ISChIA (Infezioni del Sito Chirurgico in Interventi di Artroprotesi) project has been funded by the Italian CCM (Centro Controllo Malattie, Ministry of Health) with the main aim of evaluating the role of microbial air contamination in the risk of surgical site infection (SSI) in hip and knee arthroplasties. This report describes the first results for microbial air contamination in the operating theatres (OTs) included in the survey, focusing on the role of the HVAC (Heating Ventilation and Air Conditioning) system, number of persons and number of door openings during interventions.

**Methods:** Microbial contamination was evaluated in the patient area, when empty and during operations. Passive sampling was used to determine the Index of microbial air contamination (IMA) (Pasquarella et al., 2000) and active sampling (SAS, International PBI) was used to determine CFU/m<sup>3</sup>. Tryptic Soy Agar with incubation at 37°C for 48 hours was used. SSI surveillance, which is still ongoing, is conducted according to the HELICS protocol (2004).

**Results:** From March 2010 to September 2011 a total of 1396 surgical procedures (60.4% hip and 39.6% knee arthroplasties) were included: among those 395 were performed in unidirectional airflow OTs, 256 in turbulent air ventilation OTs, 356 in turbulent air ventilation OTs with surgical team wearing Steri-Shield Turbo Helmet (Stryker). Microbial contamination values in empty OTs were: 0.0 IMA and 4 ± 3.6 CFU/m<sup>3</sup> (range 0–7) for in unidirectional flow OTs; 0.5 ± 1.1 IMA (range 0–2) and 11.7 ± 16.6 CFU/m<sup>3</sup> (range 0–23.5) for turbulent air ventilation OTs. During operations both IMA and CFU/m<sup>3</sup> increased: 7.3 ± 7.0 IMA and 50.3 ± 42.0 CFU/m<sup>3</sup> in unidirectional airflow OTs; 9.6 ± 13.5 IMA and 58.3 ± 41.2 CFU/m<sup>3</sup> in turbulent air ventilation OTs. In turbulent air ventilation OTs with surgical team wearing Steri-Shield Turbo Helmet 4.6 ± 4.1 IMA was obtained. In occupied OTs, a significant positive correlation between mean number of persons/number of door openings and microbial air contamination values was shown. A significant correlation between IMA and CFU/m<sup>3</sup> was also found.

**Conclusion:** Few data are available on air quality in OTs. Our preliminary results show a high variability in microbial air contamination values. Notably, the values obtained in unidirectional air flow OTs were higher than the recommended values (HTM03-012007; ISPEL, 2009; H+ Schweiz, 2007). The number of persons and of OT door openings are shown to affect the air bioburden.

**P1121** Emergent infection risk associated with hot water disinfection practices

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**Background:** Control measures that are effective in minimizing legionella will be effective against many, but not all other pathogens. The aim of the study was to analyze the effect of chlorine dioxide and monochloramine on the fitness of relevant waterborne pathogens.

**Methods:** During 8 years surveillance of our hospital water network, 101 *Legionella pneumophila* serogroup 1 environmental strains, isolated prior to and following the start of chlorine dioxide disinfection were genotyped according to Sequence-Based Typing (SBT) and pulsed-field gel electrophoresis (PFGE). Chlorine susceptibility was assessed in accordance with BS EN 1040:1997 and after chlorine exposure, the virulence expression profile of locus lvh, region rtx and mip gene, related to host invasion was analyzed. The intracellular growth kinetics in *Acanthamoeba polyphaga* was also evaluated.

Starting from November 2010 the monochloramine-based disinfection started in a new building; hot water samples were analyzed also for nontuberculous Mycobacteria (NTM) by using culture method and QPCR for 16S rRNA gene; presumptive culture positive for NTM were confirmed by sequencing of hsp65 gene and restriction fragment length polymorphism (RFLP) analysis.

**Results:** Our observations indicated the emergence of a prevalent clone of *Legionella pneumophila* serogroup 1 (SBT 269, pulsetype 2, 70% of isolates) isolated following the start of chlorine dioxide water disinfection. The persisting clone appeared to be more chlorine-tolerant, showing the ability to express more promptly and markedly the virulence genes and to infect more efficiently *Acanthamoeba polyphaga*.

Before the application of monochloramine, all samples resulted positive for legionella with a mean load of  $7.2 \times 10^3$  CFU/L, while NTM mean load was  $4.0 \times 10^1$  CFU/L. After disinfection, legionella was never isolated, while NTM loads increased up to  $1.1 \times 10^4$  CFU/L; *Mycobacterium gordonae* was the predominant specie.

**Conclusion:** The occurrence of emergent infection risk could be related to disinfection practices: long-term disinfection with chlorine dioxide seemed to induce an adaptative response of legionella, selecting more virulent clones; on the other hand, monochloramine reduced the risk of legionella infection, but selected opportunistic mycobacteria. The choice of an appropriate disinfection method represents a determining factor for reducing water-associated infections.

## Clinical epidemiology of nosocomial infections – general

**P1122** Are cardiac surgeons the only common denominator between surgical site infections and poor compliance to antibiotic prophylaxis in cardiothoracic surgery?

M. Hassan\*, T. Al-Badawi, L. Moorhouse, S. Staff, A. Guleri (Blackpool, UK)

**Background:** Surgical site infections (SSI) are associated with complications, increased mortality, length of stay and associated costs. Literature/guidelines suggest optimal prophylactic antibiotics reduce risk of postoperative infections. Lancashire cardiac centre (LCC), a tertiary unit within Blackpool Teaching Hospitals, serves a population of 1.5 millions population in the northwest England and undertakes 1800 cardiothoracic (CT) surgeries annually. Blackpool Teaching Hospitals operates a successful HAI programme with high emphasis on antibiotic stewardship. Regular audits; HAI surveillance (incl.SSI surveillance) and action plan help fine tune local policies. We present findings from audit of a new antibiotic prophylaxis guidance in CTsurgery, introduced as a consequence findings of SSI (incl postdischarge) surveillance in CT surgery.

**Methods:** Audit of compliance (all Cardiac/thoracic surgeries conducted over 1-week in Sept 2011) to a new antibiotic prophylaxis policy. New policy suggests use of Flucloxacillin or Teicoplanin, plus gentamicin at induction and cover 1st 24-hours post surgery as opposed to previous practice of variable duration use of cefuroxime +/- Teicoplanin subject to surgeon variation. Comprehensive SSI surveillance (sternal/leg wounds) in post CT surgery including post discharge, over January–March 2011, using health protection agency (HPA) criteria.

**Results:** Key findings from prophylaxis audit: 8-CT surgeons/9 anaesthetists involved in 29 CT surgeries (including 20 elective cardiac; two emergency open heart procedures, six thoracic and one epicardial pacing insertion via thoracotomy); patients – 23 males/six females; mean age 60.9 years; Mean hospital stay 6 days and no SSI during hospital stay; non compliance with policy in 48.2% (14/29) including no gentamicin use in 24% (7/29); extended prophylaxis in 27.5% (8/29); doxycycline use in 17% (5/29); tazocin use 3% (1/29). SSI surveillance January–March 2011 revealed a SSI rate of 9% (23/255) for sternal and leg wounds.

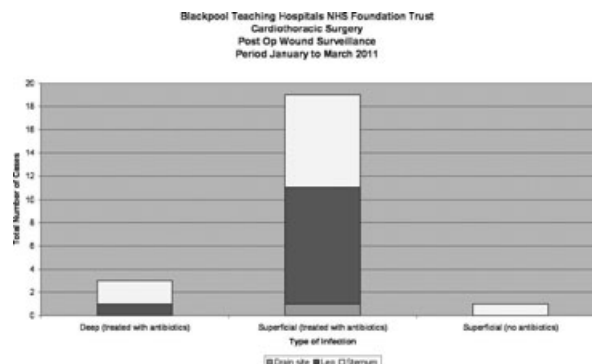
**Conclusions:** LCC is committed to reduce rates of SSI as a part of NHS quality initiatives to improve quality/patient care. The comprehensive SSI surveillance earlier this year reported a rate of 9% (sternal/leg wounds) including post discharge. Current audit has revealed issues including limited compliance to the new prophylaxis policy, poor documentation and gaps in the policy. This has been used to inform the HAI programme within CT surgery and action planned.

**P1123** Advancing quality in patient care initiative at a tertiary care cardiac unit: an innovative project drawing on surgical site infections (including post discharge) surveillance in cardiothoracic surgery to drive quality

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**Background:** Surgical site infections (SSI) are associated with complications, increased mortality, length of stay (LoS) and associated costs. Literature suggests SSI comprise up to 20% of all healthcare associated infections (HAI); 5% of all surgeries; doubles the cost of care and extends LoS upto 6.5 days. Lancashire cardiac centre (LCC), a tertiary unit within Blackpool Teaching Hospitals, serves a population of 1.5 million in the northwest England and undertakes 1800 cardiothoracic (CT) surgeries annually. We present findings from an innovative project on advancing quality. This includes gathering high quality data, a comprehensive SSI surveillance (including post discharge and both sternal/leg wounds) over 3-months to establish baseline SSI rate, assess any epidemiological links, impact of patient or healthcare factors and baseline to evaluate impact of interventions.

**Methods:** Analysis of database on 255 CT surgeries over 3-months (January–March 2011). SSI surveillance included both sternal and leg wounds. Surveillance period included up to 30 days post surgery and post discharge data. Data was collected from patient questionnaire



(posted after 30 days and postage paid), readmissions, followup clinics, GP consultations and contacting patients if no response. HPA standard criteria for definition of SSI was used.

**Results:** *Key findings:* Total of 255 CT surgeries during 3-months, 8-CT surgeons undertook between 22 and 38 surgeries/consultant; 7.8% (20/255) patients had a SSI including deep (3/20) and superficial (17/20) infections. Fifty percent (10/20) had leg wound infections; 50% (10/20) had SSI within 1st 10 days; predominant SSI noted in Males (3:1); Age range 71–80 years; BMI range 25–30; ASA scores of 3+.

**Conclusions:** LCC is committed to reduce rates of SSI as a part of commissioning for quality and innovation (CQUIN) schemes to improve quality/patient care. An innovative project was initiated with collaboration between cardiac surgery – microbiology – infection control. The aim was to gather high quality baseline data to drive a variety of interventions and measure the impact against baseline SSI rates. These interventions are in various stages and include new antibiotic prophylaxis in cardiothoracic surgery guidance, change of skinprep from povidone iodine-IPA to chlorhexidine-IPA; dedicated link nurse conducted hand-hygiene compliance audit to secret shopper style audits; enhanced Microbiology ward rounds; antibiotic compliance audits. Details to be presented.

**P1124 Debridement and implant retention in periprosthetic joint infections: could long-term antibiotic therapy improve the outcome?**

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**Introduction:** Several medical and surgical treatment strategies are currently available for the treatment of periprosthetic joint infections (PJI). Retention of the implant is gaining greater acceptance when the joint function is adequate. Debridement and implant retention (DIR) is an attractive strategy but the outcome may be not better or even could be worse than in other treatment options.

**Objective:** To evaluate the outcome of prolonged antibiotic treatment of PJI with DIR.

**Materials and methods:** A retrospective cohort analysis (from a prospective register of patients with PJI treated with DIR) was carried out from 10/91 to 9/10. Patient management was not previously standardized but on individual case evaluation and according to the criteria of the treating team physicians. STATA 10 was used for statistical analysis.

**Results:** One hundred sixty one episodes (ep) of PJI treated with DIR in 136 patients were evaluated; it accounts for 37.9% (161/424) of all PJI seen in the period considered. Mean age: 67.3 year-old (SD + 12.1), 55.9% were female. Co-morbidities (rheumatoid arthritis, diabetes, cancer) 34.7% of the ep. Prosthesis location: hip 52.2%, knee 44.1%, shoulder 3.1% and elbow 0.6%. Early PJI (<30 days after surgery) 65 ep (40.4%). Clinical findings: soft tissue swelling 65.8%; pain 64.0%, purulence discharge 44.7%, sinus tract 34.8%, fever 29.8%. Microbiology: grampositive cocci 67.1% (*S. aureus* 45.3%), gramnegative bacilli 16.7%, polymicrobial 16.8%. Antibiotic therapy: 27 weeks (median; IQR 25–75% = 16–48); route of administration: parenteral/oral 53.4%, only oral 39.1%, only parenteral 6.2%. Follow-up: 26 months (median; RIQ 25–75%; range = 17–52.5). DIR was performed by surgery in 159 ep; 2 ep. underwent arthroscopy. Successful outcome (cure/improvement) 56.5%, relapse 36.0%, failure 7.5%.

In the univariate analysis the age, gender, co-morbidities, sinus tract, prosthesis location, time of PJI since surgery and type of microorganism were not related to a better/worse outcome. Prolonged antibiotic therapy more than 24 weeks in early PJI showed to be related with a more successful outcome ( $p = 0.001$ ).

**Conclusion:** In PJI treated with DIR, a prolonged antibiotic therapy should be recommended to improve to outcome.

**P1125 Differential risk of post-surgical wound infection according to patient ethnicity**

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**Objectives:** A number of studies have identified differences in risk of infection according to patient ethnicity. Such studies have been largely focused on community-acquired infections with less work undertaken to examine risks for infections acquired in healthcare settings. As a means to investigate whether there is any evidence of excess risk, we investigated risk of wound infection surgical patients according to ethnic group.

**Methods:** Surveillance data for 2009/2010 submitted by NHS hospitals participating in the HPA surgical site infection surveillance for the following categories were extracted for analysis: coronary artery bypass graft; large bowel surgery; vascular surgery; reduction of long bone fracture. Patient preoperative health was recorded using the American Society of Anesthesiologists score. In parallel, data from the NHS Information Centre Hospital Episode Statistics for the same period and surgical categories were extracted to provide additional data fields, including patient ethnicity. Stepwise logistic regression analyses were undertaken to assess the relationship between risk of infection (detected during the inpatient stay or through readmission) and patient ethnicity with adjustment for other patient and surgical risk factors.

**Results:** Surveillance records for a total of 14 277 patients undergoing one of the four selected categories of surgery were submitted in 2009/2010, of which 73% ( $n = 10\,383$ ) were successfully matched to a corresponding HES record. Patient ethnicity was recorded for 9738 of the matched records, of which 93% were coded as being white, 4% Asian, 1% black, 1% Chinese and 0.4% of mixed ethnicity. With exclusion of the small number of patients of mixed ethnicity ( $n = 42$ ), rates of post-surgical infection across all surgical categories combined varied significantly ( $\chi^2$  (3 df) = 8.05,  $p = 0.045$ ) according to patient ethnicity, being highest in black patients (9%) followed by Asian (6%), white (5%) and patient of Chinese or other ethnic group (2%). Adjustment for additional patient factors (BMI, social deprivation, age, sex) and operational factors (category of surgery, duration of operation, wound class, ASA score) removed any significant association between ethnicity and risk of infection although odd ratios remained elevated for black (OR = 1.73, 95% CI: 0.84–3.56) and Asian patients (OR = 1.31, 95% CI: 0.82–2.07) compared to white patients.

**Conclusion:** Unadjusted risk of post-surgical wound infection was higher in non-white vs. white patients. Adjustment for additional factors diminished the association although odds ratios remained elevated suggesting a possible residual increased risk. Further analyses examining other categories of surgery should be undertaken to better characterise the association between ethnicity and risk of surgical site infection and the necessity of targeted prevention measures.

**P1126 Does preoperative statin therapy reduce postoperative infectious complications in patients undergoing cardiac surgery? A systematic review and meta-analysis**

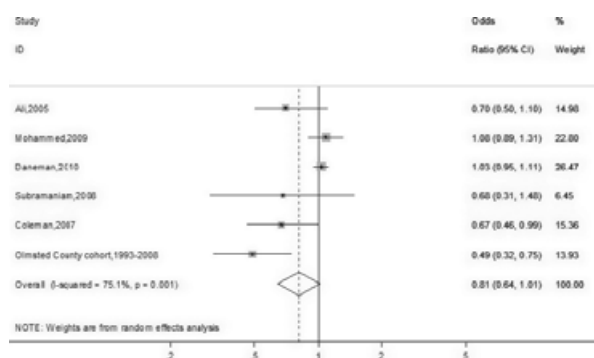
I. Tleyjeh\*, F. Alasmari, M. Riaz, A. Bin Abdulhak, L. Baddour (Riyadh, SA; Rochester, US)

**Objective:** Post cardiac surgery infectious complications are a major problem with significant morbidity and mortality. Statins have been shown to have a modulation impact on both innate and adaptive immune system and anti-inflammatory effects, as well as direct inhibitory effects on pathogenic microorganisms. Having this pleiotropic effect, statins might help in the prevention of post-surgical infections. We sought to systematically examine the association between statins use and the risk of post-operative infectious complications in patients who undergo cardiac surgeries.

**Methods:** We searched OVID MEDLINE from 1950 to 2010; EMBASE from 1988 to 2010; Scientific Web of Science (inception through February 2011), and Elsevier Scopus (inception through February 2011); for any comparative studies examining the association

between statins use and risk of post-operative infections in patients who undergo cardiac surgeries. Two reviewers independently extracted data. We contacted study's authors for missing information. We conducted a random-effects meta-analysis of individual studies' odds ratios (adjusted for potential confounders). I-squared was used to examine for heterogeneity.

**Results:** We identified six cohort studies. All studies were published between 2005 and 2010. Three studies were conducted in Canada and three in USA. Three studies were single-center studies, two were population-based, and one was unclear. Statin exposure ascertainment was based on a review of admission medication list, or prescription databases. Infectious outcomes were heterogeneous and included surgical site infections within 30 days, serious infections (sepsis and deep sternal wound infection) or any postoperative infection. Use of statins in preoperative period was associated with a trend for reduced incidence of post-operative infections in patients who underwent cardiac surgeries (OR 0.81; 95% CI [0.64–1.01]),  $I^2 = 75%$  (Figure 1).



**Conclusion:** Our meta-analysis suggests that statins use may be associated with a lower risk of post-operative infectious complications after cardiac surgeries. Given the safety of statins and the major sequelae of post-operative infections, our results merit further validation in randomized controlled trials.

#### P1127 Incidence and microbiological characterisation of infections related to implantation of joint prostheses in São Paulo, Brazil, over a 5-year period

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**Objectives:** Describe the incidence and profile of agents involved in surgical site infections secondary to implantation of joint prostheses at an orthopaedic reference centre in São Paulo, Brazil, over a 5-year period.

**Methods:** This survey included all procedures for implantation of joint prostheses of the hip, knee and shoulder between January 2006 and December 2010. All patients received antimicrobial prophylaxis consisting of cefuroxime for 24 hours. The definition of surgical site infections followed the criteria of the Centers for Disease Control and Prevention, United States (USA). For microbiological characterization, only the agents obtained from cultures of samples of bone, soft tissue, synovial fluid, joint capsule or exudate that had been collected after antisepsis and debridement were taken into consideration. The cumulative antibiograms followed the standards recommended by the Clinical and Laboratory Standards Institute, USA.

**Results:** Over the study period, 2085 procedures to implant joint prostheses were carried out. Superficial or deep surgical site infections were diagnosed in 133 cases, with an incidence of 6.4%. It was possible to isolate the causative agent in 49% of the cases, and the rates of

positive cultures were 37%, 39% and 66% for cases relating to the shoulder, knee and hip, respectively. For these cases, the mean number of isolates was 1.35 microorganisms per patient. Among the 89 isolates, 50% were Gram-positive cocci (GPC), 48% Gram-negative bacilli (GNB), 1% fungi and 1% mycobacteria. The most frequent agents were: *Staphylococcus aureus*, with 27 isolates (30%); Coagulase-negative Staphylococci, 13 isolates (15%); *Acinetobacter baumannii*, 12 isolates (14%); *Klebsiella pneumoniae*, 11 isolates (13%) and *Pseudomonas aeruginosa*, eight isolates (9%). Other species of GPC were seen in four isolates (5%) and other species of GNB, in 12 isolates (14%). Table 1 shows the susceptibility profiles for the most frequent agents.

Table 1 – Susceptibility profiles for the most frequent isolates

Pathogen/antimicrobial tested	Antimicrobial susceptibility (%)
<i>S. aureus</i> (n = 27)	
CEFOXITIN	100
CLINDAMYCIN	63
CIPROFLOXACIN	63
LINEZOLID	100
METICILLIN	75
TRIMETHOPRIM/SULFAMETOXAZOLE	77
TEICoplanin	100
VANCOMYCIN	100
CEFOXITIN	100
<i>Coagulase-negative Staphylococci</i> (n = 13)	
CLINDAMYCIN	30
CIPROFLOXACIN	37
LINEZOLID	100
METICILLIN	9
TRIMETHOPRIM/SULFAMETOXAZOLE	57
TEICoplanin	80
VANCOMYCIN	100
<i>A. baumannii</i> (n = 12)	
AMIKACIN	25
AMPICILLIN/SULBACTAM	61
CEFEPIME	17
CEFTAZIDIME	20
CIPROFLOXACIN	19
IMIPENEM	42
MEROPENEM	34
COLISTIN	100
<i>K. pneumoniae</i> (n = 13)	
AMIKACIN	91
AZTREONAM	23
CEFEPIME	34
CEFTAZIDIME	18
CEFOTAXIME	13
CIPROFLOXACIN	27
ERTAPENEM	86
IMIPENEM	100
MEROPENEM	86
PIPERACILLIN/TAZOBACTAM	29
<i>P. aeruginosa</i> (n = 8)	
AMIKACIN	50
AZTREONAM	43
CEFEPIME	63
CEFTAZIDIME	63
CIPROFLOXACIN	50
IMIPENEM	63
MEROPENEM	58
PIPERACILLIN/TAZOBACTAM	67
COLISTIN	100

**Conclusions:** Incidence of surgical site infections relating to implantation of joint prostheses was 6.4%. It was possible to isolate the causative agent in 49% of the cases. The greatest numbers of positive cultures were in cases relating to the hip and the smallest numbers, to the shoulder. Although *S. aureus* was the most frequent agent, the prevalence of GNB was high, and the high resistance profile of these isolates to the antimicrobials that were tested (including carbapenems) was highlighted.



**P1128** Prevalence and risk factors of hospital-acquired infections in intensive care units: retrospective analysis from a USA hospital database

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**Objectives:** To determine the prevalence of intensive care unit hospital-acquired infections (HAI), identify the risk factors and determine the inpatient consumptions for these infections in a U.S. hospital database.

**Methods:** A retrospective cohort study was undertaken using hospital database from the Premier Perspective of adults ( $\geq 18$  years old) admitted with a stay  $\geq 48$  hours in 2007. Three HAI known to be high drivers of hospital consumption were followed: bloodstream infection (BSI), surgical site infection (SSI), Hospital-acquired pneumonia (HAP) including ventilator-associated pneumonia (VAP). Case-subjects were defined as patients with NI. Control subjects without NI consisted of all patients who did not meet the definition for case-subject. Inpatient consumption was limited to hospital length of stay (LOS) and inpatient mortality rates. Logistic regression for prevalence odds ratio (OR) was used to assess the effects of independent variables as risk factors for the presence or absence of infection.

**Results:** Out of 5 426 276 patients meeting the entry criteria in 2007 in the Premier database, 8.5% of these patients experienced HAI. Focusing on ICU population of 463 491 patients, 119 616 (25.8%) patients developed HAI. HAP/VAP: 16.6%, BSI: 13.9%, and SSI: 1.4%. Patients with HAI were mostly elderly (57% vs. 52%), admitted via emergency room (71% vs. 56%), a high bed occupation, presented with more severe illnesses, more risk of mortality, and often underwent device procedures as: central catheter placements (49% vs. 18%) or mechanical ventilation hook-ups (42% vs. 15%) compared to patients without NI. The inpatient-mortality rate and LOS were higher in patients with HAI (18.5% vs. 4.5% and 15.8 + 17.4 days vs. 8.1 + 7.6 days), respectively compared to patients without HAI. Central catheters and mechanical ventilations were identified as the two main risk factors for HAI with OR = 3.4 CI (3.3–3.4) and OR = 2.8 (2.7–2.8), respectively.

**Conclusions:** Hospital-acquired infections are common and often associated with two main specific risk factors of mechanical ventilations and central catheters, which subsequently lead to longer LOS and higher mortality rates. These findings illustrate the changing nature of hospital patient population over the years that are more vulnerable to nosocomial infections, which demand preventive measures to reduce the prevalence and risk factors of these infections on the elderly.

**P1129** Transmission dynamics of *Staphylococcus aureus* and *Pseudomonas aeruginosa* in a burn centre during a 6-month period of patient monitoring by molecular typing

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**Introduction:** Patients hospitalized with burn wounds are at increased risk of developing microbial colonization and infection. Infections may lead to prolonged hospitalization, an increased morbidity and mortality and disturbed skin graft acceptance. Knowledge of colonization dynamics is limited and standard bacteriological techniques are not designed to detect and monitor transmission of bacterial strains. Therefore, the use of appropriate molecular typing methods may provide more insight into these dynamics and can result in the design of targeted infection prevention programs.

**Objective:** To obtain more insight into the transmission dynamics of *Staphylococcus aureus* (SA) and *Pseudomonas aeruginosa* (PA) in a burn centre.

**Methods:** From February till August 2011 all (n = 136) patients admitted at the Burn Centre, intake specimens were routinely taken from the nose, throat, and perineum on admission and from wounds and

infection sites on admission and thereafter twice a week. In addition, 56 nurse practitioners and surgeons of the Burn Centre/ICU were screened for SA and PA (nasal) carriage. A cluster was defined as a group of at least two patients carrying an identical SA or PA strain. During a 6 month period, all SA and PA isolates were typed by MLST and AFLP.

**Results:** Forty-eight out of 136 patients (35.3%) were positive for SA at any moment during hospitalization. We identified 27 multilocus sequence types and 38 AFLP types. Ten clusters of SA-positive patients were observed (range 2–8 patients). One third of patients were SA carriers at admission. Half of these carriers became infected by their own endogenous SA strain and in at least 67% of the patients, evidence for exogenous SA infection was observed. Three health care workers (HCWs) from the burn wound department shared an SA-strain with one of the clusters. PA was found in 11.6% of patients. In 50% of them, an identical PA was found in both an intake sample at admission and in a wound, indicating endogenous infections. None of the HCW were PA carrier. In 44% of patients carrying PA, evidence for exogenous infection of PA belonging to two large clusters was observed.

**Conclusions:** The results of this study show a high rate of endogenous and exogenous infections during a 6 month period in burn wound patients. The routine practice of molecular typing demonstrates the endogenous and exogenous spread in a burn centre and opens the possibility of more targeted infection prevention interventions.

**P1130** Evaluation of clinical and economic outcomes associated with infective endocarditis at an academic medical centre

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**Objectives:** Infective endocarditis (IE) is a prevalent problem in health care associated with increased mortality, resource utilization and costs. We evaluated the epidemiology, clinical outcomes, and costs associated with IE at our institution.

**Methods:** Adult patients (pts) diagnosed with IE (Duke Criteria) were evaluated between January 2009–December 2010. Demographic characteristics, ICU admission, ICU length of stay (LOS), mortality, and hospital costs were evaluated. Statistical analysis included Fisher's exact or Wilcoxon ranksum test. Data are presented as percent or median (IQR).

**Results:** One hundred twenty-eight pts were identified for inclusion: 55% male, age 56 (40–67) years, 54% were admitted to the ICU. Comorbidities included: 74% cardiovascular disease, 38% renal insufficiency, 36% diabetes mellitus, and 19% COPD. Most pts had native valve IE (97%) with the aortic (43%) and mitral (48%) valves most frequently infected. Methicillin-resistant *staphylococcus aureus* (MRSA) (27%), methicillin-sensitive *staphylococcus aureus* (MSSA) (18%), *Enterococcus faecalis* (*E. faecalis*) (10%), and *Streptococcus viridans* (*S. viridans*) (9%) were the most common organisms. Complications included: stroke (13%), embolization other than stroke (5%), heart failure (9%), intracardiac abscess (3%), and  $\geq 1$  complication (17%). Valve replacement was performed in 23%. ICU LOS and hospital LOS were 6 (3–10) and 13 (9–22) days, respectively. Overall, hospital mortality was 23%. Hospital costs were \$39 455 (20 596–82 820). Costs were further analyzed based on valve replacement and organism.

Organism	Surgery (n=29)	No Surgery (n=99)	P-value
MRSA	562,935 (45,648-80,714)	538,828 (18,006-77,870)	0.35
MSSA	581,139 (54,275-95,308)	534,297 (21,900-44,921)	0.01
E faecalis	5204,691 (136,243-439,271)	536,430 (30,188-43,539)	0.02
S viridians	595,691 (74,351-111,471)	526,285 (19,319-33,230)	0.09

**Conclusions:** IE is associated with a high complication rate, increased mortality, and significant health care costs. Successful management of IE requires a multidisciplinary stewardship and surgical approach. Cost

outcome data favored surgery for staphylococci but not for *E. faecalis* or *S. viridans*. The significance of these findings warrants further investigation.

**P1131** Characteristics of meningitis/ventriculitis associated to neurosurgical procedures at a Brazilian teaching hospital: a retrospective analysis from 2005 through 2010

M.M. Alves\*, D.B. Assis, T. Guimaraes, M.P. Freire, F.C.G. Pinto, S.F. Costa, I. Boszczowski (Sao Paulo, BR)

**Objectives:** To describe characteristics of meningitis/ventriculitis associated to neurosurgical procedures at a Brazilian Teaching Hospital. **Methods:** Patients with meningitis/ventriculitis, microbiologically confirmed were included retrospectively from January 2005 through November 2010. Hospital charts were reviewed and liquor characteristics, isolated microorganisms and resistance profile were described.

**Results:** There were 72 microorganisms isolated in a total of 66 episodes of meningitis/ventriculitis. The 66 cases represent 45.8% (66/144) of meningitis/ventriculitis reported in the period. Median between meningitis/ventriculitis occurrence and last neurosurgical procedure was 11 days (range 1–47 days). Glycorrhachia/serum glucose had a median of 0.23, (range 0.0076–0.93). In 88.8% of the episodes this ratio was below 0.66. CSF lactate had a median of 61 mg/dL, range (16–179 mg/dL). The non-fermenting Gram-negative accounted for the majority (40%) of the infections followed by Gram-positive (30%) and Enterobacteriaceae (25%). *Acinetobacter* spp. was the leading pathogen (23.6%), followed by *P. aeruginosa* (12.5%), *Staphylococcus aureus* and coagulase negative staphylococci (11.1% each one). Among *Acinetobacter* sp. isolates, only 37.5% were susceptible to imipenem and 28.6% to meropenem. Among *Pseudomonas aeruginosa*, 22.2% were susceptible to imipenem and 44.4% to meropenem. Before 7 days after hospitalization, all Gram negative isolates were susceptible to ceftazidime. After 7 days, 50% of non-fermenting Gram negatives were cephalosporin resistant and carbapenem susceptible and 50% of them, were multi-drug-resistant. The most prescribed empiric therapy was vancomycin plus meropenem. And the second one was vancomycin plus ceftazidime. The empiric therapy was correct in 65% of the cases. Twenty-eight (42.4%) patients died. Deaths related to non-fermenting Gram negatives were 57% and related to *Acinetobacter* spp. was 47%. *Acinetobacter* spp. was isolated in 25% among patients who died, and all but one were multi-drug-resistant.

**Conclusion:** Differently from other centers, there is a high prevalence of non-fermenting Gram-negative bacteria resistant to carbapenems causing meningitis after the seventh day of hospitalization at our institution. There is a high mortality rate associated to non-fermenting Gram negatives, in special related to *Acinetobacter* spp. Highly effective preventive measures related to this pathogen are needed.

**P1132** Incidence of hospital-acquired pneumonia, bacteraemia and urinary tract infection in patients with haematological malignancies, 2004–2010: a surveillance-based study

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**Introduction:** Patients with haematological malignancies are at high-risk of hospital-acquired infection (HAI). The objective was to describe the incidence trends of HA pneumonia, bacteraemia and urinary tract infection (UTI) in a university haematology department between 2004 and 2010.

**Methods:** A prospective surveillance of HAI was performed in a university haematology department composed of 42 single-bed rooms. All patients hospitalized between 1 January 2004 and 31 December

2010 for at least 48 hours were included. Definitions of HAI were based on a standardized protocol. The analysis was restricted to the first HAI. The incidence was the number of events per 1000 patient-days at risk. Univariate and multivariate Poisson regressions were fitted to assess temporal trends, adjusted on age, gender, aplasia, exposure to central venous catheter, haematological disease and treatments.

**Results:** Overall, 3355 patients accounting for 58 063 patient-days at risk were included. A total of 1055 (31.4%) patients had at least one HAI. The overall incidence of HA pneumonia, HA bacteraemia, and HA-UTI were respectively 3.3 (95% CI: 2.8–3.8), 12.0 (95% CI: 11.1, 12.9), and 2.9 (95% CI: 2.5, 3.4) per 1000 patient-days at risk. HA bacteraemia incidence increased of 11% (95% CI: +6%, +15%,  $p < 10^{-3}$ ) per year, independently of aplasia, central venous catheter and haematological disease. The incidences of HA pneumonia and HA-UTI were stable. In acute myeloid leukaemia patients, incidences of HA pneumonia, HA bacteraemia and HA-UTI were respectively 4.2, 12.9, and 2.6 per 1000 patient-days at risk. In patients with acute lymphoid leukaemia, incidences of HA pneumonia, HA bacteraemia and HA-UTI were respectively 1.8, 13.4 and 3.5 per 1000 patient-days at risk. The most frequently isolated pathogens were *Aspergillus* spp. (59.2%) and *Candida* spp. (7.8%) for pneumonia, coagulase negative *Staphylococcus* (44.2%) and *Candida* spp. (4.6%) for bacteraemia and enterobacteria (60%) for UTI.

**Conclusion:** Incidence of HA pneumonia and HA-UTI remained stable. The improvement of infection control measures could have been counterbalanced by exposure to more aggressive chemotherapies. Incidence of bacteraemia increased suggesting that other factors than central venous catheter exposure could explain this trend. More investigation are needed to explore in depth such findings.

**P1133** Surveillance of the central venous catheter-related bloodstream infections in haematological patients

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**Objectives:** Analysis of the frequency, risk factors and etiology of the central venous catheter-related bloodstream infections (CVC-BSIs) in haematological patients.

**Materials and methods:** We retrospectively analysed data related to the risk factors and etiology of CVC-BSIs for the time period from January 2006 until September 2011. The duration of catheter insertion, the number of CVC-BSIs and incidence density rates per 1000 catheter-days were calculated. Catheter tip cultures were performed according to Maki's semi-quantitative method. Bacterial strains were isolated and identified according to standard methods. All staphylococcal cultures were subsequently tested for methicillin susceptibility with a cofoxitin disc diffusion test.

**Results:** The total of 2500 central lines were analysed. The mean duration of CVC insertion decreased from 19 days in 2006 to 14.5 days in 2011. During the study period 97 CVC-BSIs were reported. The incidence density rate decreased from 4.5/1000 catheter-days in 2006 to 1.3/1000 catheter-days in 2011. Among cultured microorganisms ( $n = 93$ ) the most common were staphylococci – 67 (72.0%) strains. Methicillin-resistant *S. epidermidis* (MRSE) comprised 42/50 (84.0%) strains, while methicillin-resistant *S. aureus* (MRSA) – 4/13 (30.8%) strains. Enterococci constituted 8/93 (8.6%) isolates, enteric rods – 8/93 (8.6%), and *A. baumannii* – 2/93 (2.1%) strains. Eight strains of *C. albicans* were cultured (8.6% of all isolates).

**Conclusions:** During the observation period the mean duration of CVC insertion decreased from 19 days in 2006 to 14.5 days in 2011. At the same time the incidence density rate decreased from 4.5/1000 catheter-days in 2006 to 1.3/1000 catheter-days in 2011. The main etiological agents were staphylococci; MRSE comprised 84% of *S. epidermidis* strains. *C. albicans* strains amounted to almost 9.0% of all isolates, while no strains of *C. parapsilosis* were cultured. Gram-negative non-fermenting rods were very uncommon (2.1%).

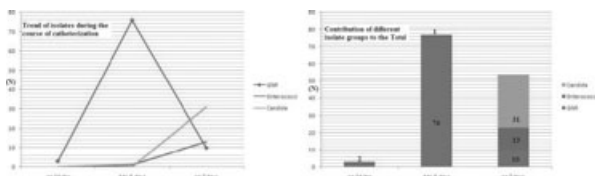
**P1134** A study of microbial aetiology of catheter-associated urinary tract infection in intensive care unit and medical wards from a tertiary care teaching hospital in New Delhi, India

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**Objectives:** The study aimed to find the rate and microbial agents of Catheter Associated Urinary Tract Infection (CAUTI), but not mere asymptomatic and insignificant bacteriuria, in Intensive Care Unit (ICU) and Medical Wards (MW) as per the CDC CAUTI guidelines.

**Methods:** Two hundred adults with urethral catheters were included with 100 each from the MW and ICU of MAMC and LN Hospital, Delhi, India, from August 2010 to September 2011. Three samples were aseptically collected from each patient – within 24 hours, in the 1st week between 4th and 6th day and beyond 1 week of catheterization. The unbiased allocation of patients was a key step. Everyday, ‘‘Allocation Concealment’’ was done by obtaining initial blanket consent from all eligible and willing patients. Blocked Randomization was performed to include a minimum of one patient each time from each of four block categories, viz. the MW men and women blocks and the ICU men and women blocks. The day for collecting the 1st sample were selected by simple randomization every week, as the patients admitted under different units in the different days of the week were hypothesized to have varying levels of stringency and vigilance regarding catheter care and antibiotic usage. Sixty-five cases were unavailable for the 3rd sample due to catheter withdrawal, death or discharge. Semi-quantitative culture followed by identification of isolates was done by conventional techniques and confirmed by VITEK2 COMPACT<sup>®</sup> (Biomerieux).

**Result:** Only three of 200 (1.5%) patients in the 1st sample, 77 (30) from ICU and 47 from MW) from the 2nd sample (38.5%) and 41 of 135 patients (14 from ICU and 27 from MW) with two isolates from 13 and one isolate from 28 cases from the 3rd sample (30%) fitted to the CDC CAUTI criteria. The isolate characteristics were quite different in 2nd and 3rd samples. 98.7% of the 2nd sample isolates were gram negative rods (N), including *Escherichia coli* (36), *Klebsiella pneumoniae* (19), *Enterobacter* spp. (7), *Pseudomonas aeruginosa* (4), *Acinetobacter baumannii* (3), *Proteus mirabilis* (2), *Providencia stuartii*, *Burkholderia cepacia*, *Serratia fonticola*, *Citrobacter freundii*, *Pantoea agglomerans* and *Strenotrophomonas maltophilia* (one each). Whereas, among the 3rd sample isolates, 57.4% were *Candida* spp. (31), 24% were *Enterococcus* spp. (13) and only 18.5% were gram negative rods.



**Conclusion:** A clear shift from gram negative predominant isolates in the first week to *Candida* and Enterococcal isolates beyond 1st week in CAUTI patients was observed with broad spectrum antibiotic usage.

**P1135** Bloodstream infections in a neonatal intensive care unit: a 2-year study

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**Objectives:** To present the experience with bloodstream infection (BSI) in a 30-bed, university-affiliated, level III-IV NICU at a large pediatric hospital in Athens.

**Methods:** The charts of all neonates with culture-proven BSI admitted to our NICU over 2 years (2009–2010) were retrieved. Clinical, epidemiologic and microbiologic characteristics were recorded.

**Results:** A total of 787 neonates (58.4% boys) were admitted during the study period. Overall, 128 episodes of BSI, due to 131 pathogens were diagnosed in 88 neonates (63.6% boys, 56.8% preterms). Of those, 28/88 (31.8%) presented congenital anomalies, 22/88 (25%) respiratory distress and 19/88 (21.6%) necrotizing enterocolitis. Average stay before infection was 20.8 days (0–195) and mean hospitalization was 60.4 days (0–396), significantly higher than overall mean hospitalization (18.4 days). The mean incidence of BSI was nine cases/1000 patient days. Overall, 128 bacteria (Gram positive 96 [75%], Gram negative 32 [25%]), and three fungi (*Candida albicans* 2, *Malassezia furfur* 1) were recovered. Analytically, the following pathogens were identified: *Staphylococcus* spp. (81/96, 84.4%), *Enterococcus* spp. (15/96, 15.6%), *Escherichia coli* (8/32, 25%), *Klebsiella pneumoniae* (7/32, 21.8%), *Enterobacter aerogenes* (6/32, 18.7%), *Enterobacter cloacae* (5/32, 15.6%), *Serratia marcescens* (2/32, 6.25%), and *Serratia liquefaciens*, *Klebsiella oxytoca*, *Proteus mirabilis*, *Alcaligenes xylosoxidans* (1/32 each, 3.1%). Polymicrobial bacteremia was diagnosed in three cases. Extended-spectrum beta-lactamases (ESBL) were detected in *K. pneumoniae* (71%), *Escherichia coli* (13%) and *E. cloacae* (80%). Overproduction of AmpC beta-lactamases was identified only in one *E. aerogenes* strain. All coagulase-negative staphylococci (CNS) were susceptible to linezolid and vancomycin, whereas 16/80 (20%) were non susceptible to teicoplanin. Multidrug resistant isolates, such as VRE, MRSA, carbapenemases producers were not identified. A cluster of five nosocomial cases due to *K. pneumoniae* ESBL was detected from February to May 2009, and a cluster of four nosocomial cases due to *E. cloacae* ESBL from July to November 2010. The crude mortality rate was 11.4%, significantly higher than the overall mortality (3.6%).

**Conclusions:** Neonatal bacteremia was mainly caused by CNS. Resistance to commonly used antibiotics was exhibited especially by Gram negative bacteria. Strict infection control measures were important to limit the spread of ESBL producers in the NICU.

**P1136** Hospital-acquired pneumonia in non-ICU patients in a rural general hospital

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**Objectives:** Hospital-acquired pneumonia (HAP) is a major subgroup of all hospital acquired infections and an important public health problem. It usually occurs in patients with underlying diseases, increases nosocomial morbidity and mortality, prolongs hospital stay and raises the cost of health care. The aims of this study were to determine the prevalence, aetiology and outcome of HAP in our rural general hospital.

**Methods:** This is a retrospective and descriptive study. We reviewed the medical files of all inpatients fulfilling clinical criteria of HAP, during last 5 years. Demographical data, co-morbidities, clinical and laboratory findings, pathogens identified and outcome were registered and analyzed.

**Results:** In a total of 3156 patients, 127 (4.02%) had HAP, 76 (59.8%) male and 51 (40.2%) female with mean age of  $74 \pm 7.3$  years. Eighty-four (66.2%) of HAP cases were diagnosed at Internal Medicine wards and 43 (33.8%) at Surgical wards. The main identified co-morbidities were: Stroke (67.7%), diabetes mellitus (42.5%), malignancy (29.9%), heart failure (26.8%), chronic renal failure (25.2%), chronic pulmonary disease (20.5%) and alcohol abuse (10.2%). Etiologic diagnosis was achieved in 62 (48.8%) patients. Mixed aetiology was considered in 7 (5.5%) cases. The most common isolated pathogens were: Methicillin Resistant *Staphylococcus* in 23 (18.1%) patients, Enterobacteria (*E. coli*, *Enterobacter* spp., *K. pneumoniae*) in 22 (17.3%), *Acinetobacter baumannii* in 6 (4.7%), *Pseudomonas aeruginosa* in 12 (9.4%) and

*Enterococcus* spp. in 7 (5.5%) patients. Complications were observed in 61 (48%) cases with respiratory failure in 33 (26%) cases, septic shock in 12 (9.5%), pleural effusion in 11 (8.7%) and renal failure in 5 (3.8%) cases. The mean duration of hospitalization was  $23 \pm 5.6$  days. The mortality rate attributed to HAP was 29.9%.

**Conclusions:** As in other European countries, in our Hospital HAP affects mostly elderly patients with severe underlying diseases predisposing them to aggressive pathogens that vary accordingly to the hospital flora of an institution. Guidelines have been developed in effort to deal with this issue, but as we know, part of the solution to this problem passes through a full commitment to the clinical rules of antibiotics management and microbiological aspects of this disease.

#### P1137 Health-care-acquired pneumonia in internal medicine departments: frequency and risk factors for difficult-to-treat micro-organisms

M. Giannella\*, J.A. Capdevila, B. Pinilla, P. Muñoz, E. Bouza on behalf of the ENEMI study group

**Objective:** To assess frequency and risk factors for difficult-to-treat (DTT) microorganisms in patients with HCAP treated in IMDs.

**Methods:** Seventy-two internal medicine departments (IMDs) reported all patients with pneumonia attended in their department during 1 week in January 2010 and 1 week in June 2010. We analyzed those who fulfilled Health-care-acquired pneumonia (HCAP) criteria (prior hospitalization  $\geq 2$  days or surgery in the past 180 days; residence in a nursing home; chemotherapy, intravenous therapy, wound care or specialized nursing care at home in the past 30 days; attending hospital or hemodialysis clinic). DTT microorganisms were *P. aeruginosa*, Enterobacteriaceae and methicillin-resistant *S. aureus* (MRSA).

**Results:** The 1002 patient records examined revealed 307 (30.6%) cases of HCAP. Prior hospitalization (56%), residence in a nursing home (55%), and attending hospital regularly due to a chronic comorbid condition (55%) were the most common HCAP criteria. An etiological diagnosis was achieved in 65 patients (21.2%): *S. pneumoniae* (38.5%), *P. aeruginosa* (17%), Enterobacteriaceae (12.3%), MRSA (12.3%), *H. influenzae* (5%) and others (11%). Overall, 41.5% of patients with an etiological diagnosis had a DTT microorganism. Differences between patients with difficult-to-treat (DTT) microorganism and those with an easy-to-treat microorganism were chronic obstructive pulmonary disease (70% vs. 43%,  $p = 0.04$ ) and attending the hospital regularly due to a chronic comorbid condition (73.3% vs. 40%,  $p = 0.01$ ). Multivariate analysis adjusted for age, Charlson index, functional status (Barthel index), residence in a nursing home, prior hospitalization, and severity of pneumonia (ATS/IDSA) showed that attending the hospital regularly due to a chronic comorbid condition was the only independent risk factor for difficult-to-treat (DTT) microorganism (OR 4.12, 95%CI 1.43–11.84;  $p = 0.008$ ).

**Conclusions:** HCAP accounts for one third of cases of pneumonia treated in IMDs; up to 40% of those with identified etiology are caused by difficult-to-treat (DTT) microorganisms. Broad-spectrum antibiotics should be considered in this setting especially in patients attending the hospital regularly due to a chronic comorbid condition.

#### P1138 Characterisation of healthcare-associated pneumonia in a Belgian university hospital

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**Objectives:** Healthcare-associated pneumonia (HCAP), referring to patients with frequent healthcare contacts and at higher risk of contracting resistant pathogens, is now recognized as a new category of pneumonia. Evidence regarding its distinct clinical course and microbiological etiology remains controversial. The aim of our study was to compare the clinical and microbiological aspects of HCAP to those of community-acquired pneumonia (CAP) in a Belgian tertiary center.

**Methods:** This prospective observational study included all adult patients hospitalized with pneumonia, admitted between 15 October 2010 and 15 August 2011. Clinical characteristics and culture data of HCAP were determined and compared to CAP.

**Results:** A total of 157 patients (83 women and 74 men) were included. Forty-nine percent of them had HCAP, 51% had CAP. HCAP differed from CAP in age ( $p < 0.001$ ), the presence of cancer ( $p = 0.003$ ), lung disease ( $p = 0.007$ ), cardiac disease ( $p = 0.021$ ), metabolic disease ( $p = 0.025$ ), neurological disease ( $p = 0.001$ ) and immunosuppression ( $p = 0.012$ ). CAP needed more frequently transfer to an intensive care unit (ICU) (27 vs. 16%,  $p = 0.048$ ). Thirty-five percent of HCAP was culture-proven vs. 22% CAP. *Streptococcus pneumoniae* was the major pathogen (32%), followed by *Staphylococcus aureus* (14%) and *Haemophilus influenzae* (14%). No differences in microbiological etiology were detected between both groups except for *Moraxella catarrhalis* (4 in HCAP vs. 0 in CAP,  $p = 0.039$ ). Adequacy of treatment was similar in both groups (80% in CAP vs. 76% in HCAP), as were length of hospital stay (23 days in CAP vs. 21 days in HCAP) and mortality rate at 28 days (9% in CAP vs. 18% in HCAP). Logistic regression analysis revealed that mortality at 28 days was independently related to age (OR = 3.63; 95% CI [0.04–1.16];  $p < 0.001$ ); the presence of cancer (OR = 2.25; 95%CI [0.16–2.40];  $p = 0.025$ ) and the presence of *S. pneumoniae* (OR = 2.17; 95%CI [1.18–3.64];  $p = 0.03$ ).

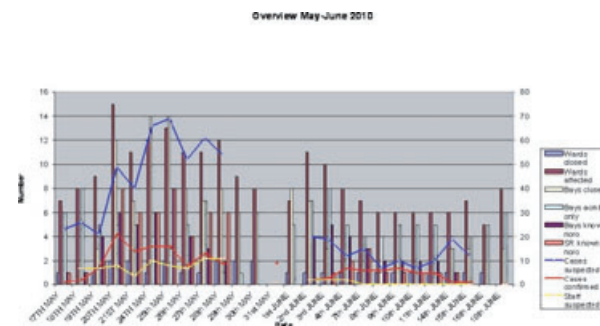
**Conclusion:** HCAP differs from CAP in age and underlying illnesses. However, the outcome and microbiological etiology were similar in both groups. Mortality was primarily related to underlying patient-related factors rather than the mode of acquisition or the presence of nosocomial pathogens. This study, held in a monocentric setting on a limited number of patients, did not indicate the need to adapt empiric antibiotic treatment in case of HCAP.

#### P1139 A review of the infection control prevention and control interventions during a large outbreak of norovirus infection at a hospital, London

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**Background:** An outbreak of norovirus gastroenteritis took place between 15 May and 18 June 2010 at St George's Hospital, a tertiary centre in London. This project describes the outbreak and the strategies implemented to identify and control the infection. Most of these strategies fell in accordance with the current Health Protection Agency (HPA) guidelines written in 2000. However certain aspects were managed differently from the current guidance in order to maintain the acute and routine services of the hospital.

The objectives of this study were to: (i) evaluate if using a 48 hour isolation period rather than 72 hours for symptomatic patients resulted in secondary cases, (ii) Ascertain if modified infection prevention and control measures used by St George's Hospital during the outbreak had a detrimental effect on the running of the hospital. This will help assess if modifying approaches to infection control in the context of a norovirus outbreak is appropriate in hospital outbreaks.



**Methods:** Number and date of clinical and laboratory confirmed diagnoses were used to plot an epidemic curve. Number of secondary hospital cases as a result of reduced isolation period and number of affected wards, closed wards, closed bays and quarantined bays were recorded. Hospital performance data were collected for the epidemic period and compared with a non-epidemic period.

**Results:** The study showed no secondary cases resulted from exposure to patients out of isolation between 48 and 72 hours. There was no reduction in Accident and Emergency attendees seen within 4 hours and patients admitted on the 18-week pathway were better during the outbreak than the following year. The clinical division most affected was Medicine, where there was an increase in last minute cancellations and mean length of stay in acute medicine was higher than the following year.

**Conclusions:** A shorter isolation period did not result in an increase in secondary cases and there was no adverse outcome in hospital performance data over the period of the outbreak other than in the hospital division with the most cases. A reduced isolation period can be recommended for similar tertiary referral centres for management of future outbreaks.

**P1140 A nosocomial outbreak of 2009 influenza A (H1N1) in a haematology ward**

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**Objective:** Data about the measures to adopt in nosocomial outbreaks of 2009 influenza A (H1N1) are scarce, specially in wards with severely immunosuppressed pts.

**Methods:** We describe the characteristics and measures adopted in a nosocomial outbreak of 2009 influenza A (H1N1) that occurred in an hematology ward from 27 January to 4 February 2011. Influenza diagnosis was made by real-time polymerase chain reaction. The hematology ward has three wings (two with rooms with a positive airflow relative to the corridor and frequent air changes).

**Results:** The outbreak involved 15 (56%) of 27 hospitalized pts and six health care workers (HCW). The likely index case was admitted with B cell lymphoma and respiratory infection in a positive pressure room and diagnosed with influenza A (H1N1) 4 days later. All pts but one were diagnosed in the wings with rooms with positive pressure and were transferred after influenza diagnosis to rooms without positive pressure. The median age of pts was 55 years (35–83); most of them had acute leukaemia and five were stem cell transplant recipients. Only 13% of the pts and 12% of the HCW had received the recommended influenza vaccine. There was one case of pneumonia. Seven asymptomatic pts were diagnosed by screening. The main measures adopted were: (i) instituting droplet and contact precautions; (ii) close the ward for new admissions; (iii) restricting visits and avoid patients, family and staff movement between wards, (iv) re-offering influenza vaccination; (v) universal screening and oseltamivir therapy for all hospitalized pts during 14 days; (vi) stop the positive airflow, and (vii) test all symptomatic health care workers. The overall case-fatality rate was 20%.

**Conclusions:** Our study suggests that airflow transmission may play a significant role in nosocomial outbreaks of influenza. The measures adopted merit consideration when facing new outbreaks occurring in hematology wards. Our report reinforces the needed to maximize prevention measures against influenza infection such as annual influenza vaccination.

**P1141 Unusual hospital-related outbreaks due to fungi: the need to improve diagnosis, treatment and prevention**

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**Objectives:** Fungi are a well known cause of severe infections in immunocompromised patients. Two or more unusual hospital related

fungi infections diagnosed in a short period should be assumed as an epidemic. Outbreak's reports are increasing: the aim of this review is to collect data to improve the management of these infections.

**Methods:** The free online worldwide database for nosocomial outbreaks of the Institute of Hygiene and Environmental Medicine-Charité of the University of Berlin (<http://www.outbreak-database.com>) was used to collect the English literature on unusual fungal infections published from 1990 to 2011 (*Aspergillus* and *Candida* were excluded). For each outbreak the following data were considered: phylum, duration (months), source of infection (identified, not identified, not searched), site of infection (organs, devices), ward (medical, surgical, ICU), risk factors (identified, not identified, not searched), number of patients infected or colonized, treatment (medical, surgical, none), related mortality and type of epidemiological study.

**Results:** Twenty-seven reports were considered: Ascomycetes caused the majority of the reported outbreaks (17/27) and the overall median duration was high (6 months). In one outbreak the infection was limited to prosthetic devices. Six centers did not look for the source of the infection and nine centers were not able to identify it. Statistically significant risk factors were searched and found in 13/27 and 11/13 outbreaks, respectively. In the majority of cases (14/27) only molecular analysis and environmental surveys were performed. The complete results are summarized in Table 1.

Table 1.

Phylum	Duration*	Source	Site	Ward
17 Ascomycetes 5 Basidiomycetes 5 Zygomycetes	6 months (0-45)	12 identified* 9 not identified 6 not searched	26 organ** 1 device	10 medicine 7 surgery 10 ICU
Risk factors	N° patients per outbreak*	Therapy	Mortality*	Type of study
11 identified** 2 not identified 14 not searched	5 (2-379) infections§	17 antifungals 1 surgery 1 no therapy 8 not reported	27% (0-100%)	13 case-control studies 14 other§§

ICU: intensive care unit; \*median and range; \*\*contaminated material or human to human; \*\*\*blood, lung, skin, bowel, central nervous system, eye; \*\*\*\*preterm birth, low birth weight, invasive devices/procedures, co-morbidities; § 12 co-infections; §§ molecular analysis and environmental surveys.

**Conclusions:** Fungal nosocomial outbreaks usually involve few patients but show high mortality (median value: 27%), especially in patients with severe co-morbidities. This fact could be explained by the delay in the diagnosis, the inability of recognizing the source of infection and the challenges of the treatment. In two cases a direct human to human transmission was proved and in seven cases was hypothesized. More efforts should be done to implement the application of proper care and hygiene practices in order to avoid human to human transmission.

**P1142 Reporting observational studies on antibiotic-resistant infections. It is time to improve it**

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**Objectives:** Several limitations affect observational studies exploring risk factors associated with infection or colonisation due to antibiotic-resistant bacteria (ARB). An attempt to improve the quality of scientific evidence was done with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement. However, we do believe that the peculiarity of the epidemiology of ARB needs specific variables to be considered and included in this score. The main objective of the study was therefore to suggest new items to be considered to improve reporting of observational studies on ARB infections.

**Methods:** The study was designed as a three-step study. Firstly, we reviewed the literature selecting all articles analysing the association between antibiotic exposure and acquisition of MRSA or multidrug-resistant *A. baumannii*. Secondly, all the articles were reviewed

according to the items of the STROBE index. Absolute and relative frequencies were used to describe the results of the judgment of quality of reporting. The impact factor of the journal where the study was published was also reported. Thirdly, a new set of items specifically focused on antibiotic-resistant infections were developed by an Expert panel and tested on the same articles. The Expert panel was composed by the authors and three external reviewers.

**Results:** Seventy-eight studies (39 case-control, 28 cohort, and 11 cross-sectional) were reviewed. Overall the quality of reporting, assessed through the STROBE statement, was poor in most areas. Statistical details, subgroup analysis, sample size calculation and the description of main study objectives were satisfied in <25% of the studies. Linear regression analysis revealed that increase in impact factor was significantly associated with the percentage of satisfaction of the STROBE statement ( $p = 0.01$ ). However, an unexpected low level of quality of high rank journals for major issues such as sample size calculation, bias and confounding analysis was observed. Twelve new items were proposed (Table 1). All the new items were reported in <25% of studies.

Table 1.

Section	New item proposal
Introduction Background /rationale	Report previous in vivo and in vitro studies.
<b>Methods</b>	
Study design	State type of study.
Setting	Specify if setting is epidemic or endemic. Specify type of hospital and characteristics of population served by the hospital. Describe infection control measures applied at the study location.
Participants	Provide clear definition for case and control and give reason for control selection. Give reason for matching criteria.
Variables	Specify antibiotic usage according to: type, dosage, duration and route of administration. Define time at risk for antibiotic exposure. Include among potential confounders: comorbidity, length of hospital stay, previous surgery, presence of devices.
Data sources/measurement	Provide definition of resistance, multidrug resistance, including pattern of co-resistance. Whether variation of resistance is seen in studied pathogens. Whether studies performed to identify location or resistance e.g plasmid, chromosome, integron, transposon.
	Definition of infection and/or colonization. If not a validated reference, evidence of robustness of definition.
Quantitative variables	Provide subgroup analyses for immunocompromised, surgical medical patients and patients in intensive care units.
<b>Results</b>	
Descriptive data	Specify among the exposure: previous stay in long term care facilities, nursing home, and other hospitals.
Other analyses	Report subgroup analysis by type of patients and type of microorganism.
<b>Discussion</b>	
Limitations	Provide description of sources of selection bias, information bias and confounding.
Generalizability	Discuss study setting for the generalizability.

**Conclusion:** There is a significant need to improve the quality of reporting epidemiological studies on infections due to ARB. Since these studies might impact on public health officers decisions to try to reduce the spread of ARB, the application of our modified STROBE score can play a role.

#### **P1143** Predicting a complicated course of *Clostridium difficile* infection using a validated scoring system at patient's bedside

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**Objective:** *Clostridium difficile* infection (CDI) is the leading cause of antibiotic associated diarrhoea. Mortality associated with CDI is high and varies from 6% to 19%. Patient characteristics such as age and comorbidity, laboratory findings and type of *Clostridium difficile* have been used to predict the outcome of CDI. However, no validated prediction rule is available. In this study, we aim to develop such a

scoring system using criteria that are available at the bedside of the patient at time of diagnosis.

**Methods:** Between March 2006 and May 2009, nine hospitals in the Netherlands included patients with a positive toxin test for *C. difficile*. Demographic characteristic, clinical and laboratory parameters were collected using patient records, the electronic medical information system and consulting the physician in charge. We performed univariable logistic regression of putative predictors of a severe course of CDI after 30 days (death, ICU admission or colectomy due to CDI). Multivariable logistic regression and subsequent reduction of the model resulted in a final model. Internal validation was done using bootstrapping techniques. External validation took place using a different cohort of CDI patients, gathered in a different time frame.

**Results:** In total, 395 CDI patients were included of whom 46 had a complicated course within 30 days (11.9%). Seventeen putative predictors were included in univariable analysis. Five variables remained in the final model: age (OR 4.96  $\geq$  85 years; OR 1.83 50–84 years), diagnosis at the ICU (OR 7.03), recent surgery (OR 0.23), hypotension (OR 3.25) and admission because of diarrhoea (OR 3.27). After internal validation, a score was developed with four risk categories on the outcome: no risk (0%), low risk (5%), medium risk (16%) and a high risk (39%). External validation among 140 CDI patients showed that the score was capable of categorizing CDI patients according to their outcome.

**Conclusion:** This prediction rule uses bedside collected criteria to predict a complicated course of CDI after 30 days. Objective categorization of patients is therefore possible, which could have major implications for guidance of treatment decisions and selection of patients for randomized trials.

## Clinical epidemiology of nosocomial infections – Gram-positive infections

### **P1144** Molecular characterisation of VRE from the hospital environment and correlation with clinical VRE samples during an outbreak in a tertiary referral centre

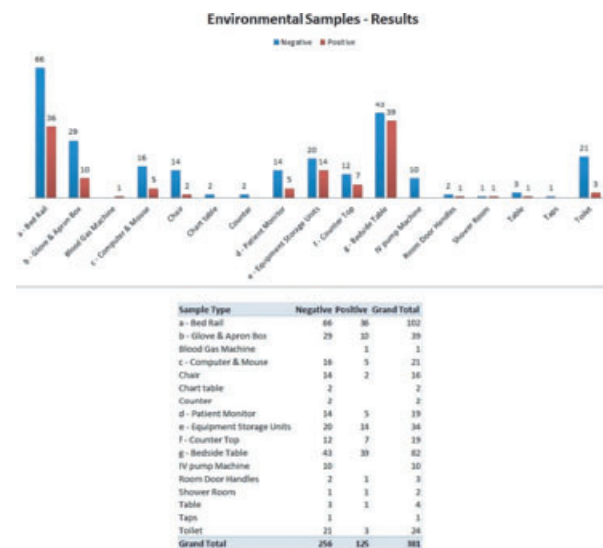
C. Fenelon\*, C. Wrenn, S. Fitzgerald, K. Schaffer, E. O'Mahony, L. Fenelon, D. Keating, D. O'Brien (Dublin, IE)

**Background:** EARS-Net data in 2011 show that Ireland had the highest proportion of enterococcal bloodstream isolates that were resistant to vancomycin (44.0%, quarter 2 2011). St Vincent's University Hospital is a 479 bedded tertiary referral centre and is the National Centre for Liver Transplantation and Pancreatic Surgery. In 2010, the proportion of enterococcal bloodstream isolates that were vancomycin resistant in our hospital was 39%. Almost all of these infections occurred in patients in the intensive care unit (ICU), general surgical and haematology/oncology wards.

**Objectives:** To establish whether there was an environmental reservoir of vancomycin resistant enterococci (VRE) in these wards and to determine whether the clinical and environmental strains were related.

**Methods:** Three hundred and eighty-one environmental samples were taken using flocked swabs (FLOQSwabs™; Copan Flock Technologies). The swabs were taken from specified frequently-touched locations in patient care areas in ICU, general surgical and haematology-oncology wards. Culture specimens were obtained by rubbing premoistened swabs repeatedly over each designated site and placing the swab in liquid transport medium. Specimens were inoculated onto blood agar and VRE chromogenic agar (Biomerieux) and incubated for 48 hours. VRE was confirmed using the VITEK II identification system (Biomerieux). Isolates were tested for antimicrobial susceptibility and typed by PFGE. PFGE was performed on 10 isolates of VRE from patients present in the clinical areas at the time that the environmental swabs were taken.

**Results:** Of the 381 environmental samples taken, 125 (32.8%) were positive for VRE (Table 1). Linezolid resistance was identified in 4%. VRE was most frequently isolated from bed rails, patient tables and



equipment storage units. PFGE analysis yielded a diverse set of isolates with six clonal complexes (EC-6/7, EC-9, EC-10, EC-11, EC-12, EC-13) identified. Five of these were found among both clinical and environmental samples.

**Conclusion:** The hospital environment plays an important role in the transmission of VRE. Frequent transfer of patients between the study areas, a paucity of single rooms and shared toilet facilities may be a factor in the molecular diversity of isolates identified. Strict adherence to infection prevention and control measures and thorough environmental cleaning is necessary to prevent spread following the establishment of successful clones.

**P1145** **Molecular epidemiologic characterisation of *Staphylococcus epidermidis* isolated from prosthetic joint infections**

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**Objectives:** *Staphylococcus epidermidis* is a commensal that comprises a substantial part of the normal human skin flora. Nevertheless, this bacterium has emerged as the most important pathogen in infections related to implanted foreign body materials, especially prosthetic joint infections (PJIs). A major problem for the clinical laboratory is to determine whether finding *S. epidermidis* in a sample from a PJI is a true infection or if it is a sampling contamination. The aim of this study was to examine the molecular epidemiology of *S. epidermidis* isolated from PJIs and compare to commensal isolates from skin of healthy, non-hospital individuals.

**Methods:** Sixty-one isolates from PJI and 24 commensal isolates were examined using multilocus sequence typing (MLST) according to the MLST-scheme published by Thomas et al. (Thomas et al., 2007). For studies of relationship between different sequence types (ST) eBURSTv3 were used (<http://eburst.mlst.net>).

**Results:** Two STs dominated among the PJI isolates; ST2 in 28 isolates (45%) and ST215 in 19 isolates (30%). The remaining PJI isolates (n = 14) were assigned as singletons, pairs or triplets of different STs. In contrast, the commensal isolates displayed a diverse picture with mainly singletons and not more than three isolates assigned the same ST. ST2 and ST297 were the only STs found among both groups of isolates.

This is in concordance with previous studies of nosocomial isolates reporting ST2 as the dominant ST among invasive isolates (Li et al., 2009; Miragaia et al., 2007). ST215 is a relatively novel ST that recently has been described in a study of MDR *S. epidermidis* obtained from patients at hospitals in northern Europe, and ST215 was identified

only in isolates from hospitals in Sweden and Norway (Widerström et al., 2009).

**Conclusion:** A correlation between the results of the MLST and the source of the isolates could be seen. ST2 and ST215 dominated among the PJI isolates while only two and no commensal isolates displayed ST2 and ST215, respectively.

**P1146** **The impact of methicillin-sensitive *Staphylococcus aureus* bacteraemia in a UK renal population**

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**Objective:** Infection is the second leading cause of death in patients with established renal failure, comprising 25% of all deaths. (i) *Staphylococcus aureus* is a common cause of bacteraemia and associated with significant mortality and morbidity in this population. (ii) In this study we examine the impact of methicillin-sensitive *Staphylococcus aureus* bacteraemia (MSSAB) in our established renal failure population.

**Methods:** We performed a retrospective study of prospectively recorded data. An electronic patient record search was conducted for all MSSABs in the established renal failure population of the Glasgow Renal Units and outpatient dialysis facilities between 1 January 2010 and 31 December 2010. Events were allied to electronic records of in-patient admissions, antimicrobial therapy, clinical events and mortality.

**Results:** Thirty-eight patients developed 43 episodes of MSSAB. 3/38 (7.9%) occurred in patients with a functioning renal transplant, 6/38 (15.8%) in patients with an arteriovenous fistula, 21/38 (55.3%) in patients with a tunneled venous catheter and 6/38 (15.8%) in patients with a non-tunneled venous catheter. 11/38 (28.9%) patients died within 6 months of MSSAB. 6/38 (15.8%) had metastatic infection including 4/38 (10.5%) with endocarditis.

Average in-patient stay increased from 11.3 to 24.5 days ( $p < 0.001$ ) comparing the periods 6 months before and after MSSAB. 33/38 (86.8%) patients were treated as in-patients. No difference in metastatic infection, hospitalisation or mortality rates was seen when comparing flucloxacillin with vancomycin based treatments.

**Conclusion:** MSSAB confers a significant burden of comorbidity, hospitalisation and death in renal patients. In this analysis, there was no discernible difference in clinical outcomes between flucloxacillin and vancomycin based therapies.

**References:** 1. United States Renal Data System. 2009 Annual data report. Bethesda, MD: National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, 2009.

2. Fitzgerald SF, O'Gorman J. A 12 year review of *Staphylococcus aureus* bloodstream infections in haemodialysis patients: more work to be done. *J Hosp Inf* November 2011; 79: 218–221.

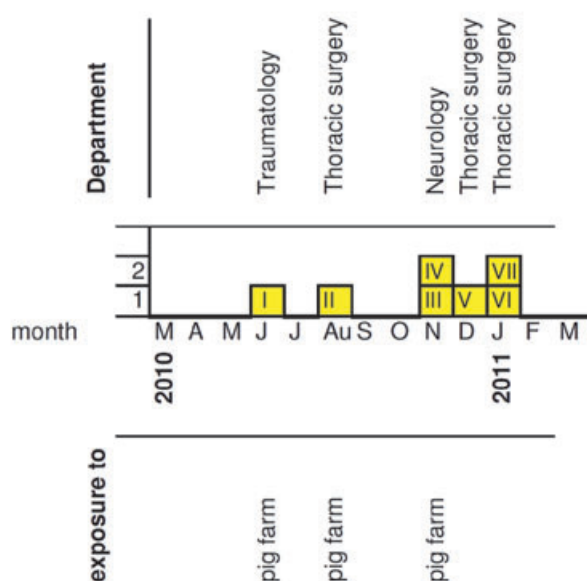
**P1147** **First documented nosocomial transmission of MRSA spa type 011 in an Austrian hospital, 2010–2011**

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Livestock-associated (1a) methicillin-resistant *Staphylococcus aureus* belonging to multilocus sequence type (ST) 398 was first associated with human disease in 2003 in the Netherlands. MRSA isolates of spa types t011, t034, t108, t567, t571, t588, t753, t898, t899, t1184, t1254, t1255, t1451, t1456, t1457, t2123, t2330, t2383, t2582, or t3013 correspond to ST 398. In December 2010 a cluster of five patients positive for t011 was identified in a 1400 bed – hospital in Austria.

Aim of our investigation was to describe the cluster, to identify the mode of spread and the source(s) of that strain. A cluster case was defined as a person, who stayed as a patient or health-care worker in hospital A between June 2010 and March 2011 with a specimen positive for MRSA t011.

Case finding was performed within routine MRSA screening or infection diagnostics. Data on demographics and livestock contact were ascertained by personal interviews. Six patients (four male, two

**Figure 1;** cases by date of MRSA detection, department (ward) of stay and pig-farm contact

female) and one male nurse fulfilled the cluster case definition (median age: 72 years). The initial MRSA t011 isolates were from nasal ( $n = 7$ ) or skin ulcer ( $n = 1$ ) swabs. None of the cases showed signs of MRSA infection. Figure 1 displays the cases by date of MRSA detection, department of stay and livestock contact. The case-patients I, II and III had a positive history of livestock contact as farmer or by living in the household of a farmer. Case-patient IV shared the patient room with case-patient III. The case-patients V and VI and the case-nurse VII were linked to each other by stay or work within 2 months at the same ward, where the case-patient II had been hospitalized 4 months earlier. In May 2011 dust samples and pig nasal swabs from the pig farm related to case-patient III yielded t011; samples from the pig farm related to case-patient I tested negative for MRSA, and case II refused testing of the involved pig farm. Our findings underline, that regular contact with pigs contributes to transmission of la-MRSA ST398 from animals to humans and to spread of la-MRSA into the health-care-setting. The first described nosocomial outbreak due to la-MRSA affected a Dutch hospital in 2007. In 2008 and 2009, in Austria MRSA t011 was detected in dust samples from pig farms, and in nasal swabs from veterinarians and pig farmers. Efforts of clinicians, infection control practitioners and veterinarians are required to prevent further spread of la-MRSA into health-care settings.

#### **P1148** Use of a novel MRSA strain-typing system to investigate a neonatal intensive care unit outbreak

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**Background:** In May 2011, two 26 week-gestation infants, developed pneumonia, due to a community-type methicillin-resistant *Staphylococcus aureus* (cMRSA) and died within days of each other. They had shared a room, in a 39-bed Neonatal Intensive Care Unit (NICU – 19 ventilator, 20 non-ventilator cots). Previously, MRSA had been isolated rarely from NICU patients. Using a novel MRSA typing system (19-target PCR, reverse line blot assay) the cMRSA was identified as PVL positive, SCCmec type IV. We hypothesised that one infant had acquired cMRSA from her mother and the other by nosocomial transmission.

**Methods:** Enhanced infection control precautions (hand hygiene, contact precautions and environmental cleaning) were implemented. All infants, staff (138 NICU staff) and selected environmental sites were screened for MRSA and routine screening of infants, on admission and weekly, was introduced. Despite this, another 11 infants (all but one of whom had been delivered by Caesarean section) were colonized or infected with MRSA in the next 5 months. One hundred and thirty-four NICU, 19 visiting staff and 52 environmental sites were re-screened and screening of relevant delivery ward and operating room staff is underway (70 screened so far).

**Results:** Ten of 13 colonised infants (including the two index cases) had the same "epidemic" strain; the mothers of two of these 10 infants carried a different strain. Three infants had non-epidemic cMRSA strains, including one whose mother carried a matching strain. Two NICU staff in the 1st and two in the 2nd round of screening were MRSA carriers; only one carried the epidemic strain, but was not thought to be the source. All were successfully decolonized. MRSA was isolated from only one environmental site (blood gas machine). Screening of junior medical staff, who had rotated to other hospitals before screening commenced is still incomplete. Although, no source has yet been found, no newly colonized infants have been identified in NICU since this rotation occurred. Further investigation of the epidemic strain showed that it is a PVL-positive ST22 MRSA, which previously has been identified rarely in Australia.

**Conclusion:** The use of a rapid, sensitive MRSA strain typing system allowed precise characterization of the epidemic and elimination of several potential sources (mothers and staff). There has been no intra-NICU transmission since September. We assume the source is an as-yet-unidentified external staff member; investigations are continuing.

#### **P1149** Is colonisation pressure a predictor of methicillin-resistant *Staphylococcus aureus* transmission in a low prevalence setting?

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**Objectives:** Colonization pressure (CP) has been described as a useful predictor of nosocomial transmission of methicillin-resistant *Staphylococcus aureus* (MRSA) in settings of high MRSA rates. This study investigates the role of CP as a predictor of nosocomial MRSA infection in a low prevalence setting.

**Methods:** Retrospective analysis of health care associated MRSA (HA-MRSA) rates from 2004 to 2009 at the Saudi Aramco Dhahran Health Center, Saudi Arabia was carried out. MRSA-patient days, susceptible patient days, nosocomial incidence and colonization pressure were calculated for each month from January 2008 to December 2009.

**Results:** In 2005 and 2006 HA-MRSA accounted for 29.6% and 31.6%, respectively, of the hospital associated infections, declining to 5.1% in 2009. During the study period, MRSA constituted 13.7% of all staphylococcal isolates and was associated with bacteremia and wound infection. All isolates were sensitive to vancomycin. The incidence of nosocomial infection per 1000 susceptible patient days was 1.17 in 2008 and 0.7 in 2009. Monthly colonization pressure ranged from 0.1 to 1.62 across the 2-year period. Nosocomial transmission was observed in 13 out of 24 months; however, no pattern of association between the preceding month's CP and the nosocomial incidence in the subsequent month was demonstrable.

**Conclusion:** These findings indicate that in settings of low MRSA prevalence, nosocomial transmission does occur at low CP levels probably due to lapses in adherence to infection control. However, CP does not appear to be a predictor of transmission and nosocomial incidence. In such settings, strict adherence to infection control policies with a low tolerance approach to lapses in adherence, coupled with adequate staffing, ongoing education, continuous surveillance and feedback measures are recommended.



**P1150** Virulence determinants among coagulase-negative staphylococci recovered from bacteraemias and device-related infections

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**Objectives:** Coagulase-negative staphylococci (CNS), mainly *Staphylococcus epidermidis* and *S. haemolyticus*, are a major etiological agent of neonatal nosocomial sepsis and a major cause of device-related infections in hospitalized patients. Among the various virulent factors potentially implicated, are the adhesion mechanisms used by the bacteria to anchor themselves to the surface of various materials, including medical devices. Of equal importance is the production of an intercellular polysaccharide adhesin (PIA), encoded by the *ica* operon, and biofilm formation. In this study, CNS isolated from bacteraemias and device-associated infections were compared in terms of antibiotic resistance, biofilm formation, *ica* and adhesin's gene carriage.

**Methods:** In total, 313 CNS (161 from infants with bacteraemia and 152 from catheters and other device-mediated infections) were identified at species level by Vitek 2 Advanced Expert System (bioMérieux, France). Biofilm formation was tested by Christensen's method. Antibiotic resistance was tested by the disk diffusion method and Etest, according to CLSI guidelines. Clonality was identified by PFGE analysis of *Sma*I chromosomal DNA digests. The presence of *mecA*, *icaA* and *icaD* (*ica* operon) and adhesin's genes (*atlE*, *fbe*, *bap*, *fnbA*) was tested by PCRs. Data were statistically analyzed using SPSS.

**Results:** One hundred and fifty-nine (98.8%) CNS from blood cultures and 136 CNS (89.5%) from medical devices were *mecA*-positive (MR-CNS). Two major clones were characterized among *S. epidermidis* and one among *S. haemolyticus*. Forty-six (30.3%) CNS from various devices and 76 (47.2%) from blood infections formed biofilm, whereas most strains were multi-resistant. There was a significant difference in biofilm formation ( $p = 0.002$ ), *mecA* ( $p < 0.001$ ), *ica* and *fnbA* ( $p = 0.001$ ) gene carriage in favor of bacteraemic CNS, whereas those from medical devices were more frequently related with the presence of *bap* gene ( $p = 0.031$ ). No difference was found in regards of the adhesins *AtlE* and *Fbe*.

**Conclusion:** In our hospital, multi-resistant CNS, mainly *S. epidermidis* and *S. haemolyticus* are a major cause of neonatal sepsis and device-mediated infections among hospitalized patients. Those isolated from blood cultures carried more pathogenic and antibiotic resistance elements, including methicillin resistance, biofilm formation, *ica* and *fnbA* gene carriage, whereas CNS from medical devices were, in comparison, more related with the adhesin *Bap*.

**P1151** Rapid bench top whole genome sequencing for investigation of a putative MRSA outbreak

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**Objective:** To investigate the relatedness of atypical methicillin resistant isolates of *Staphylococcus aureus* in an intensive care unit setting using a rapid turnaround bench top sequencer.

**Methods:** Seven cases over a 2 week period were found to be colonised with *S. aureus* on routine screening using MRSA selective agar; however the isolates had an oxacillin MIC of  $< 2 \mu\text{g/mL}$  on routine E-strip testing suggesting that they were methicillin susceptible. These were sent to a reference laboratory and were shown to be spa type t5973 and *mecA* positive by PCR. No further cases were detected on repeated screening of all patients on the unit. Two months later a case grew similar isolates from a blood culture and a screening swab. These were also t5973 and *mecA* positive. These isolates were tetracycline resistant on routine testing whereas the earlier isolates were susceptible. The Illumina MiSeq platform was used to sequence and

assess the relationship between these two later isolates to the seven cases identified 2 months earlier.

Two of the study isolates were sequenced twice and the reference isolate MRSA-252 was sequenced for validation. Four samples were sequenced per run using  $2 \times 150$  bp reads. The sequences were mapped to a reference genome with STAMPHY. Base and variant calls were made using SAMTOOLS, PICARD and bespoke Python scripts. The entire process from the time of culturing the organisms to determining relatedness of strains took  $< 5$  working days.

**Results:** All 12 samples were successfully sequenced with a mean call rate of 81% of the reference genome. No sequence differences were detected in the two pairs of replicates or between the MRS-A252 control sample and reference sequence. Among the test samples, no sequence differences were detected between isolates from six of the original seven cases, and the other isolate differed at a single site. The two isolates from the blood culture positive case yielded identical sequences that differed at two positions from the other isolates.

These data are consistent with recent acquisition from a common source.

**Conclusion:** Rapid bench top whole genome sequencing provided unambiguous evidence of an outbreak of atypical MRSA acquisition in an intensive care unit. This was achieved in a timescale sufficiently rapid to inform case management.

**P1152** Successful implementation of a new MRSA-screening concept

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**Objectives:** To reduce nosocomial infections and colonisations with MRSA at a 1600 bed secondary and tertiary care a six-hospital network in Germany, the department of infection control, in collaboration with the hospital administration and the laboratory introduced a new MRSA screening concept.

**Methods:** A checklist was created to select high risk patients for mandatory MRSA-screening based on 7 points. Instead of the physicians, the nurses were now responsible for this selection process. Patients with prior history of MRSA-colonization or infection were identified by means of a pop-up window on the computer screen with the message "CAVE" (lat.: caution) at the time of admission. To shorten turn-around time, a PCR-based assay for MRSA-detection (Light Cycler MRSA Advanced, Roche Diagnostics) was established with a throughput of up to 100 tests per day. Results were available within 24 hours after receiving the specimen. The MRSA-KISS (KISS = German Hospital Surveillance System) results were to be reported regularly. Three testing phases: (i) real observation, (ii) intensive training and (iii) inspection interval were monitored for number of screenings, nosocomial MRSA infections, nosocomial MRSA colonisations and residence time during which infection control precautions remained unchanged.

**Results:** Screening rates increased from 10% to 15% before introduction of the new screening concept to 31% after the introduction. Turn-around time was reduced for detection of MRSA

Table 1: Facts of the testing phases and development of nosocomial MRSA infections

	Phase 1 'real observation'	Phase 2 'intensive training'	Phase 3 'inspection interval'
Observation period	09/2008 – 03/2009	09/2009 – 03/2010	04/2010 – 12/2011
Number of patients	41,563	41,535	122,676
Decision for screening	Physician	Nurse	Nurse
Checklist	No	Yes	Yes
Screening rates	10-15%	31%	33%
Duration (months)	7	7	21
Number of nosocomial MRSA infections	11	3	-7
Number of nosocomial MRSA infections p.a.	18,0	4	-4,0

colonisations and MRSA infections after compared to before the introduction of the new screening concept. Most importantly, rates of MRSA colonization ( $p < 0.005$ ) and MRSA infection were markedly reduced.

The evaluation of economic data shows that the incremental costs are not fully compensated by the additional DRG (diagnosis related groups) proceeds.

**Conclusions:** The introduction of a new stringent screening concept including checklist, caution label in medical information system, and rapid diagnosis using PCR resulted in a significant reduction in the rates of nosocomial MRSA-infections and MRSA colonisations in the hospital network.

**P1153 Phenotypic and genotypic characterisation of coagulase negative staphylococci bacteraemic isolates from infected very low birth weight neonates: antibiotic and antiseptic susceptibility, biofilm production and clonality**

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Very low birth weight neonates (VLBW) are high risk patients to develop hospital acquired infections as catheter related bacteraemia (CRB). Coagulase negative staphylococci (CNS) are the leading cause of CRB.

**Objectives:** To characterize CNS isolated from blood cultures of VLBW hospitalized at the NICU of Antoine Bécélère hospital (Clamart, France).

**Methods:** Forty-nine strains of CNS responsible for clinically relevant CRB among 47 VLBW (median 830 g, range 540–1430 g) were included between January 2009 and September 2011. Species identification was performed using conventional and reference methods. Antimicrobial susceptibility was tested by disc diffusion method and MIC determination (Etest®); mupirocin susceptibility study was completed by mupA PCR. Antiseptic susceptibility was tested by broth microdilution method (chlorhexidine CHX, benzalkonium chloride BZC, acriflavine ACR) and qacA PCR. Biofilm formation was evaluated by the Ring Test method; a PCR targeting IS256 and icaADB was performed. Clonality of *S. epidermidis* and *S. capitis* was studied using PFGE analysis.

**Results:** The species distribution was: *S. epidermidis* (47%), *S. capitis* (37%), others species (16%). The proportion of rapid (<6 hours), slow (6–24 hours) and no biofilm producer was 41%, 37% and 22%, respectively. Antibiotic resistance was common: oxacillin (98%), gentamicin (88%), ciprofloxacin (29%) and mupirocin (61%). The MIC90 of vancomycin, teicoplanin, daptomycin and linezolid was 3, 4, 1 and 1 mg/L, respectively. Among mupirocin resistant strains, all but one harboured a high level resistance and were mupA positive. Antiseptic decreasing susceptibility was identified among 12% of strains for CHX, 22% for BZC and 33% for ACF. Qac A was presented among 63% of the 49 strains. IS256 or icaADB was identified in 92% and 74% of strains, respectively. PFGE analysis pointed out a significant clonality among *S. epidermidis* and *S. capitis* isolates.

**Discussion:** Among this particular population of patient, several clones of CNS were implicated in CRB. They are characterized by a high level of antibiotic and antiseptic resistance, biofilm production and a high prevalence of IS256, icaADB, qacA and mupA. Preventive policies including large use of CHX and mupirocin could have influenced these epidemiologic features. Cross contamination seem to be very common.

**Conclusion:** Some well adapted multi-resistant clones of *S. epidermidis* and *S. capitis* are responsible for the majority of CRB in this NICU.

## Clinical epidemiology of nosocomial infections – Gram-negative infections

**P1154 Multiresistant bacteria obtained from returning travellers: incidence, characteristics, influence on clinical outcome**

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**Background:** Worldwide, the burden of multiresistant bacteria is increasing with strong regional differences. Such bacteria may be introduced into the healthcare system by returning travellers requiring medical care.

**Methods:** Patients transferred from abroad to the University Hospital Zuerich (USZ) routinely undergo screening for possible colonisation with Methicillin resistant *Staphylococcus aureus* (MRSA), Extended-Spectrum Beta-Lactamase producing bacteria (ESBL) and multiresistant gram-negative bacteria (MR-gram negative). Clinical characteristics and outcome of 259 patients transferred between 1 January 2009, and 30 September 2011, were retrospectively analysed.

**Results:** Eighty-six were women (33.2%) and 173 were men (66.8%) with a median age of 56.2 (range: 17.3–96.6). One hundred and sixty-three (62.9%) were hospitalized on surgical wards and 96 (37.1%) on medical wards, respectively.

Forty-six patients (17.7%) were colonized and 9 (3.5%) were infected. Thirty-three (12.7%) were colonized with one bacterial strain, 12 (4.6%) with two different bacterial strains and three (1.2%) were colonized with three different bacterial strains. In total, 36 ESBL expressing bacteria, 21 MR-gram negative and three MRSA were detected. *Escherichia coli* (n = 18), *Klebsiella pneumoniae* (n = 14) and *Acinetobacter baumannii* (n = 14) were most frequently isolated. The most common site of detection was the skin (45 of 46 patients; 97%), and the respiratory tract (19 of 46 patients; 41%).

Travellers transferred from Europe were colonized in 12.4%, from the Americas in 20%, from Africa and Middle East in 39.1%, and from Asia and Australia in 33.3%, respectively.

Being colonized contributed independently to a significantly increased length of stay in ICU at USZ. The mortality rate of colonized patients (10.9%) during hospital stay was significantly higher than the mortality rate of noncolonized patients (2.3%) ( $p = 0.018$ ). Being colonized was independently associated with death (Odds ratio: 5.176 [1.325–20.218]).

**Conclusions:** A substantial proportion of patients transferred from abroad are colonized with multiresistant bacteria, a fact which is associated with poor clinical outcome. Gram negative bacteria with different resistance patterns occurred most frequently and were detected in travellers from every continent. These data mirror the global crisis of emerging, multiresistant gram negative bacteria and the associated clinical impact.

**P1155 Surveillance of *Escherichia coli* bacteraemia in England – preliminary results of the recently introduced mandatory surveillance scheme**

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**Objective:** In June 2011 the English Healthcare Associated Infection (HCAI) mandatory surveillance programme was extended to include the collection of all cases of *Escherichia coli* (*E. coli*) bacteraemia reported by NHS acute Trust hospitals. Surveillance was initiated in response to recent increases in *E. coli* bacteraemia observed across England with the aim of better understanding this national trend. Present work represents preliminary analysis of the early months of *E. coli* surveillance data.

**Methods:** *E. coli* bacteraemia data for the period June to August 2011 were extracted from the Health Protection Agency's mandatory surveillance system. Basic demographic information for each *E. coli* bacteraemia case is captured by surveillance. Organisations also have

the opportunity to submit additional information for each case. This record level information was used to undertake preliminary analysis of the aetiology and underlying risk factors of reported *E. coli* bacteraemia cases.

**Results:** In the first 3 months of surveillance 8165 *E. coli* bacteraemia cases were reported via mandatory surveillance. Fifty percent of cases were reported in patients aged 75 years and over with this pattern being observed for both sexes. Seven thousand nine hundred and seventy-four of the 8165 reported cases were admitted to an acute Trust at the time of specimen collection, with 60% having the specimen taken on the day of admission. A further 20% of cases had the specimen taken within a week of admission with half being taken on the day following admission. Seventy-five percent of *E. coli* bacteraemia patients were admitted from home.

Urinary Tract infections (UTI) were indicated to be the primary source of infection in 45% of cases (3682). Where UTI had been indicated and information on catheterisation was provided 85% of records indicated a catheter was present. In a quarter of cases the primary source of infection was listed as unknown.

Thirty-five percent (2882) of total records suggest one or more predisposing factors to the bacteraemia episode. Vascular access is indicated in 30% of relevant records and surgical procedures in 20%. Invasive devices, neutropaenia and wound ulcers are all cited in 10% of cases. In some instances more than one factor is indicated.

**Conclusion:** Mandatory surveillance of *E. coli* bacteraemia is currently in its infancy with the scheme only commencing in June 2011. Preliminary analysis indicates that enhanced surveillance will provide us with a wealth of useful data/information on this important and currently increasing infection.

#### **P1156** Developments in England's mandatory surveillance programme: targeting *E. coli* and MSSA bacteraemia

*E. Sheridan\*, R. Hope, A. Johnson (London, UK)*

**Background:** Reporting of MRSA bacteraemia and *C. difficile* infection by hospitals is mandatory in England (since 2001 and 2004 respectively). Following reduction in the incidence of these infections, we considered extending the surveillance programme. We describe below the decision-making process regarding which additional pathogens to focus on and which clinical and epidemiological data to collect.

**Methods:** Bacteraemia data are routinely collected by the HPA on a voluntary basis using a database (LabBase2) which enables trends to be followed. However it contains few data on whether infections are healthcare-associated (HCAI) and hence potentially preventable. The LabBase2 data were evaluated to ascertain the predominant pathogens causing bacteraemia. A web-based tool was developed to minimise reduplication of data collection, align with routine information collected by microbiologists and to capture all diagnoses and procedures based on ICD-10 and OPCS codes. Other data sources, in particular Hospital Episode Statistics (HES), a national database covering all NHS admissions, were evaluated with the intention of linking the databases, with only questions not answerable by these asked via the system. The data collection was trialled in a variety of clinical settings.

**Results:** Overall bacteraemia rates were largely static since 2006, with a paradoxical increase in *E. coli* (35% rise 2006 – 2010 to 27 055 cases annually). MSSA, the second most common organism (around 84% of 10 070 *S. aureus* bacteraemia cases), declined slightly but not as fast as MRSA. *E. coli* and MSSA were chosen for enhanced surveillance as the two leading causes of bacteraemia in England. HES data (include dates of admission, clinical diagnoses, procedures etc.) and LabBase2 antimicrobial resistance (AMR) data were identified as datasets to link. Additional data collected by the mandatory system include: probable primary focus of infection; related urinary catheter/other medical devices; clinician opinion: HCAI or not? If yes – primary or secondary care?

**Conclusion:** By establishing this additional surveillance and linking the datasets we hope to ascertain the burden of infection due to *E. coli*

and MSSA bacteraemia, develop means of determining whether the infections are HCAI, review clinical correlates of AMR and guide future interventions to reduce infection rates.

#### **P1157** An nosocomial transmission of IMP-1 producing *K. pneumoniae* in a intensive care unit at a Korean hospital

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**Objectives:** The worldwide emergence of carbapenem resistant Enterobacteriaceae is associated with life threatening infections in hospitalized patients. We describe the emergence and spread of IMP-1 producing *K. pneumoniae* in a medical intensive care unit (MICU) at a Korean hospital.

**Methods:** Carbapenem-resistant *K. pneumoniae* isolates were characterized by standard biochemical methods and disc diffusion susceptibility testing. Genes coding for beta-lactamase were sought by PCR and sequencing. Isolates were compared by PFGE. A point prevalence survey of environmental cultures and health care workers (HCW) was conducted in MICU. Surveillance rectal swab cultures were obtained from all patients and newly admitted patients.

**Results:** In August 2011, the index case was a 79-year-old man who was transferred from another hospital and at MICU admission. The index case was diagnosed of pneumonia treating with ventilator care. He was treated with piperacillin/tazobactam until carbapenemase resistant *K. pneumoniae* was isolated in the follow-up culture of sputum. During active surveillance from August to October, other two cases were colonized with isolates showing the same susceptibility pattern. Patient 1 was with the index case in the MICU about 10 days, and patient 2 was with patient 1, not the index case, in the MICU about 8 days. There was no isolate in follow-up culture in all of them and other MICU-admitted patients. PCR and sequencing identified IMP-1 gene. The PFGE typing showed that all of three isolates was genetically related. There was no isolate in environmental cultures and HCWs' hands. This transmission was controlled by isolation of all patients with IMP-1 producing *K. pneumoniae* as well as strict contract precautions.

**Conclusions:** The emergence of IMP-1 producing *K. pneumoniae* in Korean hospitals creates an important challenge for clinicians and hospital epidemiologists. Combined control measures should be immediately adopted in order to control the cross-transmission of CRE especially in ICU.

#### **P1158** Case-control analysis of Gram-negative catheter-related bloodstream infection in a tertiary care medical centre

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**Objectives:** Data on catheter-related bloodstream infections by Gram-negative pathogens (GN-CRBSI) are scarce and biased. GN-CRBSI seem to be increasingly frequent and may lead to erroneous empirical therapy. We describe the epidemiology, risk factors and clinical characteristics of GN-CRBSI in nonselected patients in a tertiary hospital.

**Methods:** From 2008 to 2010, we compared each patient with proven GN-CRBSI (positive peripheral blood culture and catheter tip or differential blood cultures) with two randomly selected controls (non-GN-CRBSI).

**Results:** We detected 81 episodes of GN-CRBSI (17% of all CRBSI). Incidence/1000 admissions was 0.46 in 2008, 0.66 in 2009 and 0.49 in 2010. The most common microorganisms were *Escherichia coli* (22.5%), *Enterobacter cloacae* (18.5%), *Pseudomonas aeruginosa* (15%) and *Klebsiella pneumoniae* (12.5%). Cases were compared with 162 controls (coagulase-negative staphylococci, 58.7%; *Staphylococcus aureus*, 18.5%; *Enterococcus* spp., 8.6%; *Candida* spp., 14.2%). There were no differences in age, sex, underlying disease, surgery, place of acquisition, type of catheter, lumens, catheter days, maximum severity or hospital stay. The comparison of cases vs. controls revealed the following: Charlson index, 1.56 ± 2 vs. 2.6 ± 2.8, p = 0.003;

neurological disease, 6% vs. 0.6%,  $p = 0.01$ ; intensive care (ICU) stay, 5% vs. 15%,  $p = 0.04$ ; immunosuppression, 17% vs. 32%,  $p = 0.02$ ; time to positive blood culture,  $10.8 \pm 16$  hours vs.  $19.8 \pm 16$  hours,  $p < 0.001$ ; origin in a lower limb catheter, 18.5% vs. 8%,  $p = 0.005$ ; subclavian catheter, 24.7% vs. 39%,  $p = 0.02$ ; previous antimicrobials, 57% vs. 19%,  $p < 0.001$ ; concomitant infection, 57% vs. 42%,  $p = 0.02$ ; empirical antimicrobial therapy, 78% vs. 48%,  $p < 0.001$ ; efficacious empirical therapy, 57% vs. 36%,  $p = 0.004$ ; removal of catheter for therapy, 96% vs. 84%,  $p = 0.004$ ; development of bacteremia-related complications, 16% vs. 8%,  $p = 0.07$ ; admission to intensive care (ICU) due to sepsis, 47% vs. 29%,  $p = 0.007$ ; and mechanical ventilation, 41% vs. 20%,  $p = 0.001$ ; Mortality was similar (21% vs. 26%). Multivariate analysis showed that GN-CRBSI were more common in the following situations: no immunosuppression, underlying neurologic conditions, previous antimicrobial therapy, non-subclavian insertion site, and blood cultures growing before 8 hours.

**Conclusion:** GN-CRBSI account for 17% of all CRBSI and should be taken into consideration in the empirical therapy of patients with the characteristics mentioned above. Mortality and need for admission to the ICU are significant.

**P1159** **Low contribution of ESBL-producing Enterobacteriaceae from long-term care facilities' residents in a tertiary medical centre**

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**Objectives:** New Slovenian guidelines published in mid-2010 have recommended stricter screening policies in order to contain the spread of ESBL-producing Enterobacteriaceae. National guidelines recommend screening of patients upon admission into intensive care units, patients transferred from long-term care/nursing facilities (LTCs), travelers and all of the patients who have previously had an ESBL-producing Enterobacteriaceae isolated from a clinical or surveillance site. It has been suggested that LTCs may act as a reservoir for ESBL-producing Enterobacteriaceae within the community and may significantly contribute to the spread within hospitals. The objective of this study was to determine the mode of acquisition of ESBL-producing *E. coli* (EC) and *K. pneumoniae* (KPN) (community, LTCs, nosocomial) and potential differences in antimicrobial susceptibility.

**Methods:** During the study period from June 2010 to October 2011 data regarding the mode of acquisition was collected prospectively. Hospital and laboratory records of patients with ESBL-producing EC and KPN isolates were reviewed. Antibiotic susceptibility tests were performed using a disk diffusion method or an automated system and interpreted according to CLSI standards.

**Results:** A total of 433 patients with EC-ESBL and 427 patients with KPN-ESBL isolated from a clinical or surveillance sites were included in the study. EC-ESBL was first recovered from surveillance samples in 59.1% of patients and subsequently detected in blood cultures in 2.7%, tracheal aspirate in 5.8% and urine in 10.9% of patients. KPN-ESBL was first recovered from surveillance samples in 53.2% of patients and subsequently detected in blood cultures in 2.2%, tracheal aspirate in 8.8% and urine in 17.6% of patients. The differences in antimicrobial susceptibility test results of community and nosocomial isolates of EC-ESBL and KPN-ESBL are presented in Table 1.

Table 1. Cumulative antimicrobial susceptibility test results of community and nosocomial isolates\*

		AMC	TZP	CAZ	CIP	CTX	FEP	ETP	IPM	AN	GM	SXT
EC-ESBL	community	28.3	80.2	55.7	26.4	0.0	72.6	100.0	100.0	90.6	66.0	27.4
	nosocomial	19.6	78.6	41.1	25.9	0.0	55.4	100.0	100.0	86.6	50.9	21.4
KPN-ESBL	community	6.7	45.0	6.7	5.0	0.0	36.7	95.0	100.0	93.3	48.3	8.3
	nosocomial	5.6	46.9	5.6	5.6	0.0	32.7	95.1	96.8	87.0	66.0	9.9

\* Cumulative antimicrobial susceptibility test results for LTCs isolates are not shown due to small number of isolates

**Conclusions:** Our results suggest that while LTCs may be reservoirs of ESBL-producing Enterobacteriaceae they contributed only 8.3% and 3.3% of the newly discovered patients with ESBL-producing EC and KPN isolated from clinical or surveillance cultures during the study

period. EC-ESBL was community-acquired in 42.7% and hospital-acquired 48.7% of cases. Majority of KPN-ESBL were hospital-acquired (75.9%), while only 20.6% were community-acquired.

**P1160** **Carriage of antibiotic-resistant bacteria in the respiratory tract during SDD and SOD: preliminary results of a cluster-randomised cross-over study**

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**Objectives:** Selective Digestive tract Decontamination (SDD) and Selective Oropharyngeal Decontamination (SOD) aim to eradicate Gram-negative bacteria (GNB) from the respiratory tract in intensive-care-unit (ICU) patients. In both regimens tobramycin and colistin are administered in topical form in the oropharynx four times daily. In a previous study the unit-wide prevalence of ARB in the respiratory tract was lower during SDD/SOD as compared to standard care, but significantly increased in time during these measures (Oostdijk et al. AJRCCM 2010;181:452). We conducted a 16-center cluster-randomized cross-over (CRCO) study in the Netherlands, comparing 12 months periods of SDD and SOD to determine effects on carriage with antibiotic-resistant bacteria (ARB). This is a preliminary analysis on the ecological unit-wide effects of SOD and SDD on respiratory tract carriage with antibiotic-resistant bacteria (ARB).

**Methods:** All patients with an expected ICU-stay of >48 hours were eligible to receive SDD/SOD. Prevalence of respiratory tract carriage with ARB was determined once monthly in all ICU patients (receiving or not receiving SDD/SOD), through inoculating swabs on selective media supplemented with either colistin or tobramycin and a chromogenic ESBL agar. This preliminary analysis includes 265 of the planned 384 point prevalence surveys (69%): 130 during SDD and 135 during SOD, from 14 out of 16 hospitals. Trend analysis was performed of consecutive point prevalence surveys during 24 months of study.

**Results:** Respiratory samples were obtained from 2359 patients (1110 during SOD and 1249 during SDD) of which 236 patients (10%) had growth on selective media with Enterobacteriaceae (209 cultures) or *Pseudomonas aeruginosa* (72 cultures). Completeness of culture taking was 89% based on 100% quality control checks. ESBL production was detected in 1.2% ( $n = 28$ ) of all cultures (SOD vs. SDD  $p = 0.66$ ). Resistance to aminoglycosides, ciprofloxacin and colistin was detected in 88 (3.7%), 71 (3.0%) and 15 (0.6%) cultures, respectively ( $p = 0.22$ ,  $p = 0.38$  and  $p = 0.11$  for SOD vs. SDD). In time, a gradual decrease in the prevalence of aminoglycoside resistance and ciprofloxacin was observed (beta-coefficients  $-0.14$  ( $p < 0.05$ ) and  $-0.83$  ( $p < 0.05$ )), whereas resistance prevalence for ESBL and colistin remained stable.

**Conclusion:** Longitudinal trends in respiratory tract carriage of antibiotic-resistant bacteria (ARB) in patients in 14 Dutch ICUs reflected low prevalence to beta-lactam antibiotics, aminoglycosides and colistin, and no determinable increase of resistance during 24 months of study.

**P1161** **Comparison of MALDI-TOF mass spectra analysis and XbaI-restriction of genomic DNA (PFGE) in evaluation of nosocomial transmission and outbreaks of Escherichia coli and Klebsiella pneumoniae**

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**Objectives:** Two suspected cases of intra- and interdepartmental outbreak and nosocomial transmission of *E. coli* – ESBL and intra- and interdepartmental nosocomial transmissions of *K. pneumoniae* – ESBL were analyzed. A total of 20 strains of *E. coli* – ESBL from 19 patients and 11 strains of *K. pneumoniae* – ESBL from 10 patients were analyzed.

Genomic DNA restriction presented with pulsed-field gel electrophoresis (PFGE), a genotyping method with a high discriminatory power is

considered a golden standard for determination of genetic relatedness of isolates in investigation of outbreaks; however, the method is time consuming. The aim of this study was to investigate the potential of matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) for rapid preliminary evaluation of strain relatedness in investigation of an outbreak by comparing both methods.

**Methods:** Two methods were applied. XbaI-digested genomic DNA (PFGE) banding patterns were used for genotyping of the strains. Isolates were also analyzed by whole cell MALDI-TOF MS as recommended by the manufacturer (Bruker Daltonics, Germany). GelComparII software (Applied Maths, Ghent, Belgium) was used to compare banding patterns of PFGE and mass spectra.

**Results:** PFGE analysis has confirmed an outbreak within two affected units and transmission of *E. coli* – ESBL from one unit to the other. In the case of *K. pneumoniae* – ESBL intra- and interdepartmental nosocomial transmissions were confirmed using PFGE analysis. In both cases analysis based upon mass spectra has shown some agreement between the two methods, however there were some notable exceptions. Some strains from non-related PFGE clusters were grouped together while PFGE analysis demonstrated <60% relatedness.

**Conclusions:** Based upon our data MALDI-TOF-based method did not produce satisfactory results in our preliminary evaluation of strain relatedness in investigation of an outbreak. More outbreak strains must be analyzed with both methods for optimization and definitive evaluation of the use of MALDI-TOF for outbreak investigations.

**P1162** **Molecular profiling of nosocomial isolates of *Klebsiella pneumoniae* expressing AmpC beta-lactamase in a tertiary referral hospital of northern India**

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**Objective:** *Klebsiella pneumoniae* is considered an important pathogen causing nosocomial and community-acquired infections, often associated with the production of plasmid mediated AmpC beta-lactamases, which are frequently described as a part of complex integrons, facilitate their horizontal transfer to other related and unrelated microbes. The present study was designed to determine the occurrence of AmpC producing clinical isolates of *K. pneumoniae*, their molecular characterization, genetic environment, plasmid profile and transmission dynamics in a tertiary referral hospital in northern India.

**Methods:** A total of 132 consecutive, clinical isolates of *K. pneumoniae* were collected from inpatients and outpatients of S.S. Hospital, Banaras Hindu University, Varanasi India, during April 2008 to September 2009. All the isolates were investigated for the presence of AmpC beta-lactamase. Molecular characterization and genetic association of plasmid mediated AmpC producing isolates was studied by performing multiplex PCR for blaAmpC and integrase gene PCR for the presence of integron among AmpC producers. All the AmpC positive isolates were typed by random amplification of polymorphic DNA. The plasmid-mediated AmpC gene blaCIT was further sequenced. Amplicons of EBC gene were digested with Cfr9I to determine their clonal types. All the ampC harbouring isolates resistant to third generation cephalosporins were selected for plasmid profiling, transformation, and transconjugation.

**Results:** A total of 104 isolates were phenotypically positive for AmpC beta-lactamase. On performing their genotypic characterization, 40 isolates were harbouring different ampC gene. Among them 37 were harbouring class 1 integron. Sequencing results confirmed the presence of CMY-2 variant of AmpC. RFLP pattern demonstrated the presence of blaACT among EBC harbouring isolates. CMY-2 and ACT gene on a 16 kb conjugative plasmid was consistently present in all the screened isolates of *K. pneumoniae*, and was inferred to encode cefoxitin and cephalosporin resistance based on transconjugation experiments. RAPD showed presence of 24 different clones.

**Conclusion:** The presence of integron in AmpC producers and their genetic association with the ampC gene underlines the potential risk of their dissemination in hospital as well as in community. Thus the

acquisition of plasmid-mediated ampC genes by *K. pneumoniae* restricts therapeutic alternatives for combatting infections caused by these organisms.

**P1163** **An outbreak of OXA-48 carbapenemase-producing *Klebsiella pneumoniae* in an Irish tertiary referral centre in 2011**

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**Background:** Between January and October 2011, 13 isolates of OXA-48 producing *Klebsiella pneumoniae* (*K. pneumoniae*) were detected in the laboratory. The first five cases were found in clinical specimens of inpatients on general surgical wards.

**Methods:** After the second and third isolate had been confirmed as an OXA-48 producing *K. pneumoniae* by an external reference laboratory, the general surgical wards were closed for admissions and an outbreak investigation undertaken. Case patients were isolated with full contact precautions. Weekly screening for rectal carriage of carbapenem resistant Enterobacteriaceae (CRE), which had already been initiated in the ICU, was commenced on all affected surgical wards. A screen of the general and the near patient environment on the ward was undertaken. Intensified cleaning and disinfection was implemented. The isolate was not detected on environmental screening. Six additional patients were identified as carriers on rectal screening. A review of previous *K. pneumoniae* blood culture isolates stored in the laboratory revealed one additional OXA-48 positive *K. pneumoniae* isolate from a patient not linked to the surgical wards.

**Results:** Typing of all isolates by pulse field gel electrophoresis (PFGE) showed the 10 isolates from the general surgical wards to be identical by PFGE. The OXA-48 *K. pneumoniae* from the patient on the medical ward had a unique typing pattern.

Multi-locus sequence typing (MLST) performed demonstrated a unique sequence type (ST 13). To our knowledge, no other OXA-48 producers associated with this ST have been found.

**Conclusion:** The aim of this presentation is to highlight the emergence of this highly resistant organism in the Irish healthcare setting and to discuss the challenges it presents. The experience of our laboratory underlines the importance of maintaining a high level of alert in Irish laboratories to detect carbapenem resistant isolates. As molecular confirmation of CRE isolates is required, the availability of timely laboratory services will be elementary to control further outbreaks.

**P1164** **Risk factors for locally acquired New Delhi metallo-beta-lactamase-1 Enterobacteriaceae in Singapore**

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**Objective:** New Delhi metallo-beta-lactamase-1 (NDM-1) was reported in India and the United Kingdom in August 2010. The first case of NDM-1 in this hospital was detected in October 2010. Contact screening by rectal swab and strict isolation was immediately instituted. From October 2010 to October 2011, we detected seven NDM-1 patients with no overseas travel in last 2 years. The objective of this study is to assess risk factors for and treatment outcome of NDM-1 Enterobacteriaceae.

**Method:** We conducted a retrospective case-control (1:4 ratio) study from October 2010 to October 2011 for risk factors and treatment outcome. NDM-1 isolates were screened phenotypically and genotypically for acquired metallo-beta-lactamases (MBL), serine carbapenemases and extended spectrum-beta-lactamases (ESBL).

**Results:** We detected eight NDM-1 bacteria in six patients from clinical cultures (six in urine, one in bile) and one from contact screening; five were *Escherichia coli*, two *Klebsiella pneumoniae* (KP) and one *Enterobacter cloacae*. All isolates were positive for at least two ESBL's (TEM-type, SHV-type and CTX-M-type) and plasmid AmpC (DHA-1 or CMY-type) and negative for VIM-type, IMP-type, KHM-1;

OXA-48, KPC-1 and GES-type beta-lactamases or 16S rRNA methylases. Our NDM-1 isolates were distinct from the Indian NDM-1 by multilocus sequence typing. Cases and controls had similar demographic data, with no cases of Indian race. NDM-1 patients had more dementia (37.5% vs. 6.3%,  $p = 0.05$ ). On univariate analysis, cases were more likely to be exposed to carbapenems within 30 days (OR 1.61, 95%CI 1.6–216.1,  $p = 0.019$ ) and fluoroquinolones within 90 days (OR 7.67, 95%CI 1.3–45.3,  $p = 0.025$ ), have other multi-drug resistant organisms (MDRO) (OR 5.95, 95%CI 1.1–31.2,  $p = 0.035$ ) and ESBL KP (OR 9.67, 95%CI 1.5–60.0,  $p = 0.015$ ) within 12 months. All isolates were resistant to all tested carbapenems, cephalosporins, penicillin-inhibitor combinations, and ciprofloxacin; 100% were susceptible to polymyxin B, 87.5% to amikacin, and 25% to gentamycin and ciprofloxacin. Three cases of urinary infections were cured with amikacin or polymyxin B.

**Conclusions:** Carbapenem and fluoroquinolone exposure, and a history of MDRO and ESBL KP were risk factors for NDM-1 at our centre. Amikacin or polymyxin B may be effective in treating NDM-1 urinary infections.

**P1165** Epidemiological, microbiological and clinical characteristics of an outbreak of colistin-resistant KPC carbapenemase-producing *Klebsiella pneumoniae*

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**Objectives:** We aimed at describing the characteristics of an outbreak of KPC carbapenemase – producing *Klebsiella pneumoniae* (KPC-KP), occurring in Pisa Hospital in Italy and still going on.

**Methods:** A boronic acid-inhibition test method was used directly on rectal swabs to detect colonization. Direct Polymerase Chain Reaction (PCR) was also performed on a subset of samples. Susceptibility was tested by the E-test method according to the breakpoints of the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Periodical screening of patients admitted in affected wards was conducted and colonized patients were isolated. Clinical records and laboratory databases were revised.

**Results:** The first case was recorded on April 2010 and data up to the 31st of October 2011 have been revised. One hundred and eighty-eight strains of KPC-KP were isolated from 128 patients. Out of them, 49 had a clinically evident infection: 23 sepsis, 13 respiratory infections, nine abdominal infections, six urinary infections, two other infections. In 79 patients, intestinal KPC-KP colonization occurred asymptotically. Nine patients died. Almost all of them had a long history of hospitalization and serious comorbidity.

PCR confirmed the presence of blaKPC gene. Patients infected by KPC-KP were admitted in the following wards: Intensive Care Units 45, Surgery 18, Neurorehabilitation 9, Infectious Diseases 11, Pneumology 17, Diabetology 8, Haematology 4, others 16.

Number of cases per quarter increased over time with a linear trend. MIC50 and MIC90 of relevant antimicrobials is reported in Table 1. At least half of the strains showed a full or intermediate susceptibility to tygecycline, gentamicin and fosfomicin. Only 30% of strains were non-resistant to colistin or imipenem. Tygecycline and gentamicin were generally used often in association with a third drug, generally fosfomicin. Mortality among patients with sepsis was around 40%.

	Pisa, Italy, 2010-2011		Range		breakpoint EUCAST (mg/L)	
	MIC50 (mg/L)	MIC90 (mg/L)	min (mg/L)	max (mg/L)	≤	>
amikacin	32	>256	1	>256	8	16
gentamicin	1	64	0.19	>1024	2	4
imipenem	24	>32	0.25	>32	2	8
meropenem	>32	>32	4	>32	2	8
ertapenem	>32	>32	0.25	>32	0.5	1
colistin	4	12	0.125	24	2	2
fosfomicin	16	1,024	3	>1024	32	32
tigecycline	1.5	3	0.5	12	1	2
cotrimoxazole	>32	>32	0.049	>32	2	4

**Conclusion:** KPC-KP is spreading in our hospital, especially in ICUs, and is a relevant cause of morbidity and mortality. We suggest treatment to be based on three drugs including imipenem or colistin whenever possible.

**P1166** In vitro activity of colistin and tigecycline against carbapenemase producing *Klebsiella pneumoniae* clinical isolates during 2005–2010 in a university hospital

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**Objective:** *Klebsiella pneumoniae*, a pathogen frequently involved in nosocomial outbreaks, constitutes a serious problem because of its virulence and multidrug resistance. Tigecycline (TIG) and colistin (COL) are antibiotics with broad-spectrum activity; appear to be the only therapeutic option for infections of carvapenemases producing *K. pneumoniae* (Kpcp). The aim of this study was to define the susceptibility of such isolates to TIG and COL, during the last 6 years, in Patras University Hospital.

**Methods:** A total of 493 Kpcp isolates were collected during 2005–2010, from inpatients (313 men), hospitalized in ICU (221), Internal Medicine Units (159) and Surgical wards (113), one isolate per patient. Isolates were recovered from cultures of clinical specimens (177 pus, 149 blood, 100 urine, 67 BAL). Identification was performed by standard methods. Antimicrobial susceptibility was carried out by disk diffusion method according to CLSI. Besides standard antibiotics, gentamicin (GEN) was included, whereas, MIC to imipenem (IMP), TIG and COL were determined by E-test. Isolates were tested applying Hodge Test for the presence of carvapenemases according to CDC and by meropenem-EDTA-Boronic acid synergy disk test, for presence of MBL and/or KPC. The presence of blaVIM and blaKPC genes was confirmed by PCR. Molecular typing was performed by PFGE of XbaI restricted genomic DNA.

**Results:** During the study period, Kpcp isolates were 14%, 22%, 33%, 27%, 34% and 46%, accordingly. Resistance rate among standard antibiotics was very high (>90%), while 42% were resistant to GEN, 20% to COL and 7% to TIG. From 2005 to 2008, all Kpcp isolates (197) were MBL producers, sensitive to TIG with 5% resistant to COL. Three KPC producing isolates were isolated in 2008, sensitive to COL. During 2009, 41MBL/66KPC Kpcp were isolated, whereas, in 2010 20 MBL/186KPC. The resistance rates in 2009 were 25% to COL and 7% to TIG, whereas, in 2010 were 31% and 15%, accordingly. blaVIM gene was detected in all MBL (+) isolates, whereas, blaKPC2 was found in all KPC (+). Three isolates carried both genes. All KPC (+) isolates belonged to pulsotype A.

**Conclusion:** Isolation of Kpcp isolates shows a significant increase during the last 6 years ( $p < 0.001$ ). Only GEN, COL and TIG are effective. During 2009–2010 resistance to COL and TIG also rose significantly ( $p < 0.001$ ). These results emphasize the urgent need for implementation of infection control measures and new antibiotic strategies.

**P1167** Gram-negative bacteraemias in elderly ICU patients

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**Objectives:** Bloodstream infections (BSI) from Gram negative pathogens are among the most frequently encountered nosocomial infections in Intensive Care Unit (ICU) patients representing an important cause of morbidity, mortality and excess cost. Aim of this study was to investigate the epidemiology of these infections in ICU elderly patients and identify potential risk factors.

**Methods:** Retrospective study (January 2007–December 2010) including patients >65 years old with ICU-acquired Gram negative bloodstream infection, as identified by positive blood cultures obtained

>48 hours following ICU admission. Patients were classified into two groups, according to BSI diagnosis. Group A included patients who developed BSI (n = 72) and Group B patients who did not (n = 182). Other data collected included: Age, gender, APACHE II score, septic shock on admission, history of chronic obstructive pulmonary disease (COPD), diabetes mellitus (DM), chronic renal failure (CRF), immunosuppression, prior use of antibiotic or surgery, hypoalbuminaemia, preceding ICU length of stay on day of BSI diagnosis, as well as isolated pathogens. We also evaluated the impact of BSI on duration of mechanical ventilation and central venous catheter (CVC) catheterization, ICU length of stay (LOS) and mortality. Data were analyzed using Mann-Whitney rank sum test, Chi-square and Fisher Exact test.

**Results:** Ninety-four BSIs (10 polymicrobial) were identified in 72 patients. Median time for a positive blood culture was 18 days following admission. Isolated pathogens included *Acinetobacter baumannii* (32), *Klebsiella pneumoniae* (30), *Pseudomonas aeruginosa* (10), *Proteus mirabilis* (7), other Gram negative bacteria (25). Demographic data, evaluation of risk factors and impact of BSIs on ICU morbidity and mortality are depicted on Table 1.

	Group A (n=72)	Group B (n=182)	p-value
Age	74 (71-79)	77 (72-81)	0.022
Gender (male)	45 (62.5)	111 (61)	0.796
APACHE II score	21.5 (16.5-25.5)	20 (16-25)	0.293
Septic shock on admission	32 (44)	57 (31)	0.067
Hypoalbuminaemia	43 (60)	99 (54)	0.528
COPD	26 (36)	54 (30)	0.398
CRF	8 (11)	33 (18)	0.194
DM	24 (33)	62 (34)	0.971
Prior surgery	28 (39)	57 (31)	0.315
Immunosuppression	7 (10)	18 (10)	0.847
Prior antibiotic use	30 (42)	58 (32)	0.183
Days on mechanical ventilation	24 (16-39)	7 (5-12)	<0.001
Days on central venous catheterization	33 (20-53)	11 (7-21)	<0.001
ICU length of stay	28 (19-43)	11 (7-17)	<0.001
ICU mortality	32 (44)	32 (21)	<0.001

TABLE 1: Demographic data, risk factors and impact of BSIs on ICU morbidity and mortality  
Data are number (%) of patients, or median value

**Conclusions:** Gram negative bacteraemias were significantly correlated with age. Prolonged ICU stay was identified as risk factor for BSI acquisition. However, no other risk factor was determined. Gram negative bacteremic episodes significantly prolonged ICU length of stay and increased ICU mortality.

**P1168** Virulence factors and clones of multidrug-resistant *Pseudomonas aeruginosa* isolates disseminated among patients in a University hospital

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**Objectives:** *Pseudomonas aeruginosa* is a cause of serious infections, especially in immunocompromised patients. The high resistance rate to antibiotics, the large number of virulence factors, including the type III secretion system (TTSS) responsible for exotoxins Y, U, T, S, and the frequent spread of endemic strains, constitute this microorganism a major nosocomial pathogen. The association of *P. aeruginosa* isolates carrying TTSS genes recovered from clinical samples of different hospitalized patients during a 2 year period, with respect to antibiotic resistance patterns, serotype and clone dispersal, was investigated.

**Methods:** A total of 220 *P. aeruginosa* isolates recovered from inpatients during 2006-2007 were identified at the species level by standard methods (Oxiferm, BD, BBL). Antibiotic susceptibility testing was performed by the agar disk diffusion method according to CLSI

guidelines. MIC of colistin was determined by the Etest (AB Biodisk). Serotyping was performed using 16 monovalent antisera against the O antigen, according to the International Antigenic Typing Scheme. Clones were defined by PFGE of chromosomal DNA SpeI digests. Exotoxins-gene detection was performed by PCR.

**Results:** Among 220 isolates, 64% were multi or pan-resistant but colistin susceptible. Most isolates were recovered from patients hospitalized in the ICU (55%), followed by the Department of Internal Medicine (26%), Surgery (11%), Outpatients (6%) and Pediatrics (2%). By PFGE, 40 clones were identified, with three predominant: a (50%), d (19%) and b (5%). The majority of isolates (37%) were recovered from respiratory tract samples, followed by wounds (19%), bacteremia (19%), urinary tract (13%), catheters (8%) and stool specimens (4%). The main serotype was O11 (68%) followed by O12 (10%). Most multi pan-resistant isolates (87%) produced one or more toxins: Y (94%), U (94%) and T (87%), while only 37% produced the S toxin.

**Conclusion:** Multi pan-resistant, toxin Y, U, T producing *P. aeruginosa* strains, belonging to two major clones, a and d, have disseminated principally in ICU. Spread of such strains constitute *P. aeruginosa* infections problematic and difficult to treatment, forcing continuous infection control measures and appropriate antibiotic policy.

**P1169** The impact of antimicrobial therapy on survival in patients with nosocomial *Acinetobacter baumannii*-associated infections

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The objective of the present study was to assess the impact of appropriate antimicrobial treatment on clinical outcome in patients with *Acinetobacter baumannii*-associated infections.

**Material and methods.** Eighty seven patients treated at nine multi-field hospitals between December, 2008 and November, 2010 with clinically and laboratory confirmed nosocomial infections caused by *A. baumannii* were included in the study. Among them 39 patients had favorable outcome, while 48 patients died within 30 days after pathogen isolation. Both groups were comparable by age, sex and proportion of ICU-hospitalized patients.

Antimicrobial therapy was considered to be appropriate if the list of administered antibiotics included at least one agent that was active in vitro against isolated strain of *A. baumannii* and when the route of administration and the dosage were adequate.

Antimicrobial susceptibility testing was performed by disk-diffusion method. The results were interpreted following the guidelines of the CLSI, 2011. Intermediately susceptible isolates were regarded as resistant.

In order to assess the impact of antimicrobial therapy on the outcome, cases with appropriate antimicrobial treatment from both studied groups were compared with those who receive inappropriate therapy using the odds ratio model. The Chi-squared or Z-test was used to assess differences in categorical variables, as appropriate. Continuous variables were compared using the Mann-Whitney test. The Shapiro-Wilk's test was used to assess normality. Results were considered statistically significant at  $p < 0.05$ . Data was stored and analyzed using Statistica software v.6.0 (StatSoft Inc., USA).

**Results:** Among patients with favorable outcomes four persons received appropriate empirical antimicrobial treatment, while in the other group – only three patients. Odds ratio for favorable outcome was 1.7 (95% confidence interval [CI] 0.4–8.2;  $p = 0.77$ ).

Appropriate antimicrobial agents were administered as a part of causal treatment to 27 patients who survived and to 11 patients who died within 30 days after pathogen isolation. Odds ratio for favorable outcome was 6.7 (95% CI 2.6–17.3;  $p < 0.001$ ).

**Conclusion:** Our study has revealed that appropriate causal antimicrobial therapy has a positive influence on 30-day survival rate in patients with nosocomial infections caused by *A. baumannii*, confirming in this way the statement that *Acinetobacter baumannii* is a real pathogen but not a witness of the lethal outcome.

**P1170 Clinical characteristics and outcome of post-neurosurgical *Acinetobacter baumannii* meningitis: a multicentre cross-sectional study**

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**Objectives:** *Acinetobacter baumannii* is an important cause of post-neurosurgical meningitis. The emergence of carbapenem-resistant strain in this setting has caused a therapeutic challenge. We investigated the clinical characteristics and therapeutic outcome of the patients with post-neurosurgical *A. baumannii* meningitis.

**Methods:** We retrospectively reviewed medical records of patients with post-neurosurgical *A. baumannii* meningitis diagnosed in five university-affiliated hospitals between January 2005 and May 2011. Only patients older than 16 years of age with positive CSF culture and clinical features compatible with meningitis were included.

**Results:** Forty episodes of post-neurosurgical *A. baumannii* meningitis were reviewed. The mean hospital stay before neurosurgical procedure was 23 days. The most frequent underlying diseases were brain hemorrhage (67.5%), brain neoplasm (7.5%), head trauma (5.0%), and hydrocephalus (5.0%). Eleven patients (27.5%) were treated with intrathecal antibiotics. Thirty-three patients (82.5%) received surgical therapies such as removal of ventricular shunt and lumbar drain. The mortality from meningitis was 35%, which was significantly related to carbapenem resistance ( $p = 0.002$ ). In 22 patients with carbapenem-non-susceptible *A. baumannii* meningitis, colistimethate containing regimen ( $p = 0.040$ ) and intrathecal administration of antibiotics ( $p = 0.001$ ) were significantly associated with recovery from meningitis. During the study period, two patients treated with intravenous colistimethate developed nephrotoxicity. Toxicity related to local administration was not reported in patients treated with intrathecal antibiotics.

**Conclusion:** Carbapenem-non-susceptible *A. baumannii* meningitis showed high mortality in post-neurosurgical patients. Use of colistimethate and intrathecal administration of antibiotics should be considered for the treatment of this infection.

**P1171 Predictors of mortality in multidrug-resistant *Acinetobacter baumannii*: a descriptive study in a long-term acute care hospital**

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**Objectives:** To determine the risk factors for 30-day mortality of multidrug-resistant *Acinetobacter baumannii* (MDRAB) during an outbreak occurred in a non-traditional setting, a 50-bed long-term acute care hospital (LTACH).

**Patients and methods:** From January 2010 to June 2011 an outbreak of 38 MDRAB occurred in a LTACH in Northern Italy. In this descriptive observational study a retrospective chart review was performed to analyse patient demographics, comorbidities and outcome. *Acinetobacter* was identified by automated Vitek2 system (Biomérieux, France). Antibiotic susceptibility was evaluated according to CLSI criteria. A Cox regression model was used for determining risk factors of mortality and a Kaplan–Meier analysis for comparing 30-day survival among subgroups.

**Results:** Mean age of the population was 81.5 years. 94.7% had a comorbidity. Source of isolates was respiratory tract (44.7%), urine (39.5%), blood (7.9%) and wounds (5.2%). All the isolates were resistant to beta-lactams, fluoroquinolones and carbapenems; amikacin and colistin susceptibility was 62% and 100%, respectively. 73.6% had a bacterial coinfection and 47.3% received antibiotic treatment. Colistin monotherapy was the most common (88.9%) antibiotic provided followed by combination therapy with rifampicin and amikacin monotherapy. In bivariate analysis (Figure 1) risk factors significantly associated with 30-day mortality (36.8%) were ischemic encephalopathy or stroke ( $p = 0.041$ ) and presence of central venous catheter (CVC) ( $p = 0.0344$ ). Similarly, the multivariate analysis

exhibited the same variables as the independent risk factors for mortality. Patients with ischemic encephalopathy or stroke and presence of CVC had worse survival than those without ( $p = 0.0286$  and  $p = 0.0252$ , respectively). In addition, there was no statistically significant association between mortality and treatment or coinfection.

Graphic 1. Risk factors for 30-day mortality of MDRAB

Variable	Regression Coefficient	Bivariate analysis 95% confidence Interval		P
		lower	upper	
Age	-0.01	-0.03	0.01	0.62
Sex	0.02	-0.36	0.83	6.95
<b>Underlying conditions</b>				
cardiopathic disease	-0.08	-0.64	0.47	0.08
chronic pulmonary disease	0.46	-0.08	1.02	0.40
ischemic encephalopathy or stroke	1.36	0.69	2.03	0.04
diabetes	-0.17	-0.74	0.39	0.76
hepatic failure	-0.01	-0.02	-0.01	0.99
renal failure	-0.04	-0.64	0.56	0.94
tumor	0.09	-0.50	0.70	0.87
<b>Risk factors</b>				
entrapment syndrome	0.04	-0.33	1.21	0.56
discontinuity of hygienic care	-0.32	-1.37	0.72	0.75
previous ICU-stay	-0.64	-1.41	0.12	0.40
steroid therapy	-0.83	-1.87	0.20	0.42
immunosuppressive therapy *				
broad-spectrum antibiotics	0.53	-0.50	1.58	0.60
central venous catheter	1.19	0.63	1.76	0.03
total parenteral nutrition	-0.18	-0.02	-0.00	0.99
bronchoscopy	0.10	-0.44	0.66	0.84
gastroscopy	-0.18	-0.19	-0.17	0.99
mechanical ventilation	-0.76	-1.81	0.27	0.46
non-invasive ventilation	0.34	-0.22	0.90	0.54
urinary catheter *				
nasogastric tube or gastrostomy	0.03	-0.56	0.64	0.95
<b>Severe infection index</b>				
fever > 38°	0.44	-0.32	1.21	0.56
leucocytosis (>12000/mm <sup>3</sup> )	1.08	0.31	1.85	0.16
C-reactive protein increase	0.17	-0.17	-0.16	0.99
<b>Antibiotic therapy</b>				
Bacterial coinfection	-0.25	-0.30	0.81	0.65
	-0.95	-1.54	-0.36	0.10

\* excluded from the analysis

**Conclusions:** Rare MDRAB outbreaks are described in LTACHs. Our results suggest that neurological comorbidity and invasive procedures, as presence of CVC, have a significant negative impact on survival in MDRAB infections. The treatment and the presence of bacterial coinfection were not associated with mortality, although this study was not powered to assess these covariates adequately. We assume that MDRAB isolation in long-term and post-acute care settings can reflect colonization rather than infection. On the contrary, implementation of infection control measures is mandatory in LTACHs.

**P1172 A multicentre prospective observational study for risk factors and outcome of carbapenem-resistant *Acinetobacter baumannii* bacteraemia**

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**Background:** Risk factors and outcome in patients who acquired healthcare-associated bacteremia due to carbapenem-resistant *Acinetobacter baumannii* (CRAB) are rarely investigated. The aim of this study was to analyze the risk factors and outcome of carbapenem resistance in patients with *A. baumannii* bacteremia.

**Methods:** A multicenter prospective observational study was conducted in 15 teaching hospitals around South Korea from February 2010 to August 2011. Patients who were  $\geq 18$  years old and had healthcare-associated bacteremia due to *A. baumannii* were enrolled. Only one bacteremic episode from one patient was included in the



study. Comparisons were made between patients with CRAB bacteremia and patients with carbapenem-susceptible *A. baumannii* (CSAB) bacteremia.

**Results:** One hundred and ninety-seven patients with CRAB and 69 patients with CSAB were enrolled. Risk factors associated with CRAB bacteremia included prior exposure to carbapenem (Odds ratio [OR] = 5.07; 95% confidence interval [CI] = 1.05–24.62;  $p = 0.044$ ), lower serum albumin concentration prior bacteremia (OR = 0.15; 95% CI = 0.04–0.59;  $p = 0.006$ ), and bacteremia due to pneumonia (OR = 4.58; 95% CI = 1.16–18.07;  $p = 0.030$ ). Patients with CRAB bacteremia had a higher in-hospital mortality rate than patients with CSAB bacteremia (92.4% vs. 7.6%,  $p < 0.001$ ). Multivariate analysis showed that carbapenem resistance (OR = 55.48; 95% CI = 5.05–609.45;  $p = 0.001$ ), prior central venous catheterization (OR = 6.49; 95% CI = 1.19–35.25;  $p = 0.030$ ), PITT bacteremia score (OR = 1.84; 95% CI = 1.20–2.82;  $p = 0.006$ ), and APACHE II score at onset of bacteremia (OR = 1.16; 95% CI = 1.02–1.33;  $p = 0.028$ ) were independent variables associated with in-hospital mortality in patients with *A. baumannii* bacteremia.

**Conclusion:** Prior exposure to carbapenem, lower serum albumin concentration, and pneumonia were associated with the development of CRAB bacteremia. Carbapenem resistance was one of independent risk factors associated with in-hospital mortality in patients with *A. baumannii* bacteremia.

#### **P1173** Bacteraemia caused by multiresistant *Acinetobacter baumannii*: an observational study

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**Background:** Multiresistant *Acinetobacter baumannii* is a problem worldwide. The aim of this study is deepening in the knowledge of the risk factors, clinical features, and treatments of this infections.

**Methods:** A observacional retrospective study was performed to describe the characteristics of bacteremia caused by multiresistant *A. baumannii* in Hospital Universitario Central of Asturias, a university hospital in the north of Spain, from January 2000 to December 2010. Antimicrobial susceptibilities were tested using a microdilution commercial system.

**Results:** During the period of study 103 episodios of bacteremia in 103 patients were included. Sixty-eight patients belonged to intensive care unit. The mean time of stay at hospital was 32 days (range 1–213). The most frequent underlying diseases were neoplasm (14.6%), trauma (12%), diabetes (11%), renal insufficiency (11%), trauma (12%), CNS disease (9%) and kidney transplant (7%). Seventy-nine patients had undergone surgical procedures. Ninety-one percent cases had intravascular catheters, 86% had a bladder catheter and 73% cases had undergoing mechanic ventilation. All patients had received previous antibiotic therapy for other infections. The most frequent origin of bacteraemia were: ventilator-associated pneumonia (28%), surgical wound infection (23%), abdominal abscess (17%), catheter (17%), urinary tract infection (11%), meningitis (4%). Forty-nine patients (47.6%) died due to infection. The mortality were associated to inadequate treatment ( $p = 0.0001$ ). In 67 cases, the microorganism isolated was only susceptible to colistin; in 36 cases, the microorganism was susceptible to colistin and carbapenems. All the patients had received empirical antibiotic treatments that were adequate in 42% of the cases. After receiving the antibiogram, the antibiotic therapy was reassessed and was considered inadequate in five patients. Seven patients died before the instauration of a definitive treatment. Forty-three patients received iv monotherapy with colistin, 35 with carbapenems, three ampicillin/sulbactam. In 15 cases, a combined parenteral therapy was used with imipenem (10 cases) or colistin (five cases) plus aminoglycosides.

**Conclusions:** Bacteremia due to multiresistant *A. baumannii* is a frequent problem in intensive care units. The most frequent origin is the respiratory tract. The mortality is highest and associated with inadequate empiric treatment.

#### **P1174** Impact of delayed appropriate antibiotic therapy on mortality in patients with carbapenem-resistant *Acinetobacter baumannii* bacteraemia: a multicentre prospective observational study

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**Background:** The incidence of carbapenem-resistant *Acinetobacter baumannii* (CRAB) infections is increasing and therapeutic options are limited. However, controversy exists regarding the mortality attributable to delay in starting appropriate antibiotic treatment. The aim of this study was to analyze the clinical features and outcomes of patients with CRAB bacteremia and assess the factors influencing in-hospital mortality, especially in terms of early appropriate antimicrobial therapy.

**Methods:** A multicenter prospective observational study was conducted at 15 teaching hospitals around South Korea from February 2010 to August 2011. Patients who were  $\geq 18$  years old and had nosocomial bacteremia due to *A. baumannii* were enrolled. Only one bacteremic episode from one patient was included in the study. Early appropriate antimicrobial therapy was defined as one or more agents active against CRAB, given with adequate dosage and route of administration, not later than 24 hours after the blood culture was obtained.

**Results:** Of 197 patients with CRAB bacteremia who enrolled in this study, 167 patients were evaluable. The in-hospital mortality rate was 80.2% (134/167). According to univariate analysis, older age, previous central venous catheterization and mechanical ventilation before bacteremia, primary bacteremia, pneumonia, lower serum albumin concentration prior bacteremia, development of severe sepsis or septic shock, higher PITT bacteremia score and APACHE II score, application of mechanical ventilation, and delayed ( $>24$  hours after blood culture obtained) appropriate antibiotic treatment were associated with in-hospital mortality. Multivariate analysis showed that serum albumin concentration prior bacteremia (Odds ratio [OR] = 0.086; 95% confidence interval [CI] = 0.01–0.85;  $p = 0.036$ ) and PITT bacteremia score (OR = 18.14; 95% CI = 2.76–119.40;  $p = 0.003$ ) were independent risk factors for in-hospital mortality.

**Conclusion:** A delay in starting appropriate antibiotic therapy was not associated with higher mortality in our analysis. Our data suggest that severities of baseline condition and infection presentation were major risk factors for in-hospital mortality.

#### **P1175** Risk factors for infection development following an ultrasound guided prostate biopsy procedure and effects of resistant bacteria in the intestinal flora

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**Introduction:** This study is aimed to define the risk factors of post prostate biopsy infections and to determine whether the intestinal colonization of the resistant bacteria against antibiotics used for prophylactic purposes tend to increase the risk of urinary tract infections (UTI).

**Materials and methods:** The study involved 168 patients who undergone prostate biopsy procedure accompanied by transrectal ultrasonography (TRUSPB) while receiving ciprofloxacin and gentamycin for prophylaxis. Fecal cultures were taken from all of the patients before the prophylaxis and presence of ciprofloxacin resistant bacteria was inspected by antibiogram. Odds ratio was used to evaluate whether the questioned risk factors increase UTI incidence.

**Results:** Ciprofloxacin resistant bacteria were detected in 81 out of 168 stool samples (48.2%) obtained before the procedure. From these 81 isolates, 74 were *E. coli*, five were *Enterococcus*, two were *Klebsiella*. Among the 74 ciprofloxacin resistant *E. coli* strains, 39.2% were detected to produce extended spectrum beta lactamase, and the

sensitivity rates of nitrofurantoin, fosfomycin, and amycacin were found to be relatively high (95%, 98.8% and 100% respectively).

UTI developed in 10.1% of the patients (17/168) who had had TRUSPB. Six patients (3.6%) had the infection accompanied by high fever and urosepsis was found to occur in two patients (1.2%). The findings of the risk analysis revealed that the history of urolithiasis (OR = 2.23, 95% CI 1.06–9.56  $p = 0.03$ ), presence of long term urinary catheterization (OR = 10.64, 95% CI 1.40–81.45,  $p = 0.05$ ), hospitalization preceding the prostate biopsy procedure by maximum 1 year (OR = 3.91, 95% CI 1.10–14.05,  $p < 0.03$ ), history of UTI  $\geq 3$  times in the last 1 year (OR = 4.39 95% CI 1.02–18.84,  $p = 0.03$ ) and recent history of antimicrobial agent utilization (OR = 4.85, 95% CI 1.34–17.59,  $p = 0.009$ ) were determined as the risk factors for infections to develop following a TRUSPB. None of the patients who were not detected to have intestinal fluoroquinolone resistant bacteria colonization developed an infection following TRUSPB ( $p < 0.001$ ).

**Conclusion:** The study conducted shows that the intestinal colonization of ciprofloxacin resistant bacteria increases the risk of infection development following a prostate biopsy procedure under ciprofloxacin prophylaxis.

**P1176 Risk factors for *Burkholderia cepacia* complex bacteraemia among intensive care unit patients in a Greek general hospital**

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**Objective:** We performed a case-control study to investigate risk factors of bacteremia in non-CF patients and describe the epidemiology of BCC infection in our institution.

**Methods:** In a 16 month-period between January 2010 and April 2011 BCC was recovered from 13 patients without CF admitted to the Intensive Care Unit (ICU) of our hospital. A case of BCC bacteremia was defined as a positive blood culture for the bacteria combined with deterioration of clinical status. Controls (N = 52) were defined as patients who had spent at least 7 days in the ICU within 2 weeks of the primary episode of BCC bacteremia of their matched case but did not have BCC isolated during the study period (N = 52). Risk factors that were investigated were the presence of tracheostomy, malignancies, administration of colistin during the last 10 days, administration of propofol 3 days before the onset of symptoms, administration of central venous catheters, recent abdominal surgery, length of hospitalization, preexisting comorbid conditions, presence of percutaneous feeding tube and renal failure. Multivariate logistic regression analysis (stepwise selection procedure) was performed in order to determine independent risk factors of acquisition of BCC bacteremia. Covariates were included in the model if univariate logistic regression analysis revealed that were significantly associated with BCC bacteremia at an  $p$  value of  $< 0.10$ .

**Results:** Multiple regression analysis (table attached) revealed the presence of tracheostomy, malignancies, length of hospitalization, administration of colistin during the last 10 days and administration of propofol 3 days before the onset of symptoms as independent risk factors for BCC bacteremia. Meropenem and ciprofloxacin and piperacillin-tazobactam were the most active agents against BCC.

RISK FACTOR	ODDS RATIO	ODDS RATIO (95% CI)	P VALUE
Presence of tracheostomy	2,23	1,01-3,45	0,044
Malignancies	3,01	1,4-4,61	0,023
Administration of colistin	4,12	1,53-6,81	0,019
Administration of propofol 3 days before the onset of symptoms	5,23	2,2-8,46	0,012
Length of hospitalization	1,9	1,01-2,78	0,049

**Conclusion:** Because BCC colonizes the respiratory tract, it is not surprising that factors associated with compromised respiratory status, such as tracheostomy emerged as significant risk factor for BCC bacteremia. Underlying malignancy is associated with immunosuppression. The continuous infusion of propofol supports bacterial growth because it is a lipid based emulsion. Other researchers had concluded that administration of antibiotics decrease the risk of BCC bacteremia. However, because of the intrinsic resistance to colistin of BCC, the administration of the antibiotic eliminates the other bacteria responsible for bacteremia and allows the growth of BCC.

**P1177 Outbreak of *Burkholderia multivorans* in non-cystic fibrosis patients hospitalised in intensive care units**

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**Objectives:** The *Burkholderia cepacia* complex (Bcc) comprises a large group of opportunistic pathogenic bacteria. The epidemiology and role of Bcc as an etiological agent in non-cystic fibrosis (non-CF) patients is poorly understood.

**Methods:** Between 1 February 2011 and 30 September 2011, all Bcc isolates obtained from clinical samples from patients hospitalized in the University Hospital Olomouc were collected. All Bcc isolates were identified and speciated using the Phoenix automated system (Becton Dickinson), the RapID™ System biochemical tests (Remel) and PCR analysis. Genetic relationships between isolates were identified by a random amplified polymorphic DNA (RAPD) method. Multilocus sequence typing (MLST) was used with selected epidemic isolate.

**Results:** A total of 74 Bcc isolates were acquired with 52 non-duplicate isolates from separate patients. Forty five strains (86.5%) were genomovar II (*Burkholderia multivorans*) and all these strains were isolated from patients without cystic fibrosis (CF). RAPD showed 25 different clonal groups (I–XXV) amongst these strains, the largest number of identical strains was in group III (23 strains, 44.2%). In this group, 15 (65.2%) patients were hospitalized in intensive care units (ICU) and strains were most frequently isolated from the lower respiratory tract (53.3%) and upper respiratory tract (26.7%). A phylogenetic analysis of *gyrB* of the epidemic strain showed this strain has previously been isolated from the United Kingdom, USA, New Zealand and recently the Czech Republic and found in environmental samples, CF and non-CF patients.

**Conclusion:** We investigated 52 strains from distinct patients. Most frequently isolated was *Burkholderia multivorans* (86.5%) isolated from patients without CF. Over 44% of these strains were identical by RAPD suggesting an outbreak and more than 65% of them were isolated from ICU patients. Deaths directly associated with group III *Burkholderia multivorans* infection were noted in four cases. Genetic analysis of the epidemic strain suggested a global distribution which now includes the Czech Republic. The rate of 86% of Bcc isolates being *Burkholderia multivorans* is unusual and is a novel observation. The source of the epidemic strains has not been found as yet.

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**International and national resistance surveillance data**

**P1178 Prevalence of extended-spectrum beta-lactamases in Europe: 2004–2011**

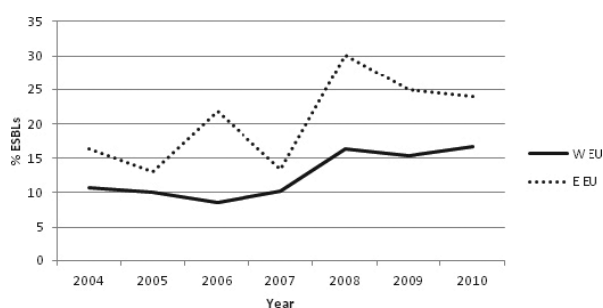
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**Background:** Infection by extended-spectrum beta-lactamase (ESBL)-producing bacterial pathogens is increasing worldwide, with the

prevalence of ESBLs in Europe varying greatly from country to country. Patients with infections caused by an ESBL-producing organism are at increased risk of treatment failure. The Tigecycline European Surveillance Trial (TEST), a longitudinal surveillance study, determined the ESBL status and antibiotic susceptibilities to tigecycline and comparator compounds for 21 149 *Escherichia coli*, *Klebsiella oxytoca*, and *Klebsiella pneumoniae*, from 25 European countries from 2004 to 2010.

**Methods:** Twenty-one thousand and seventy-three isolates (10 814 *E. coli*, 2648 *K. oxytoca*, 7687 *K. pneumoniae*) from 14 Western European and 11 Eastern European countries were analyzed in this survey. The isolates were identified to the species level at the participating sites and confirmed by the central laboratory. MICs were determined by each site using supplied broth microdilution panels and interpreted according to EUCAST guidelines. ESBL testing was performed by Laboratories International for Microbiology Studies (LIMS), a subsidiary of International Health Management Associates, Inc. (IHMA, Schaumburg, IL, USA) following CLSI guidelines.

**Results:** Results are shown in the following figure.



**Conclusions:** Linear trends in the prevalence of ESBLs in Eastern and Western Europe for the years 2004 to 2010 continue to increase significantly ( $p < 0.00001$ , Cochran-Armitage test). The percentage of ESBL+ *E. coli*, *K. oxytoca*, and *K. pneumoniae* isolates in 2010 was 16.7% in Western Europe and 24% in Eastern Europe. The percentage varies greatly from country to country, ranging from 0% in Finland to 41% in Italy.

**P1179** Global trends in frequency and susceptibility of extended-spectrum beta-lactamase positive *E. coli*, *K. pneumoniae*, and *K. oxytoca* Isolated from intra-abdominal infections since 2005 – the SMART Study

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**Objectives:** The Study for Monitoring Antimicrobial Resistance Trends (SMART) program has been monitoring the activity of ertapenem (ETP), amikacin (AK), cefepime (CPE), ceftazidime (CAZ), ceftazidime (CAZ), ceftriaxone (CAX), cefotaxime (CFT), ciprofloxacin (CP), imipenem (IMP), levofloxacin (LVX), ampicillin/sulbactam (AS), and piperacillin/tazobactam (PT) against gram-negative bacteria isolated from intra-abdominal infections (IAI) since 2002. This report evaluates changes in frequency of ESBL+ *E. coli* (Ec), *K. pneumoniae* (Kp), and *K. oxytoca* (Ko) since 2005, and their impact on susceptibility levels.

**Methods:** Seventy-six (2005) to 155 (2010) labs per year in Asia/Pacific (AP), Latin America (LA), Middle East/Africa (MEA), Europe (EU), and North America (NA) collected 32 605 isolates of Ec/Kp/Ko from IAI. Microdilution minimum inhibitory concentrations (MICs) and extended spectrum beta-lactamase (ESBL) confirmation were done by Clinical and Laboratory Standards Institute (CLSI) methods. % ESBL+ were determined each year, and percent of susceptible strains (%S) to study drugs were compared for ESBL+ vs. ESBL- strains.

Region	2005	2006	2007	2008	2009	2010
AP <sup>1</sup>	23% (782) <sup>2</sup>	27% (1289) <sup>2</sup>	38% (1935)	32% (1871)	35% (2298)	29% (2400) <sup>2</sup>
LA <sup>2</sup>	18% (514)	23% (579)	30% (680)	29% (659)	24% (1176)	33% (1493)
MEA <sup>2</sup>	16% (257)	16% (283)	15% (330)	14% (204)	16% (206)	22% (404)
EU <sup>2</sup>	7% (1635)	8% (1839)	10% (1661)	12% (1938)	12% (2183)	13% (2210)
NA	4% (257)	8% (293)	8% (311)	6% (477)	7% (1240)	8% (1202)

<sup>1</sup> Significant increasing trend ( $p < 0.05$ , Cochran-Armitage test).  
<sup>2</sup> No isolates available from India.

**Results:** Global % ESBL+ (Ec + Kp + Ko) by year:

**Conclusions:** 1 ESBL+ Ec/Kp/Ko have increased sharply since 2005, with statistically significant trends in Latin America, Middle East/Africa, and Europe and decreased %S to many drugs (incl. CP, LVX). Even the increases in North America ( $p = 0.054$ ) and Asia/Pacific ( $p = 0.06$ ) were almost significant, with the trend in Asia/Pacific being confounded by the availability of isolates from India (where ESBL rates are very high) only in 2007–2009.

2 ETP and IMP remain the most effective of the study drugs against ESBL+ strains.

**P1180** Susceptibility of intra-abdominal pathogens and occurrence of extended-spectrum beta-lactamase producers in 13 European countries – the SMART study 2010–2011

R. Badal\*, S. Lob, S. Bouchillon, D. Hoban, S. Hawser, A. Johnson, M. Hackel (Schaumburg, US; Epalinges, CH)

**Objectives:** The Study for Monitoring Antimicrobial Resistance Trends (SMART) has been monitoring the antimicrobial susceptibility of aerobic gram-negative bacteria from intra-abdominal infections (IAI) since 2002. This report compares susceptibility levels of key IAI pathogens in 13 countries Europe to ertapenem (ETP), amikacin (AK), cefepime (CPE), ceftazidime (CAZ), ceftriaxone (CAX), ciprofloxacin (CP), imipenem (IMP), levofloxacin (LVX), ampicillin-sulbactam (AS), and piperacillin/tazobactam (PT) during 2010–2011.

**Methods:** Forty-six labs in 13 European countries each collected up to 100 consecutive gram-negative bacteria/year from IAI in 2010–2011. MICs were determined by broth microdilution, and interpreted using EUCAST guidelines.

**Results:** Four thousand and twenty isolates were collected. The rate of extended spectrum beta-lactamase (ESBL) producers was 9% in Europe overall, with country-specific rates ranging from 1% and 4% in Estonia and France to 16% and 26% in Latvia and Turkey, respectively. The table below shows % susceptible for each drug for all isolates combined for which EUCAST breakpoints were available; values at least 5% less than the European average are shaded.

	n	ESBL <sup>1</sup>	AS <sup>2</sup>	AK	CAX	CAZ	CFT	CP	CPE	ETP	IMP	LVX	PT
Estonia	104	1%	59%	98%	83%	86%	83%	90%	90%	95%	95%	95%	89%
France	406	4%	52%	95%	84%	84%	84%	86%	89%	97%	98%	88%	89%
Germany	390	10%	51%	96%	78%	80%	79%	81%	85%	96%	95%	85%	86%
Greece	182	12%	37%	65%	71%	73%	70%	61%	76%	83%	72%	63%	73%
Hungary	298	13%	40%	82%	67%	69%	67%	58%	74%	92%	91%	64%	79%
Italy	461	15%	33%	85%	65%	65%	64%	65%	72%	94%	90%	68%	73%
Latvia	146	16%	50%	82%	71%	70%	70%	64%	76%	98%	86%	65%	81%
Lithuania	98	8%	58%	92%	84%	90%	84%	80%	89%	99%	100%	83%	92%
Portugal	242	7%	47%	93%	84%	86%	83%	79%	90%	99%	89%	81%	85%
Romania	90	13%	48%	88%	76%	77%	78%	82%	82%	99%	92%	83%	88%
Spain	1155	5%	48%	96%	89%	88%	88%	79%	92%	98%	96%	81%	88%
Turkey	284	26%	34%	80%	59%	66%	58%	61%	64%	90%	85%	61%	68%
UK	160	8%	53%	95%	84%	84%	84%	81%	89%	99%	93%	83%	86%
EU	4,020	9%	46%	90%	79%	80%	78%	75%	84%	96%	92%	77%	83%

<sup>1</sup> % of all species of IAI isolates that were ESBL+.

<sup>2</sup> Only species for which breakpoints exist were included in the %S calculations for each drug; eg, *P. aeruginosa* was not included in the AS, CAX, CFT, or ETP %S calculations as no breakpoints exist.

**Conclusions:** 1 The susceptibility of IAI pathogens varied dramatically among the 13 European countries that participated in this study, with AK, ETP, and IMP showing the highest % susceptible for all species combined for which EUCAST breakpoints exist.

2 ESBL rates also varied by country, and countries with high rates showed lower overall susceptibilities against IAI pathogens.

3 Surveillance studies such as SMART highlighting current regional ESBL rates and susceptibility patterns are important for guiding empiric therapy for IAI.

**P1181 Susceptibility of intra-abdominal pathogens in Europe, Africa, and the Middle East – SMART 2010/2011**

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**Objectives:** The Study for Monitoring Antimicrobial Resistance Trends (SMART) has monitored susceptibility of intra-abdominal infection (IAI) pathogens since 2002. During that time, antimicrobial resistance has increased alarmingly in many regions of the world. This report summarizes European, African, and Middle Eastern data from SMART 2010 and 2011.

**Methods:** Fifty-four hospitals in 18 countries each collected up to 100 consecutive isolates of gram-negative aerobic bacilli from IAI. Isolate identification and susceptibility testing was done at a central laboratory, and interpreted using CLSI M100-S21 guidelines.

**Results:** Four thousand seven hundred and thirty-six isolates were collected, of which almost 70% were *E. coli* (49%), *K. pneumoniae* (11%), and *P. aeruginosa* (8%). Ten percent of all IAI pathogens (13% of *E. coli* and 28% of *K. pneumoniae*) were ESBL+ with the reduced susceptibility profiles normally associated with that phenotype. Regional ESBL+ *E. coli* rates ranged from 3% (Africa, n = 238) to 13% (Europe, n = 1944) and 38% (Middle East, n = 130), with the difference between Europe and Middle East being statistically significant (p < 0.05, Fisher's exact test). Susceptibility of organisms with n > 50, listed by frequency of occurrence, are shown in the table below. Shading denotes % susceptible values ≥90%.

Organism	n	Ak*	AS	Cpe	Cft	Cfx	Caz	Cax	Cp	Ep	Imp	Lvx	PT
<i>E. coli</i> , all	2312	98	45	89	85	94	89	86	75	99	99	75	91
<i>E. coli</i> , ESBL+	302	90	9	21	1	88	30	2	21	96	99	22	77
<i>E. coli</i> , ESBL-	2010	100	51	100	98	95	98	98	83	100	99	83	93
<i>K. pneumoniae</i> , all	530	94	54	75	69	83	72	69	72	99	94	75	77
<i>K. pneumo.</i> , ESBL+	150	83	1	15	4	69	11	3	23	7	83	31	46
<i>K. pneumo.</i> , ESBL-	380	98	74	98	95	88	96	95	91	97	98	92	89
<i>P. aeruginosa</i>	380	80	na	77	na	na	70	na	70	na	76	78	86
<i>E. cloacae</i>	304	99	13	89	56	4	63	55	85	74	93	92	78
<i>P. mirabilis</i>	211	100	73	93	87	94	94	86	75	100	26	86	99
<i>P. mirabilis</i> , ESBL+	15	100	20	13	7	93	80	7	27	100	13	47	93
<i>P. mirabilis</i> , ESBL-	196	100	77	99	93	94	95	92	79	100	27	89	99
<i>K. oxytoca</i>	189	98	70	95	90	95	97	87	87	97	99	97	87
<i>K. oxytoca</i> , ESBL+	13	85	8	46	0	85	69	8	77	91	92	77	38
<i>K. oxytoca</i> , ESBL-	176	99	74	99	97	95	99	93	98	99	99	99	91
<i>A. baumannii</i>	163	39	19	14	11	na	18	12	16	na	39	24	14
<i>C. freundii</i>	141	100	53	97	68	8	70	67	96	97	97	96	87
<i>M. morgani</i>	97	99	5	98	64	88	76	80	88	99	15	94	98
<i>E. aerogenes</i>	77	100	35	97	56	6	60	55	94	86	83	94	71
<i>S. marcescens</i>	76	95	14	92	68	43	89	75	83	99	97	95	91

\*Ak=amikacin, AS=ampicillin-sulbactam, Cpe=cefepime, Cft=cefotaxime, Cfx=cefotaxin, Caz=ceftazidime, Cax=ceftriaxone, Cp=ciprofloxacin, Ep=ertapenem, Imp=imipenem, Lvx=levofloxacin, PT=piperacillin-tazobactam, na=no breakpoint defined.

**Conclusions:** ESBL rates varied between the regions with particularly high rates found in the Middle East. Overall only three of the study drugs, ertapenem, imipenem, and amikacin, showed an average % susceptible of ≥90% for the two species accounting for almost 2/3 of all IAI pathogens (*E. coli* and *K. pneumoniae*). These three agents remained effective against most ESBL+ isolates. Although *A. baumannii* represented only 3% of IAI isolates, this species was <40% susceptible to all drugs for which it has breakpoints. Increasing resistance requires ongoing monitoring to help control the rapid spread of multi-drug resistant pathogens.

**P1182 Susceptibility of Gram-negative pathogens isolated from urinary tract infections in Portugal between 2009 and 2010 – the SMART Study**

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**Objectives:** The Study for Monitoring Antimicrobial Resistance Trends (SMART) has been monitoring activity of ertapenem (Etp), amikacin (Ak), cefepime (Cpe), cefotaxime (Ctx), ceftazidime (Caz), ceftriaxone (Cax), ciprofloxacin (Cp), imipenem (Imp), levofloxacin (Lvx) and piperacillin/tazobactam (PT) vs. gram-negative bacteria from urinary tract infections (UTI) since late 2009. This report summarizes the ESBL prevalence in Enterobacteriaceae as well as susceptibility levels for key UTI pathogens in Portugal during 2009–2010.

**Methods:** Three Portuguese community hospital laboratories, representing North, Center and South of Portugal, each collected up to 25 (in 2009) and 50 (in 2010), consecutive gram-negative bacteria from UTI. MICs were determined by broth microdilution, and interpreted using CLSI guidelines M100-S20U (2010). ESBL production was determined for *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae* and *oxytoca* using the CLSI phenotypic method.

**Results:** Two hundred and forty-nine isolates were collected from UTI during the last 2 years. Susceptibility results are shown in Table 1. As expected the most prevalent species were *E. coli* (n = 120) and *K. pneumoniae* (n = 46), composing about 70% of all UTI pathogens. The ESBL rate was very high at 30.3% and 44.6% for those two species.

Drug	Breakpoints (S/I/R)	Susceptibility results 2009			Susceptibility results 2010		
		<i>E. coli</i>	<i>K. pneumoniae</i>	<i>P. mirabilis</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>P. mirabilis</i>
Amikacin	≤16   ≥2   =68	92/88	100	100	97/48	0	95
Cefepime	≤8   ≤8   =82	88/1	100	83/33	100	55/13	0
Ceftriaxone	≤2   ≤4	88/1	100	83/33	100	52/56	0
Cefotaxime	≤4   ≤8   =89	95/48	100	83/33	100	93/18	100
Ceftazidime	≤2   ≤4	88/1	100	83/33	100	52/56	0
Ciprofloxacin	≤1   ≤2   =4	65/9	100	66/67	100	48/72	0
Ertapenem	≤4/25   8/5   =6	100	100	83/33	100	100	100
Imipenem	≤2   ≤4	100	100	100	0	100	100
Levofloxacin	≤2   ≤4   =8	63/9	100	66/67	100	48/72	0
Piperacillin/Tazobactam	≤16/8   ≤2/8/4/4   =128/8	95/34	100	50	100	85/9	0

**Conclusions:** *E. coli* (~50% of all UTI pathogens) susceptibility decreased to almost all the antibiotics tested in 2010. *K. pneumoniae* (~18% of all UTI pathogens) susceptibility also decreased in 2010. The ESBL rates in these two species are disturbing, considering that it causes ineffectiveness of the majority of the antibiotics tested and used in clinical practice. Until definitive identification and susceptibility testing results are known, options for effective empirical therapy of UTI in Portugal have diminished to include very few (e.g., carbapenems, amikacin) of the agents evaluated in this study.

**P1183 Susceptibility of Gram-negative pathogens isolated from intra-abdominal infections in Portugal between 2008 and 2010 – the SMART Study**

S. Ferreira\*, J. Diogo, A. Castro, H. Ramos, E. Ramalheira (Aveiro, Lisbon, Porto, PT)

**Objectives:** The Study for Monitoring Antimicrobial Resistance Trends (SMART) has been monitoring activity of ertapenem (Etp), amikacin (Ak), cefepime (Cpe), cefoxitin (Cfx), ceftazidime (Caz), ceftriaxone (Cax), ciprofloxacin (Cp), imipenem (Imp), levofloxacin (Lvx) and piperacillin/tazobactam (PT) vs. gram-negative bacteria from intra-abdominal infections (IAI) since 2002. This report summarizes the ESBL prevalence as well as susceptibility levels for key IAI pathogens in Portugal during 2008–2010.

**Methods:** Three Portuguese community hospital laboratories, representing North, Center and South of Portugal, each collected up to 100 consecutive gram-negative bacteria/year from IAI during 2008–2010. MICs were determined by broth microdilution in a central laboratory, and interpreted using CLSI guidelines M100-S20U (2010). ESBL production was determined for *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae* and *oxytoca* using the CLSI phenotypic method.

**Results:** Seven hundred and sixty-eight isolates were collected from IAI during the last 3 years. The results are shown in Table 1. As expected the most prevalent species were *E. coli* (n = 325) and *K. pneumoniae* (n = 104), representing >50% of all IAI pathogens, and the ESBL rates were, respectively, 12% and 14%; no ESBL was detected for *K. oxytoca* or *P. mirabilis*.

Organism	S (n/N)	Susceptibility results 2008					Susceptibility results 2009					Susceptibility results 2010													
		AS	AK	AUG	CAX	CAZ	CFT	CP	CPE	ETP	IMP	LVX	PT	AS	AK	AUG	CAX	CAZ	CFT	CP	CPE	ETP	IMP	LVX	PT
<i>A. baumannii</i>	57	80											25		25	25									
<i>A. hydrophila</i>	14	42.9	100	50.0	78.6	92.9	85.7	100	100	57.1	64.3	100	78.6												
<i>C. freundii</i>	190	46.3	96.8	10.1	65.8	67.4	66.8	91.1	90.5	99.0	98.4	91.7	84.7												
<i>C. koseri</i>	39	96.9	100	96.7	100	100	100	100	100	100	100	100	100												
<i>E. aerogenes</i>	126	27.8	99.2	7.7	46.8	47.6	49.2	95.2	88.9	92.1	95.2	96.8	61.1												
<i>E. cloacae</i>	385	17.1	99.2	4.7	60.0	60.3	60.0	91.7	81.0	86.8	98.7	93.8	75.6												
<i>E. coli</i> , all	3771	35.4	97.6	22.4	90.1	90.0	89.6	73.1	92.5	95.8	99.9	74.9	91.0												
<i>E. coli</i> ESBL+	308	13.0	87.3	71.8	5.2	19.5	4.2	25.6	20.8	99.0	99.7	33.4	80.2												
<i>H. alvei</i>	22	28.2	100	0	31.8	22.7	45.5	100	95.5	100	100	100	77.3												
<i>K. oxytoca</i>	307	73.3	99.4	88.0	92.2	97.1	95.4	95.8	95.8	100	99.7	96.1	93.8												
<i>K. pneumoniae</i> , all	684	67.7	97.4	87.6	90.8	89.2	91.2	85.5	92.3	99.0	99.6	87.7	85.2												
<i>K. pneumoniae</i> ESBL+	61	11.5	82.0	47.7	23.0	11.5	21.3	39.3	36.1	96.7	100	44.3	39.3												
<i>M. morgani</i>	163	3.7	96.9	2.1	84.7	66.9	66.3	80.4	98.2	99.4	66.9	85.3	97.6												
<i>P. mirabilis</i>	347	80.4	91.6	92.5	95.7	97.4	96.3	77.0	99.1	99.7	84.2	89.1	99.4												
<i>P. vulgaris</i>	58	68.8	98.3	94.4	56.9	86.2	87.9	98.3	95.6	100	81.0	98.3	98.3												
<i>P. aeruginosa</i>	423	80.1				76.4			73.1	74.7		72.3	68.8	74.2											
<i>S. marcescens</i>	56	34.3	100	7.1	85.7	89.3	78.6	89.3	94.6	98.2	100	96.4	89.1												

**Conclusions:** There was no visible alterations in the susceptibility along the timeframe studied (2008–2010) in both *E. coli* and *K. pneumoniae*. Until definitive identification and susceptibility testing results are known, options for effective empirical therapy of IAI in Portugal remain a wide range of antibiotics of the agents evaluated in this study.

**P1184 Comparative in vitro activity of amoxicillin/clavulanate against aerobic Gram-negative pathogens in Spain – SMART 2005–2010**  
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**Objectives:** The Study for Monitoring Antimicrobial Resistance Trends (SMART) has been monitoring worldwide activity of ertapenem (ETP), amikacin (AK), cefepime (CPE), cefoxitin (CFX), ceftazidime (CAZ), ceftriaxone (CAX), cefotaxime (CFT), ciprofloxacin (CP), imipenem (IMP), levofloxacin (LVX), ampicillin/sulbactam (AS), and piperacillin/tazobactam (PT) against gram-negative intra-abdominal infection (IAI) pathogens since 2002, and from urinary tract infection (UTI) starting in late 2009. Additionally, amoxicillin/clavulanate (AUG) was tested specifically against Spanish isolates. This report compares the in vitro activity of these drugs in Spain during this period.

**Methods:** Nine thousand four hundred and sixty-one isolates were collected by 11 Spanish hospitals from IAI (2005–2010) and UTI (2009–2010). Extended spectrum beta-lactamase (ESBL) confirmation and MIC determinations for all drugs except AUG were done following Clinical and Laboratory Standards Institute (CLSI) methods; AUG was tested by Etest using the same inoculum as the other drugs. EUCAST breakpoints were used to interpret MICs.

**Results:** % Susceptible (%S) for all species with n > 10 is shown below. Values ≥90% are shaded.

Note: blanks = no breakpoint established.

ESBL+ rates in 2010 for *E. coli* and *K. pneumoniae* were 8.8 and 13.6%, respectively.

Organism	N	AS	AK	AUG	CAX	CAZ	CFT	CP	CPE	ETP	IMP	LVX	PT
<i>A. baumannii</i>	57												
<i>A. hydrophila</i>	14	42.9	100	50.0	78.6	92.9	85.7	100	100	57.1	64.3	100	78.6
<i>C. freundii</i>	190	46.3	96.8	10.1	65.8	67.4	66.8	91.1	90.5	99.0	98.4	91.7	84.7
<i>C. koseri</i>	39	96.9	100	96.7	100	100	100	100	100	100	100	100	100
<i>E. aerogenes</i>	126	27.8	99.2	7.7	46.8	47.6	49.2	95.2	88.9	92.1	95.2	96.8	61.1
<i>E. cloacae</i>	385	17.1	99.2	4.7	60.0	60.3	60.0	91.7	81.0	86.8	98.7	93.8	75.6
<i>E. coli</i> , all	3771	35.4	97.6	22.4	90.1	90.0	89.6	73.1	92.5	95.8	99.9	74.9	91.0
<i>E. coli</i> ESBL+	308	13.0	87.3	71.8	5.2	19.5	4.2	25.6	20.8	99.0	99.7	33.4	80.2
<i>H. alvei</i>	22	28.2	100		31.8	22.7	45.5	100	95.5	100	100	100	77.3
<i>K. oxytoca</i>	307	73.3	99.4	88.0	92.2	97.1	95.4	95.8	95.8	100	99.7	96.1	93.8
<i>K. pneumoniae</i> , all	684	67.7	97.4	87.6	90.8	89.2	91.2	85.5	92.3	99.0	99.6	87.7	85.2
<i>K. pneumoniae</i> ESBL+	61	11.5	82.0	47.7	23.0	11.5	21.3	39.3	36.1	96.7	100	44.3	39.3
<i>M. morgani</i>	163	3.7	96.9	2.1	84.7	66.9	66.3	80.4	98.2	99.4	66.9	85.3	97.6
<i>P. mirabilis</i>	347	80.4	91.6	92.5	95.7	97.4	96.3	77.0	99.1	99.7	84.2	89.1	99.4
<i>P. vulgaris</i>	58	68.8	98.3	94.4	56.9	86.2	87.9	98.3	95.6	100	81.0	98.3	98.3
<i>P. aeruginosa</i>	423	80.1				76.4			73.1	74.7		72.3	68.8
<i>S. marcescens</i>	56	34.3	100	7.1	85.7	89.3	78.6	89.3	94.6	98.2	100	96.4	89.1

**Conclusions:** 1 AUG was the second least active drug in the study, inhibiting ≥90% of only three relatively minor species causing IAI and UTI (*P. mirabilis*, *P. vulgaris*, and *C. koseri*); only AS was less active. 2 AUG retained marginal activity (72%) vs. ESBL+ *E. coli*, but it only inhibited 48% of ESBL+ *K. pneumoniae*. With nearly 14% of *K. pneumoniae* being ESBL+ in Spain, use of AUG to treat IAI may be unwise until ESBL status of the pathogen is known. 3 Only ETP and IMP inhibited >90% of ESBL+ isolates. ESBL+ *K. pneumoniae* were especially troubling, as other than the carbapenems and AK, no other drug inhibited >50% of these isolates.

**P1185 In vitro susceptibilities of aerobic and facultative Gram-negative bacilli isolated from patients with intra-abdominal infections and urinary tract infections in China: the 2010 Study for Monitoring Antimicrobial Resistance Trends (SMART)**

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**Objectives:** The Study for Monitoring Antimicrobial Resistance Trends (SMART) is an ongoing study to monitor the worldwide in vitro susceptibilities of aerobic and facultative gram-negative bacilli (GNB) isolated from intra-abdominal infections (IAI). In 2010, isolates from urinary tract infections (UTI) were also included. This report summarizes the most recently completed data in 2010 from SMART study in China.

**Methods:** In 2010, GNB isolates were collected from 13 teaching hospitals in 11 cities of China. Antimicrobial susceptibilities were tested by CLSI broth microdilution method (M07-A8). Extended-spectrum beta-lactamases (ESBLs) were tested by phenotypic confirmatory test recommended by CLSI. Modified Hodge Test (MHT) and PCR amplification for GES-, KPC-, SME-, NMC-, IMP-, VIM-, SPM-, GIM-, SIM- and NMD-encoding genes were performed for Enterobacteriaceae with imipenem MICs ≥ 4 µg/mL and/or ertapenem MICs ≥ 1 µg/mL.

**Results:** One thousand one hundred and forty-two isolates from IAIs and 530 isolates from UTIs were collected. Enterobacteriaceae composed 80.6% of IAI isolates and 88.3% UTI isolates. *E. coli* (53.1%/66.8%), *K. pneumoniae* (13.6%/11.5%) and *P. aeruginosa* (9.5%/5.8%) were the most common isolates from both IAIs and UTIs. Imipenem and ertapenem were the most active agents against Enterobacteriaceae (>96% susceptible) according to the breakpoints of CLSI M100-S20, followed by amikacin and piperacillin/tazobactam. The percentages of ESBL-producers in *E. coli*, *K. pneumoniae*, *K. oxytoca* and *P. mirabilis* for IAIs and UTI were 66.1%/64.5%, 35.7%/56.9%, 52.9%/28.6% and 16.7%/28.6%, respectively. Of the 181 isolates with elevated carbapenem MICs, only 11 were MHT positive. One isolate of *E. coli* and three isolates of *K. pneumoniae* produced KPC-2. One isolate of *M. morgani* produced both IMP-1 and VIM-2. One isolate of *K. oxytoca* produced VIM-1. One isolate of *E. cloacae* produced IMP-2. All four KPC-2-producers and one IMP-2-producer were MHT positive.

**Conclusion:** Enterobacteriaceae were still the main gram-negative pathogens for IAIs and UTIs in China in 2010. The carbapenems were the most active agents in vitro against Enterobacteriaceae. The percentage of ESBL-producers was higher than the previous SMART data in China, especially for *P. mirabilis*. Carbapenem-producing Enterobacteriaceae were still rare in mainland of China.

**P1186 Frequency and antimicrobial resistance of Gram-negative bacilli isolated from Latin America hospitals: results from SENTRY Antimicrobial Surveillance programme, 2008–2010**

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**Objective:** To provide an update on the frequency and antimicrobial resistance (R) of Gram-negative bacilli (GNB) isolated from SENTRY Program Latin American medical centers (LAMC). This program has monitored antimicrobial R in LAMC since 1997.

**Methods:** Twelve thousand eight hundred and eleven organisms, including 5704 GNB (44.5%), were consecutively collected (one per patient) between January 2008 and December 2010 from 10 LAMC located in Argentina (ARG; 2), Brazil (BRA; 4), Chile (CHI; 2) and Mexico (MEX; 2). The isolates were susceptibility (S) tested by the CLSI broth microdilution method at a central laboratory. *E. coli* (EC),

*Klebsiella* spp. (KSP) and *Enterobacter* spp. (ESP) isolates with MIC,  $\geq 2$  mg/L for imipenem (IMI) or meropenem (MER) were screened for carbapenemases by the Modified Hodge test, PCR for blaIMP, blaVIM, blaKPC, blaSME, blaGES, blaIMI, blaNMC-A, and blaOXA-48, and DNA sequencing. Selected IMI-R *Acinetobacter* spp. (ASP) strains were screened for blaOXA-23, 24, 51, 58, blaKPC and MBLs by PCR.

**Results:** The infection sites included bloodstream (BSI; 52.6%), skin and soft tissue (22.9%) and pneumonia (13.1%), among others. The most common causes of BSI were *S. aureus* (SA; 22%), EC (21%) and KSP (13%); while *P. aeruginosa* (PSA; 33%), SA (21%), ASP (18%), KSP (11%) and ESP (5%) represented the top five organisms from pneumonia. ESBL rates were 18.0%, 12.8%, 23.8% and 48.4% among EC and 60.5%, 49.9%, 59.2% and 33.3% among KSP from ARG, BRA, CHI and MEX, respectively. MER-non-S KSP was highest in BRA (11.1%), followed by ARG (8.2%), CHI (5.0%) and MEX (0.8%). KPC-producing KPN was not detected in 2008, but emerge in 2009 (10 strains) and increased significantly in 2010 (44;  $p < 0.0001$ ). blaKPC-2 was detected in 54 of 85 (65.9%) carbapenem-non-S *K. pneumoniae* (KPN). MER-non-S PSA was observed in 53.8%, 46.7%, 33.3% and 28.8% of strains from ARG, BRA, CHI and MEX, respectively. IMI-S ASP decreased from 38.6%, 83.2% and 91.8% in 2003–2005 to 14.5%, 28.6% and 47.4% in 2008–2010 in ARG, BRA and CHI, respectively. OXA-producing ASP was documented in ARG (OXA-23), BRA (OXA-23 and -24), CHI (OXA-58) and MEX (OXA-24). Colistin was the only compound with reasonable activity against ASP and PSA (Table 1).

Antimicrobial	% susceptible <sup>a</sup> (no. tested)				
	EC (1517)	KSP (1052)	ESP (451)	PSA (1099)	ASP (845)
Ceftazidime	82.1	54.9	63.6	61.1	12.8
Cefepime	84.1	58.8	82.0	61.9	14.8
Piperacillin/Tazobactam	90.5	60.9	74.1	55.6	10.2
Meropenem	99.9	92.2	98.2	57.1	30.3
Ciprofloxacin	59.7	55.9	76.3	56.7	12.5
Gentamicin	81.2	63.1	78.7	63.2	37.8
Amikacin	98.6	86.8	92.2	73.3	29.4
Colistin	99.8 <sup>b</sup>	97.0 <sup>b</sup>	82.7 <sup>b</sup>	99.7	98.1

a. Based on CLSI breakpoint criteria (2011); b. PSA breakpoint was applied.

**Conclusions:** Only COL showed >77% overall coverage against the five most frequently isolated GNB from LAMC participating in the SENTRY Program. Empiric antimicrobial therapy for serious infections caused by GNB requires the combination of two or more agents for adequate coverage.

#### **P1187** Resistance patterns in multi-resistant Enterobacteriaceae: data from the German Antibiotic Resistance Surveillance System (ARS) from 2008 to 2010

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**Objectives:** Multiresistant gramnegative bacteria have emerged as a serious public health problem worldwide requiring consistent and intensified surveillance efforts. Data from ARS, the German Antibiotic Resistance Surveillance system are presented.

**Methods:** ARS is a voluntary laboratory based surveillance system collecting resistance data of all clinical pathogens and sample types. Data are transmitted electronically to the central data-base of the national public health institute (Robert Koch Institute). The development of susceptibility-rates and of resistance patterns of *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*) from 2008 to 2010 is presented. The panel of antibiotics and antibiotic classes used for the estimation of resistance patterns is presented in Table 1.

**Results:** In hospital care, susceptibility testing was performed in more than 24 000 *E. coli* -isolates and more than 4500 *K. pneumoniae* isolates throughout all 3 years.

From 2008 to 2010, non-susceptibility against cefotaxim (CTX) in *E. coli* rose from 6.9% to 9.3% and in *K. pneumoniae* from 10.4% to

11.4%. In CTX-non-susceptible isolates of both species the most frequent resistance pattern includes non-susceptibility against fluoroquinolones, aminoglycosides, trimethoprim/sulfamethoxazol and tetracyclines. From 2008 to 2010, CTX-non-susceptible *E. coli* isolates comprising four additional antibiotic classes (all betalactams excluded, except carbapenems) have decreased (33.5–27.3%), while non-susceptibility patterns with only one additional non-susceptible antibiotic class increased (12.5–16.6%). In contrast, in *K. pneumoniae* non-susceptibility-patterns of CTX-non-susceptible isolates comprising three additional antibiotic classes have increased (34.3–40.5%) and the patterns with one additional non-susceptible antibiotic sank (11.7–8.3%). In the observed time period non-susceptibility against carbapenems in both species remained under 1%.

Table1: Panel of antibiotics and antibiotic classes used for the estimation of resistance patterns

Antibiotic or antibiotic class	<i>E. coli</i>	<i>Klebsiella pneumoniae</i>
Aminopenicillines	x	
Aminopenicillin-betaactamase inhibitors	x	x
Piperacillin	x	
Piperacillin/Tazobactam	x	x
3. Generation-cephalosporins	x	x
Carbapenems	x	x
Ciprofloxacin	x	x
Trimethoprim/Sulfamethoxazol	x	x
Tetracyclines	x	x
Aminoglycosides	x	x

**Conclusion:** While the overall CTX-non-susceptibility rates in *E. coli* and *K. pneumoniae* show an increase, the patterns of CTX-non-susceptibility combined with non-susceptibility to additional antibiotic classes show different directions with *K. pneumoniae* isolates showing a trend toward more resistant isolates. The analysis of resistance patterns provides a deeper insight into strain characteristics and allows a more differentiate tracing of the development of antibiotic resistance.

#### **P1188** Epidemiology of ESBL-producing Enterobacteriaceae extended-spectrum beta-lactamases in Belgian hospitals: results of nationwide multicentre survey in 2010

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**Objectives:** We assessed the species distribution and diversity of extended-spectrum beta-lactamases (ESBLs) genes found in Enterobacteriaceae isolates prospectively collected in Belgian hospitals.

**Methods:** Hospital-based laboratories participating to the national surveillance network were requested to send to the reference center five nonduplicate clinical Enterobacteriaceae isolates resistant to 3rd and/or 4th generation cephalosporins (putative ESBL producers), collected during the first trimester of 2010.

Identification was confirmed by MALDI-TOF MS. The presence of ESBL was detected by a positive disk synergy test with clavulanic acid and with phenyl boronic acid (400 µg) disks for AmpC hyperproducers. ESBLs were identified by DNA micro-array (Check-Points, The Netherlands). Typing of selected isolates was performed by PFGE and by semi-automated rep-PCR.

**Results:** Ninety laboratories (43 in Flanders, 30 in Wallonia, 17 in Brussels) sent 433 isolates (400 confirmed as ESBL) from patients (mean age 66 years; range 1–99 years) hospitalized in medical (52%), surgical units (19%) or ICU (18%). ESBLs were isolated from urine (62%), respiratory tract (16%), pus (13%) and blood (6%). *E. coli*, *K. pneumoniae*, *E. aerogenes*, and *E. cloacae* represented 64%, 14%, 13% and 5% of the ESBLs, respectively. *E. coli* accounted for 75% of the ESBLs collected in a community-acquired setting and the proportion and incidence of ESBL-producing *E. coli* rose from 4.1% and 2.2/1000 admissions in 2006 to 6.3% and 4.1/1000 admissions in 2010. The majority of ESBL types were CTX-M (71%; of which 80% were CTX-M-15). Coresistance to ciprofloxacin, cotrimoxazole and

aminoglycosides among ESBL was 75%, 72% and 40% respectively. Spread of CTX-M-15 *E. coli*, *K. pneumoniae* and SHV-12/CTX-M-9 *E. cloacae* belonging to identical or closely related rep-PCR and/or PFGE types was observed in several hospitals in different cities.

**Conclusion:** In comparison to previous surveys carried out in 2006 and in 2008, we observed an increase in the proportion and incidence of CTX-M-15 producing *E. coli*. The spread of epidemic clones of ESBL-producers in Belgium is disquieting and highlights the need for adapted national guidelines in order to limit the cross-transmission of these organisms in Belgian hospitals.

**P1189** Nationwide survey of extended-spectrum beta-lactamases, metallo-beta-lactamases, and oxacillinases produced by multidrug-resistant *Pseudomonas aeruginosa* strains in Belgium in 2010

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**Objectives:** The increasing prevalence of nosocomial infections produced by multidrug-resistant (MDR) *Pseudomonas aeruginosa* (PA) strains severely compromises the selection of appropriate treatments and is associated with significant morbidity and mortality. The aim of this study was to determine the frequency and the different types of acquired (and transferable) beta-lactamases in a collection of 111 MDR clinical PA isolates that were isolated in Belgian hospitals in 2010.

**Methods:** During a 3-month period, 54 hospital laboratories participating in the NSIH national surveillance network collected 1080 nonredundant strains of PA. MICs of 12 antipseudomonal antibiotics were determined centrally by microbroth dilution according to CLSI guidelines against MDR PA isolates (defined by resistance to at least three classes of antimicrobials among beta-lactams, carbapenems, aminoglycosides and quinolones). Beta-lactam resistance mechanisms of MDR strains were analyzed by IEF, PCR and sequencing with specific primers targeting most class A, B and D beta-lactamase coding genes. The epidemiological relatedness of the strains was studied by O serotyping, semi-automated Rep-PCR, PFGE and MLST.

**Results:** Overall, 111 MDR PA isolates (10.3%) were found in 36 hospitals (in 24 different cities). Colistin was the only agent tested consistently active against MDR PA (85% susceptibility by CLSI breakpoints). Metallo-beta-lactamases (MBLs) were detected in 55 isolates (VIM-2, n = 47; VIM-4, n = 7; VIM-1, n = 1). ESBLs were found in 13 isolates (BEL-1, n = 7, PER-1, n = 4, SHV-2a and VEB-1, n = 1 each) and OXA/CARB penicillinases in 44 strains (OXA-2, n = 14; OXA-10, n = 11 (9 in association with VIM-2); OXA-31/-35, n = 7; OXA-9, n = 2; CARB-1/-4, n = 6).

Overall, MDR PA strains were distributed among 30 distinct PFGE types and 21 rep-PCR patterns. The 47 VIM-2 producing isolates clustered in three major PGFE types (Q, X and W) were all of serotype O12 and belong to ST111 or ST244. On the other hand, VIM-4 as well as the BEL-1, PER-1 and VEB-1 clustered in several distinct PFGE and rep-PCR types, were predominantly serotype O11 and of ST235 type.

**Conclusion:** The emergence and epidemic diffusion of several clones of MDR PA isolates with acquired and transferable resistance mechanisms in Belgian hospitals is a matter of concern and underlines the need for continuous epidemiological monitoring.

**P1190** Prevalence of plasmid-encoded fosfomycin resistance gene among clinical isolates of extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in Korea and the genetic environment of fosA3

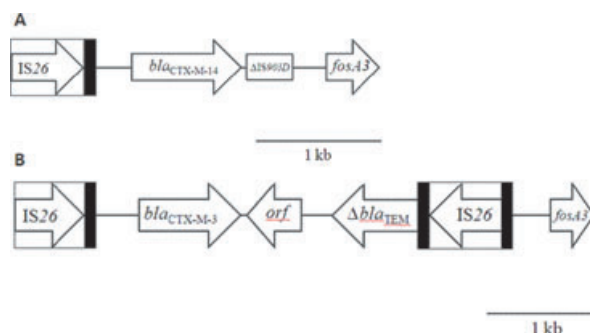
Y.J. Kim\*, S.Y. Lee, J.K. Yu, K.G. Park, Y.J. Park (Seoul, KR)

**Objectives:** The rising rate of multi-drug resistance in Enterobacteriaceae reduces the number of effective drugs that can be used. Fosfomycin, although known for nearly four decades, has been

rarely used in Korea. Although fosfomycin resistance is mostly due to mutation in the chromosomal locus including *glpT*, several, plasmid mediated resistance genes are reported. In this study, we evaluated the prevalence of plasmid-mediated fosfomycin resistance determinants among the ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* and their genetic environments.

**Methods:** A total of 347 non-duplicate ESBL-producing clinical isolates, including *E. coli* (165) and *K. pneumoniae* (182), collected at 25 hospitals in Korea, from June 2009 to July 2009. The fosfomycin MICs were determined by an agar dilution method according to the CLSI guideline using MH agar supplemented with 25 µg/mL glucose-6-phosphate. PCR was used to detect the presence of ESBLs (*bla*TEM, *bla*SHV, *bla*CTX-M) and plasmid-encoded fosfomycin resistance genes (*fosA*, *fosA3*, *fosB*, *fosC2*). The PCR products were sequenced on a 3730 DNA Analyser (Applied Biosystems, Foster City, CA, USA). The transferability of resistance was studied by the transformation experiments using *E. coli* TOPO10 (Invitrogen, USA) as the recipient strain. From plasmid recovered from the transformants, the sequences of DNAs adjacent to *fosA3* were determined with a series of PCR primers and plasmid replicon typing was performed as described by Carattoli et al.

**Results:** The susceptibility to fosfomycin was 92.9% in *E. coli*, and 95.2% in *K. pneumoniae*. Of the 21 isolates non-susceptible to fosfomycin (eight *E. coli* and 13 *K. pneumoniae*), seven (five *E. coli* and 2 *K. pneumoniae*) isolates harbored *fosA3* and all of them co-harbored *bla*CTX-M-1group or *bla*CTX-M-9group. The transformants were successfully obtained from six isolates and the *bla*CTX-M was always co-transferred to the recipient *E. coli* strain. The replicon type of transformants co-harboring *bla*CTX-M-1group and *bla*CTX-M-9group were IncF and IncN type, respectively. The genetic environment of the *fosA3* carried by IncN and IncF type plasmids are described in Figure 1.



**Conclusions:** The fosfomycin resistance rate in ESBL-producing *E. coli* and *K. pneumoniae* was low in Korea (4.2%, 5.5%, respectively). However, *fosA3* was already indwelling in the transferable plasmid of *bla*CTX-M harboring clinical isolates.

**P1191** Resistance to colistin in *Klebsiella pneumoniae* – a slowly emerging problem in England

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**Objectives:** We sought evidence for resistance to colistin amongst all isolates of *Klebsiella pneumoniae* submitted to the national reference service from January to November 2011 and similarly reviewed all isolates collected in 2003 onwards.

**Methods:** Clinical isolates of *K. pneumoniae* from patients throughout England were referred to the HPA reference service for evaluation. Patient details were collated and repeat specimens excluded. MICs of 27 antibiotics, including beta-lactams, aminoglycosides and colistin, were determined for each isolate by agar dilution (BSAC method). Current BSAC/EUCAST breakpoints for all antibiotics tested (e.g. MIC > 2 for colistin) were applied to isolates collected from 2003 to

2011 inclusive. Resistance mechanisms present in 2011 isolates were compared to those of the first colistin-resistant isolates within the 8 previous years (period 2003–2011).

**Results:** There were 28 colistin-resistant *K. pneumoniae* from 29 patients in 19 hospitals during the first 10 months of 2011 compared to three isolates from three patients in two hospitals in 2004 and none in 2003. MICs of colistin were from 4 to >32 in 2011 but all were >32 in 2004. In 2011, there were 18 male (aged 4 weeks to 82 years) and 10 female (aged 18–83 years) patients. Nine isolates were from urine, five from wounds, four each from blood or faeces, two from line tips and one each from umbilicus, sputum or nose. Five patients were from one hospital and two different isolates from one patient in another hospital. Of the 28 isolates, 14 had an ESBL whilst 18 had a carbapenemase of which three were metallo-beta-lactamases (MBL) plus an ESBL. Nine were pan-aminoglycoside resistant and only seven had sensitivity to one aminoglycoside; due to beta-lactamase action + reduced permeability, only two were sensitive to ertapenem (MICs  $\leq$  0.5). Five were sensitive to tigecycline only and one was non-susceptible. One of the 2004 isolates had a ceftazidimase whilst the other two a CTX-M. In 2005 there were nine colistin-resistant isolates (MIC = 32) from six hospitals – two identical strains from one hospital and three from another; all had CTX-Ms and one an MBL. All isolates were pan-resistant to cephalosporins (cefotaxime, ceftazidime, cefpirome; MICs 32 to >256). **Conclusion:** The first isolates of *K. pneumoniae* resistant to colistin occurred in 2004, with an increase of almost ten times that in 2011. Isolates had one or more other significant resistance mechanism. Resistance to Colistin in *Klebsiella pneumoniae* appears to be a slowly emerging problem in England.

#### **P1192** Evolution of *Escherichia coli* susceptibility in the French community from 2004 to 2010

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**Objectives:** The aim of this study was to determine the susceptibility of bacterial strains isolated from community-acquired infections. Survey was carried out by a network with 100 medical analysis laboratories in France in 2010.

**Methods:** All the strains of *Escherichia coli* isolated in these laboratories over a 7-year period, from January 2004 to December 2010, were included in the investigation.

**Results:** 200276 antibiograms were collected: 97.7% of *Escherichia coli* were isolated from urine. Among fluoroquinolones, the susceptibility of ciprofloxacin was higher than for ofloxacin/norfloxacin. Indeed, during the study period, the susceptibility to ofloxacin or norfloxacin decreased gradually from 91.4% in 2004 to 85.9% in 2010 ( $p < 0.001$ ) and the susceptibility rate to ciprofloxacin has slightly decreased since 2006 (92.7% in 2006 to 90.1% in 2010,  $p < 0.001$ ).

The rate of susceptibility to amoxicillin decreased gradually and significantly over the study period: 60.7% in 2004 and 56.4% in 2010 ( $p < 0.001$ ). Moreover, a slight decrease was observed in the susceptibility rates to amoxicillin-clavulanic acid during the study period, from 77.3% in 2004 to 72.0% in 2010 ( $p < 0.001$ ).

Cefotaxime, Ceftazidime and Ceftriaxone were the most active agents, with more than 97.6% and 97.0% of susceptibility in 2005 and 2010 respectively ( $p < 0.05$ ). The susceptibility of cefixime was stable 96.8% in 2004 and 96.3% in 2010. 2.4% *E. coli* isolated were shown to produce extended spectrum beta lactamases (ESBL).

The susceptibility to Nitrofurantoin was relatively stable over the studied period (98.2% in 2004 and 98.6% in 2010). For the susceptibility to cotrimoxazole, the rates decreased during the studied period from 85.6% in 2004 to 81.7% in 2010 ( $p < 0.01$ ).

**Conclusion:** This study confirms the worrying character of the evolution of resistance of *E. coli* to the quinolones. Quinolones should not be any more recommended to treat urinary infections in the community. A better knowledge of the bacterial epidemiology in the community should contribute to adapt the antibiotics strategies.

MedQual conduct a communication plan with Social Security alongside general practitioners to adapt the prescription of antibiotics to the resistance profile of the community Multi-Resistant Bacteria.

#### **P1193** Phenotypic and genetic screening of beta-lactamases mediated resistance amongst clinical isolates of Gram-negative bacilli from Romanian hospitals

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The purpose of this study was the detection of various types of beta-lactamases in Gram-negative rods (GNR) isolated from intensive care units (ICU).

**Material and methods:** A number of 531 GNR strains (334 Enterobacteriaceae and 197 Pseudomonadaceae) were isolated from 1166 positive clinical samples collected from patients hospitalized during 2011, in the ICUs of two big hospital from Buhcarest, Romania. Their resistance phenotypes were established using: disk diffusion test, double-disk diffusion test (DDST) with amoxicillin-clavulanic acid (AMC), cefotaxime (CTX) and ceftazidime (CAZ), DDST with AMC plus EDTA, imipenem (IPM) and IMP plus EDTA, Modified Hodge Test (MHT) and E-test ESBL, MBL and AmpC. The genetic support of the beta-lactam resistance mediated by enzymatic mechanisms was investigated by simple (blaampC, blaTEM grup, blaSHV grup, blaPSE grup, with PSE-1, PSE 4, CARB-3 alleles, blaIMP) and respectively multiplex PCR (blaVIM, blaSIM si blaSPM).

**Results and discussion:** GNR strains constituted the most frequent etiology of infections occurred in hospitalized patients, representing 45.5% from the total number of positive cases. Out of these, 64% of the Enterobacteriaceae and 36% of the Pseudomonadaceae strains expressed resistance to beta-lactam antibiotics. The extended spectrum beta-lactamases (ESBL) phenotype was present in 35.8%, while the metallo beta-lactamases (MBL) one in 34% of the tested GNR strains. A significant percentage (9.4%) of the tested strains exhibited simultaneously ESBL and MBL profiles. Some of the *Enterobacter* sp. strains expressed inducible cephalosporinases and extended spectrum AmpC cephalosporinases. The molecular study revealed, in decreasing frequency order, the presence of blaSHV, blaTEM and blaPSE genes. More than half of the *Pseudomonas aeruginosa* strains resistant to carbapenems exhibited the blaVIM gene.

**Conclusion:** The beta-lactam resistance profiles of Enterobacteriaceae and Pseudomonadaceae strains, mediated by beta-lactamases production, become more complex, especially in hospital units with high selective pressure, such are the ICUs. The high percent of ESBL and MBL producing strains suggests the necessity of resistance genotyping of key Gram-negative pathogens isolated from our geographical area, that will substantially contribute to the understanding of the genetics of antibiotic resistance and of the spread of resistance determinants in the hospital environment.

#### **P1194** Microbiological characteristics of Gram-negative bacteria isolated from patients with intra-abdominal infections in Latvia, 2007–2010

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**Objective:** This study was conducted in Pauls Stradins Clinical University Hospital in order to determine the most common Gram-negative bacteria causing intra-abdominal infections and their susceptibility to commonly used antimicrobials.

**Methods:** Data presented is part of the Study for Monitoring Antimicrobial Resistance Trends (SMART). Microbiological isolates were obtained from intra-abdominal samples by conventional/automated methods. In vitro antibacterial susceptibility was detected using MIC panel against ampicillin/sulbactam, amikacin, ceftriaxone, ceftazidime, cefotaxime, ceftoxitin, ciprofloxacin, cefepime, ertapenem, imipenem, levofloxacin, piperacillin/tazobactam and interpreted according to the CLSI standard.



**Results:** A total of 382 Gram-negative bacteria were isolated in 2007–2010, of which Enterobacteriaceae accounted for 88.7% (339), *Pseudomonas* spp. – 7.1% (27) and *Acinetobacter baumannii* – 4.2% (16) of the species. Majority of Enterobacteriaceae were *Escherichia coli* – 60.5% (205 isolates) and *Klebsiella pneumoniae* – 10.6% (36), extended-spectrum beta-lactamase producers (ESBL-positive) were 12% (25) and 42% (15), respectively. Equally high prevalence of ESBL positive Gram-negative bacteria was observed over the period from 2007 to 2010. Most antimicrobials were effective against non-ESBL *E. coli* and *Kl. pneumoniae* displaying susceptibility >85%, except ampicillin/sulbactam with susceptibility 65.6% and 71.4%, respectively. ESBL-positive *E. coli* and *Kl. pneumoniae* susceptibility to imipenem was 100%, to amikacin – 88% and 86.7%, to ertapenem – 88% and 80%, to ceftoxitin – 84% and 66.7%, to piperacillin/tazobactam – 84% and 53.3%, but <36% and 7% to other tested antimicrobials. The most resistant Gram-negative bacteria isolated from intra-abdominal samples were *Ac. baumannii* (just 62.5% susceptible to amikacin, 25% to imipenem, 18.8% to ampicillin/sulbactam and <13% to other antibiotics).

**Conclusions:** Persistently high prevalence of ESBL producing Gram-negative bacteria in intra-abdominal samples from patients in Latvian multidisciplinary hospital was observed, while high susceptibility to carbapenems was still recorded.

**P1195 Epidemiological data of VIM- and IMP-producing *P. aeruginosa* prevalence in Novosibirsk in patients undergoing cardiosurgery**

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**Objectives:** At present day the prevalence of multi-drug resistant strains of *P. aeruginosa* is an increasing therapeutical problem. The acquisition of metallo-beta-lactamases is one of the most common causes of carbapenem resistance in gram-negative bacteria. VIM and IMP-producing *P. aeruginosa* is widespread in Europe. In this work we report the epidemiological data of prevalence and types of metallo-beta-lactamase (MBL) producing strains of *P. aeruginosa*.

**Methods:** Clinical isolates of *P. aeruginosa* obtained from patients admitted in ICU after cardiosurgery from August 2009 to August 2011. Identification and susceptibility of *P. aeruginosa* were tested with the Phoenix device (Becton Dickinson). MBL production was detected by the imipenem-EDTA disk synergy test. Expression of VIM and IMP genes were tested with PCR using melting curve analysis.

**Results:** On routine microbiological examination 19 isolates of *P. aeruginosa* were resistant to all beta-lactams. Presence of MBL production was proven by positive imipenem-EDTA disk synergy test in all 19 cases. Using PCR we found that 11 isolates (58% of MBL-positive strains) were expressed by blaVIM gene and eight isolates (42%) were expressed by blaIMP gene.

**Conclusions:** During the study period 19 carbapenem-resistant MBL-producing strains of *P. aeruginosa* were found. Carbapenem-resistance was defined by blaVIM gene in 58% cases and by blaIMP gene in 42% cases. Surveillance and typing of carbapenem-resistant strains of *P. aeruginosa* is necessary to prevent further spread of these strains.

**P1196 Surveillance of carbapenemase producing Enterobacteriaceae in the Netherlands**

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**Objectives:** Carbapenemase producing Enterobacteriaceae are an important threat to health care. Besides resistance to the class of beta-lactam antibiotics, associated resistance genes to other classes of antibiotics leave very little possibilities for antibiotic therapy for infections with these strains. In several countries, outbreaks in hospitals

and rapid dissemination through the health care system have been observed; a reason to start surveillance in The Netherlands.

**Methods:** Medical microbiological laboratories in the country were advised to submit all Enterobacteriaceae with an MIC for meropenem >0.25 for phenotypical and genotypical confirmation for carbapenemases. Carbapenemase genes tested by PCR were: KPC, NDM, VIM, GIM, SIM, IMP, SPM and OXA-48. Meropenem MIC was determined with E-test. Further strain and epidemiological information was collected.

**Results:** A large outbreak in one hospital with an OXA-48 positive *Klebsiella pneumoniae* was detected. Data from this hospital is excluded from the description of the surveillance results.

For 2011, up to November, 123 isolates from 97 patients were submitted by 35 hospitals. Twenty-two different CPE were detected from 20 patients. Twice, a patient harboured two different bacterial species (*E. coli* and *K. pneumoniae*) with the same carbapenemase (OXA-48, respectively NDM).

Carbapenemases found were OXA-48 (15x), NDM (4x), KPC (2x) and VIM (1x). Species involved were *K. pneumoniae* (18x), *Escherichia coli* (2x), *Citrobacter freundii* (1x) and *Enterobacter cloacae* (1x). Meropenem MICs ranged from 0.38 mg/L (OXA-48) to >32 mg/L (7x). MICs were within sensitive range ( $\leq 2$  mg/L) in eight isolates and resistant (>8 mg/L) in nine isolates.

Risk factors reported in 13 cases were admission to a foreign hospital in eight cases (Marocco 4x, Turkey, Pakistan) or the Dutch outbreak hospital in five cases.

**Conclusion:** Besides the large OXA-48 outbreak, no other CPE outbreaks were detected in 2011 though the surveillance and a limited number of CPE were seen in The Netherlands. The surveillance is on voluntary base and may therefore not have complete national coverage yet. As far as known, cases could either be related to the outbreak or to admissions in foreign hospitals. *Klebsiella pneumoniae* was the predominant species (82%) and OXA-48 the most frequently encountered (68%) carbapenemase.

**P1197 Evolving epidemiology of carbapenem-resistant Enterobacteriaceae: molecular characterisation of KPC-positive strains and circulating plasmids**

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**Objectives:** The worldwide dissemination of carbapenemase-producing MDRO is becoming a crucial public health problem. A large number of plasmid-mediated enzymes with carbapenemase activity has been identified in Enterobacteriaceae, KPC (*Klebsiella pneumoniae* carbapenemase) being the most frequent. The aim of this study was to characterize both at the phenotypic and molecular level carbapenem-resistant strains that caused outbreaks in the Padua Teaching Hospital from January 2009 to October 2011. In particular, we focused on detection of clonal spreading of resistant strains and on sequence modification of KPC-encoding plasmids, monitoring and updating the epidemic spreading for almost 3 years.

**Methods:** Non-repetitive Enterobacteriaceae isolates with an imipenem MIC  $\geq 1$  mg/L were identified by automated systems and collected from intensive care units, surgical and medical wards. The presence of ESBL and carbapenemase genes belonging to Ambler class A, B or D was assessed by PCR. Molecular typing of positive strains was performed by PFGE, MLST and ERIC-PCR. Plasmids conferring carbapenem resistance were extracted and compared by RFLP, southern blot and deep sequenced.

**Results:** The initial outbreak in 2009 of carbapenem-resistant strains was caused by KPC-positive *K. pneumoniae* strains mostly collected from intensive care units. In particular, KPC-3 was associated with ST258 and KPC-2 with ST147. Since the end of 2010, spreading of non-clonally related KPC-3/KPC-2-positive strains (ST37, ST527, ST512, ST554, ST307, ST510 and ST437) was observed in different wards, including general medical ones. All KPC-positive plasmids were collected and sequenced: we found two different plasmid backbones

encoding KPC-3 and one presenting KPC-2. In addition, most of the strains presented TEM-1, SHV-11/12 and OXA-9. Interestingly, four KPC-2 isolates were additionally positive for the metallo-beta-lactamase VIM-1.

**Conclusion:** Our data indicate that few KPC-positive plasmids have rapidly spread in our region with a worrisome highly efficient horizontal transfer. Importantly, carbapenem-resistant strains have not been confined to intensive care units, but have extended to medical wards as well, leading to involvement of the non-hospitalized population with subsequent dangerous community acquisition. Early identification of carbapenemase producers in clinical infections, also at the carriage state, is thus becoming mandatory to prevent and contain further resistance spreading.

#### P1198 Intestinal colonisation by imipenem-resistant Gram-negative bacilli in ICU patients

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**Objectives:** The intestinal flora is a major reservoir of Gram negative bacilli potentially pathogenic for ICU patients. Their increasing resistance to cephalosporins leads to increase use of carbapenems. Here, we evaluated the intestinal carriage of Imipenem resistant Gram negative bacilli (IR-GNB) in ICU patients.

**Methods:** During a 6-month period, 523 consecutive ICU patients were screened for rectal IR-GNB colonization upon admission and weekly thereafter. Swabs were plated on Drigalski agar with imipenem E-test strips. MIC to carbapenems were determined in all GNB growing in the inhibition ellipse. Underlying resistance traits of IR-GNB were characterized. Genetic relatedness between IR-GNB was determined using the semi-automated repetitive-sequences-based PCR (rep-PCR) DiversiLab system® (Biomérieux). A case-control study was performed to identify risk factors for IR-GNB acquisition.

**Results:** IR-GNB colonization rate was 2.7% upon admission, increased to 5.6%, 15.1%, 29.7%, 36.8%, 44.7%, and reached 58.6% after 1–6 weeks of ICU stay, respectively. Overall, 56 IR-GNB were collected from 50 patients: 36 *P. aeruginosa*, 12 *S. maltophilia*, 3 *K. pneumoniae*, 2 *A. baumannii*, 1 *E. aerogenes*, 1 *E. cloacae* and 1 *H. alvei*. Imipenem resistance in *P. aeruginosa* was due to (i) an inactivation of the OprD gene alone (n = 19), associated with an overproduction of the MexAB efflux system (n = 6), a hyperexpression of AmpC (n = 6) or a GES-9 production (n = 1) or to (ii) a VIM-2 production (n = 4). Imipenem resistance in Enterobacteria was due to (i) hyperproduction of AmpC alone or with TEM-24 or SHV-12 or to (ii) DHA-1 (n = 2) or CTX-M-15 production, with porin loss. High genetic diversity was observed among the 56 IR-GNB, except for one clone of four VIM-2 producing *P. aeruginosa*, two DHA-1 producing *K. pneumoniae* and two *S. maltophilia* isolates with indistinguishable patterns. In multivariate analysis, the main risk factor for IR-GNB carriage was imipenem exposure with an increased link according to the duration of treatment: aOR = 5.9 (95% CI, 1.5–25.7) for 1–3 days and aOR = 7.8 (95% CI, 2.4–29.8) for >3 days.

**Conclusion:** Intestinal carriage of IR-GNB steadily increased over time. Imipenem resistance determinants were diverse. Exposure to imipenem was the major risk factor for IR-GNB colonization even after short exposure.

#### P1199 Antibiotic resistance trends of urinary ESBL-producing *E. coli* and *Klebsiella* spp. at a teaching hospital in Istanbul, 2004–2010

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**Objectives:** Urinary tract infections treatment has become more complex, not only because of an increasing bacterial resistance to fluoroquinolones, trimethoprim-sulfamethoxazole, or other standard

antibiotics, but also because of the emergence of ESBLs in Enterobacteriaceae. The aim of this study was to detect ESBL prevalence, antimicrobial resistant rates of *E. coli* and *Klebsiella* spp. in hospitalized patients and alterations in years.

**Methods:** This is a retrospective study conducted at a teaching hospital in Istanbul. From 2004 to 2010, we obtained a total of 12092 *E. coli* and 716 *Klebsiella* spp. strains from urine samples. Antibiotic susceptibilities of isolated strains were investigated by disk diffusion method according to CLSI criteria. ESBL production was examined with the double-disk synergy test.

**Results:** From 2004 to 2010 the prevalence of ESBL-producing *E. coli* increased from 5% to 12.2%. The prevalence of ESBL-producing *Klebsiella* spp. increased from 13% to 21.9%. Resistance to certain antibiotics of ESBL(+) *E. coli* was revealed as follows: 42.6%, 100% (p < 0.0001) for amoxicillin/clavulanic acid, 27.8%, 93% (p < 0.0001) for gentamicin, 1.8%, 14.1% (p = 0.004) for amikacin, 44.4%, 100% (p = 0.01) for ciprofloxacin, 27.8%, 72.4% (p < 0.0001) for levofloxacin, 27.8%, 100% (p < 0.0001) for trimethoprim/sulphamethoxazole and 7.4%, 17.2% (p = 0.05) for nitrofurantoin respectively. The resistance rates of ESBL(+) *Klebsiella* spp. were as follows: 60%, 100% (p < 0.0001) for amoxicillin/clavulanic acid, 20%, 64.8% (p < 0.0001) for gentamicin, 20%, 37.8% (p = 0.008) for amikacin, 40%, 91.8% (p < 0.0001) for ciprofloxacin, 20%, 81.4% (p < 0.0001) for levofloxacin, 20%, 100% (p < 0.0001) for trimethoprim/sulphamethoxazole and 20%, 29.7% for nitrofurantoin respectively. For ESBL(–) *E. coli* and *Klebsiella* spp. resistance to quinolones, trimethoprim/sulphamethoxazole decreased. Although nitrofurantoin resistance have increased in ESBL(–) *Klebsiella* spp.; it has decreased for ESBL(–) *E. coli* in years. None of the strains were resistant to carbapenems.

**Conclusion:** The prevalence of ESBL(+) organisms in our hospital was high. Over a time period of 7 years significant changes in the antibiotic resistance of ESBL(+) *E. coli* and *Klebsiella* spp. were observed. A high resistance rate was seen for fluoroquinolones, trimethoprim/sulphamethoxazole and amoxicillin/clavulanic acid.

#### P1200 The trend of selected antimicrobial-resistant pathogens in a tertiary care hospital, Istanbul

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**Background:** The objective of the present study was to analyse secular trends in antimicrobial resistant bacteria (ARB) from a tertiary care hospital from Istanbul-TR.

**Methods:** ARB identified from patients in the ICU with nosocomial infections. For each antimicrobial/pathogen pairs; the pooled mean rate of resistance for January through December of 2003–2010 was recorded and documented. The number of special antimicrobial/pathogens detected along the years of 2003–2010 was analysed and increase (or decrease) in the resistance rates during the 2010 compared with previous 8 years.

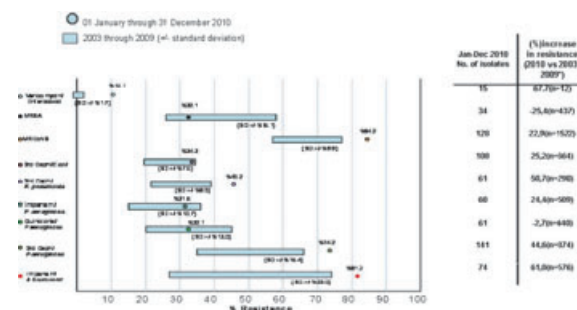


Fig. 1 Antibiotic resistance rates among selected pathogens associated with health care related infections in medical ICU and surgical ICU patients, comparing the periods 01 January through 31 December 2010 to 2003 through 2009. MRSA, Methicillin-resistant *Staphylococcus aureus*; MRSE, Methicillin-resistant coagulase-negative staphylococci; MSSA, Methicillin-sensitive *Staphylococcus aureus*; MRSA, Methicillin-resistant *Staphylococcus aureus*; MRSE, Methicillin-resistant coagulase-negative staphylococci; MSSA, Methicillin-sensitive *Staphylococcus aureus*. The percentage increase in resistance rate calculated as (2010 rate - mean rate in 2003-2009) / mean rate in 2003-2009 x 100.

**Results:** The rate of the carbapenem resistant *A. baumannii* (CRAB) was detected very high as well as 81.3% at the year of 2010. There has been up to 61% increase in CRAB when compared with previous years. The rate of the third generation cephalosporin (TGC) resistant *K. pneumoniae* was detected as 45.2%. There has been a nearly 50.7% increase in nonsusceptible *K. pneumoniae* isolates to TGC according to the previous years. The rate of the TGC resistant *E. coli* was detected as 34.3%. There has been nearly 25.2% increase in non-susceptible *E. coli* isolates to TGC according to the previous years. Our clinical and laboratory based data showed the decreasing trend for MRSA. The year of 2010s data set showed a 22.9% reduction in MRSA rates.

**Conclusions:** There is a dangerous trend about ARB in our institution. Especially CRAB, TGC resistant *E. coli* and *P. aeruginosa* are the most important examples. This 8-year study in an institution showed a data of significantly increasing antibiotic resistant pathogens which is limiting the antimicrobial therapy options especially for ICU patients. Strict implementation of infection control policy including appropriate antimicrobial prescription and simple infection control measures are needed. Decreasing resistance trend of MRSA is another reality in our institution as well as widespread phenomenon in a lot of European country but it is difficult to explain this phenomenon at least at this moment.

**P1201** Activity and spectrum of antimicrobial coverage against pathogens collected from patients with hospital-acquired and ventilated-associated bacterial pneumonia in USA medical centres

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**Objective:** To evaluate the frequency of occurrence and antimicrobial susceptibility of bacterial organisms collected from patients with hospital-acquired (HABP) and ventilated-associated bacterial pneumonia (VABP) in USA hospitals. We also evaluated the expected empirical coverage for broad-spectrum antimicrobials alone and two drug combinations.

**Methods:** Organisms were consecutively collected from patients hospitalized with pneumonia in 65 hospitals from all USA nine Census regions in 2010–2011 directed by a common prevalence style protocol. Susceptibility testing was performed by reference CLSI broth microdilution methods.

**Results:** Two thousand six hundred and fifty-seven organisms were evaluated, 2187 from HABP and 470 from VABP. The proportion of Gram-negative/Gram-positive was nearly 60%/40%. 63.6% of patients were male and 36.4% female. The median age values were 57 and 54 for HABP and VABP, respectively. Overall, 53.1% of patients were from ICU, including 44.5% of patients with HABP and 83.9% of patients with VABP. The five most common organisms were (% of total for HABP/VABP): *S. aureus* (36.3%/33.4%), *P. aeruginosa* (20.8%/17.9%), *Klebsiella* spp. (10.1%/10.6%), *Enterobacter* spp. (5.5%/8.3%) and *E. coli* (5.5%/4.9%), and these accounted for 77.6% of the total. 47.1% of *S. aureus* were oxacillin-resistant (MRSA) and 30.1% of *P. aeruginosa* were non-susceptible to imipenem. Among *Klebsiella* spp., 12.6% had an ESBL phenotype and 1.5% of strains were non-susceptible to imipenem. None of the antimicrobials alone provided adequate spectrum against the five most common organisms as a group. The two drug combination with best coverage (susceptibility to at least one of the compounds) for the top five organisms was amikacin plus vancomycin (VAN) or linezolid (LZD); 98.6% coverage), gentamicin plus VAN or LZD (94.9%), meropenem plus VAN or LZD (93.0%) and cefepime plus VAN or LZD (90.4%). The 6th and 7th most frequently isolated organisms were *S. maltophilia* (4.0%) and *Acinetobacter* spp. (3.8%), and exhibited high resistance rates to all antimicrobial agents tested.

**Conclusion:** Empirical antimicrobial therapy for HABP/VABP requires at least two agents to provide adequate coverage for the most common organisms. The best antimicrobial coverage was obtained with the combination of an aminoglycoside (amikacin or gentamicin) with VAN or LZD.

**P1202** Carbapenem-resistant *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* clinical isolates in a tertiary hospital

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**Objectives:** Carbapenemase-producing Gram-negative bacteria have increased substantially. The aim of this study was to evaluate carbapenem-resistant *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolates derived from clinical samples in a tertiary hospital.

**Methods:** A total of 310 strains of *K. pneumoniae*, 224 of *P. aeruginosa* and 324 of *A. baumannii* were studied. Isolates were recovered from blood cultures, body fluids, catheters, pus, bronchial secretions and urine samples derived from hospitalized patients (ICUs and surgical and medical wards), during 1 year, from 1 November 2010 to 31 December 2011. One hundred and eighteen out of 310 *K. pneumoniae* isolates were recovered from ICU patients' samples while the number for *P. aeruginosa* isolates was 96 out of 224 and for *A. baumannii* 209 out of 324 respectively. All isolates had meropenem and/or imipenem MICs > 1 mg/mL. The identification and susceptibility testing was performed via the Vitek II automated system (Biomérieux, France), and when necessary susceptibility results were confirmed with the use of E-test strips (AB Biodisc, Sweden) according to CLSI guidelines. Metallo-beta-lactamase (MBL) production was evaluated using disks containing meropenem with and without EDTA and KPC-production with boronic acid combined-cisk tests, using disks containing meropenem with and without boronic acid.

**Results:** All isolates were multidrug resistant. *K. pneumoniae* isolates revealed 11% resistance to gentamicin, 8% to tigecycline and 40% to colistin. With reference to carbapenemase production, 61 isolates were producing MBL and the rest 249 were producing KPC. Seventy-five out of 310 *K. pneumoniae* isolates were recovered from blood samples. As for *P. aeruginosa* (38 out of 224 being recovered from blood samples), resistance to gentamicin was at 75%, and to colistin at 4%. One hundred and eighty-one out of 224 isolates were producing MBL. *A. baumannii* isolates (72 out of 324 being recovered from blood samples), revealed resistance to gentamicin at 58%, to tigecycline at 39% and to colistin at 2%.

**Conclusion:** It is of great importance to monitor the emergence and prevalence of carbapenem resistant Gram-negative bacteria depending not only on susceptibility testing but on phenotypic detection of carbapenemase production as well, in order to choose appropriate empiric therapy according to existing information and adjust individual antimicrobial chemotherapy when necessary.

**P1203** In vitro activity of fosfomicin in well characterised carbapenem-nonsusceptible *Pseudomonas aeruginosa* isolates recovered from 16 Spanish hospitals

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Multiresistance in clinical *Pseudomonas aeruginosa* isolates is worrying, mainly in severe infections in which few options remain available. Fosfomicin, an inhibitor of peptidoglycan precursor's synthesis, shows no cross-resistance with other antimicrobials and may act synergistically with many antimicrobial compounds. Data suggest that IV fosfomicin can be used in combination to treat infections caused by multidrug-resistant *P. aeruginosa*.

**Objective:** The shortage of new antimicrobials constitutes a challenge that mandates the re-evaluation of existing compounds. With such an aim, fosfomicin MICs against a genetically unrelated collection of clinical carbapenem-non susceptible *P. aeruginosa* from 16 Spanish hospitals were evaluated.

**Methods:** Fosfomicin (FOS) MICs (agar dilution-CLSI; breakpoints-EUCAST) of 63 contemporary carbapenem-nonsusceptible *P. aeruginosa* isolates (16 Spanish hospitals, COMPACT Surveillance

Study) exhibiting 55 PFGE- and 37 MLST-different patterns, were determined. Carbapenem resistance mechanisms (molecular level) and co-resistances were addressed in a preceding study. Results were compared with those obtained in 151 carbapenem-susceptible isolates.

**Results:** A total of 33% of carbapenem-non susceptible isolates were fully susceptible to FOS (MIC  $\leq$  32 mg/L) while median MIC value was 64 mg/L. These figures almost entirely superimposed to that of EUCAST distribution for FOS-*P. aeruginosa* (<http://www.eucast.org>). All sequence types (STs), even the most represented (ST175, ST646, and ST532), were distributed along the whole range of MIC values (1–1024 mg/L). Consequently, the activity of FOS appears to be maintained against *P. aeruginosa* isolates irrespective of their carbapenem resistance status. This trait was reinforced as the same behavior of FOS was observed against the counterpart carbapenem-susceptible *P. aeruginosa* population recovered along the same cited study.

**Conclusion:** Difficult-to-treat infections caused by carbapenem-nonsusceptible *P. aeruginosa* (endocarditis, cystic fibrosis, foreign-body-associated infections) are communicated worldwide. Re-evaluation of already existing antimicrobials like FOS is constantly addressed due to scarcity of treatment options and results appear to be promising as synergy has been confirmed between FOS and betalactams, aminoglycosides and fluoroquinolones. Figures obtained in the present study reinforce the previous statements and warrants further research to confirm the utility of FOS.

**P1204** Impact of ertapenem utilisation on *Pseudomonas aeruginosa* susceptibility to imipenem. Trends in the last decade in a tertiary teaching hospital

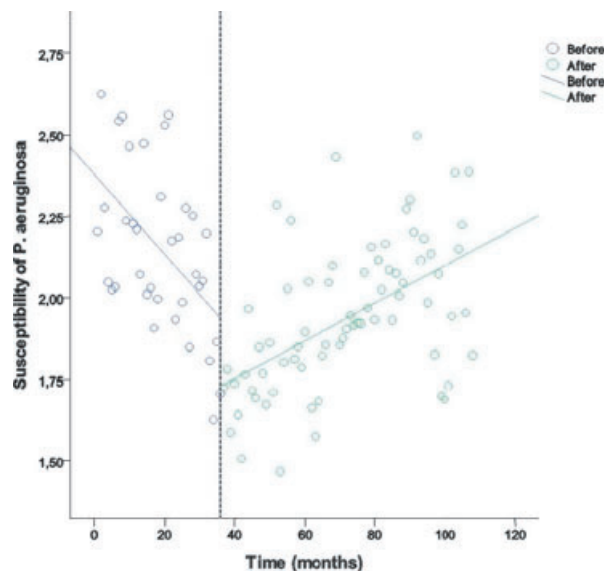
D. Sousa\*, J.M. Gutierrez, F. Molina, B. Lopez, L. Carrajo, L. Castelo, E. Sanchez, E. Miguez, G. Bou, P. Linares (La Coruña, ES)

**Objectives:** We sought to determine the impact of ertapenem use on *Pseudomonas aeruginosa* susceptibility to imipenem and on hospital antimicrobial utilization.

**Methods:** Antimicrobial consumption was recorded monthly in defined daily doses (DDD)/100 patient-days from January 2002 to December 2010 in A Coruña Hospital, a tertiary teaching hospital in Spain. Ertapenem was introduced in January 2005. The incidence of susceptibility (CLSI criteria) of *Pseudomonas aeruginosa* to imipenem (proportion of imipenem-susceptible isolates/1000 patient-days) was evaluated. An interrupted time series with segmented regression analysis was performed to determine the change in *Pseudomonas aeruginosa* susceptibility to imipenem pre- and post-ertapenem introduction.

**Results:** During the whole period of study, we documented an imipenem mean consumption of  $3.05 \pm 0.87$  DDD/100 patient-days, of meropenem  $1.14 \pm 0.71$  DDD/100 patient-days and of ertapenem (after introduction)  $0.85 \pm 0.54$  DDD/100 patient-days. Ertapenem use rose steadily once adopted, with a mean of  $0.09 \pm 0.08$  DDD/100 patients-day in 2005 and a mean of  $1.41 \pm 0.20$  DDD/100 patient-day in year 2010 ( $r = 0.919$ ;  $p < 0.001$ ). There was also a steady increase of imipenem ( $r = 0.620$ ;  $p < 0.001$ ) and meropenem ( $r = 0.851$ ;  $p < 0.001$ ) over the study period. After introduction of ertapenem, there was a significant increase in median consumption of imipenem (before  $2.32 \pm 0.52$  vs.  $3.41 \pm 0.77$  after;  $p < 0.001$ ) and meropenem (before  $0.55 \pm 0.25$  vs.  $1.44 \pm 0.68$  after;  $p < 0.001$ ).

We evaluated a total of 5 343 *P. aeruginosa* isolates, of which 3 791 were susceptible to imipenem. Mean incidence of imipenem-susceptible *P. aeruginosa* was  $2.01 \pm 0.25$ . There was a positive correlation between ertapenem consumption and the incidence of imipenem-susceptible *P. aeruginosa* ( $r = 0.462$ ;  $p < 0.001$ ). No correlation was found with the use of Imipenem ( $r = -0.106$ ;  $p = 0.273$ ) or Meropenem ( $r = -0.031$ ;  $p = 0.750$ ). By segmented regression analysis, the susceptibility of *P. aeruginosa* to imipenem increased after the introduction of ertapenem. The increasing trend was significant (slope = 0.018;  $p < 0.001$ ) (Figure)



**Conclusions:** The overall carbapenem consumption steadily increased over the study period. Ertapenem use was associated with improved susceptibility of *P. aeruginosa* to imipenem.

**P1205** Rapid spread of OXA-23-positive *Acinetobacter baumannii* in Greece marking the end of OXA-58 predominance

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**Objectives:** To study the spread of carbapenem-resistant *Acinetobacter baumannii* isolates, collected in Greece during 2011 and identify the respective carbapenemases.

**Methods:** A total of 143 randomly selected carbapenem-resistant *A. baumannii* isolates were included in the study. They were collected during 2011 from various clinical specimens (blood, bronchial secretions, pus, catheters) in four tertiary care hospitals located in Athens (2), Larisa (1) and Patra (1). Identification and susceptibility testing were performed with the Vitek-2 System (BioMerieux, France). Identification of blaOXA-58, blaOXA-23, blaVIM and blaIMP was carried out by PCR and sequencing. Molecular typing was performed by the three loci-sequencing typing scheme according to the protocol described in the *A. baumannii* ST website <http://www.hpa-bioinformatics.org.uk/AB/home.php>.

**Results:** The vast majority (88–98%) of *A. baumannii* isolated in the participating hospitals exhibited resistance to carbapenems. The isolates studied represented 20% of the total isolations. None of the isolates harboured metallo-beta-lactamase genes. One hundred one isolates (70.6%) were blaOXA-23 carriers. The remaining isolates were blaOXA-58-positive. The higher frequency of OXA-23 isolates was observed in Larisa and the lower in the Athens. Molecular typing showed that OXA-23-positive isolates belonged to either the ST101-European clone II or the ST201-European clone I. OXA-58-producers were classified into the ST106-European clone II and into ST201. One isolate of the OXA-58 group displayed a novel allelic profile (2-1-2).

**Conclusion:** Up to 2009, OXA-58 producers were predominant among carbapenem-resistant *A. baumannii* circulating in Greek hospitals. Data presented here indicate a relatively rapid change due to the dissemination of at least two distinct *A. baumannii* clones carrying the blaOXA-23 gene that had not been previously seen in this setting.

**P1206** First identification of colistin and tigecycline-resistant *Acinetobacter baumannii* producing KPC-3 carbapenemase in Portugal

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**Objectives:** Nowadays the carbapenems resistance among *Acinetobacter baumannii* and *Klebsiella pneumoniae* are serious therapeutic and infection control challenge. The aim of this study was to analyse the clinical characteristics and to investigate the genetic basis of the carbapenem resistance of *K. pneumoniae* and *A. baumannii* clinical strains isolated from the same patient.

**Methods:** Antimicrobial susceptibility was determined by disk diffusion (amoxicillin/clavulanic acid, cefotaxime, ceftazidime, imipenem, meropenem, ciprofloxacin, gentamicin, tigecycline and colistin), and interpreted according to CLSI guidelines. The presence of blaTEM, blaSHV, blaCTX, blaKPC, blaIMP and blaVIM was screened by PCR using specific primers. Amplicons were sequenced and compared to sequences available in the GeneBank database.

**Results:** A female patient, with 35 years old, had renal insufficiency, being an immunocompromised host. She had been submitted to ciprofloxacin and meropenem therapy before the identification, in February 2011, of KPC-3 producing *Klebsiella pneumoniae*. *Acinetobacter baumannii* 86982FF was isolated in March 2011. Both isolates were recovered from sputum at surgical ward. *K. pneumoniae* showed susceptibility to colistin, intermediate susceptibility to ciprofloxacin and gentamicin, and resistance to the other antimicrobials, including tigecycline. *A. baumannii* was resistant to all studied antimicrobials and was identified a KPC-3 carbapenemase. The blaKPC-3 gene was part of a plasmid in both isolates and was included in transposon Tn4401.

**Conclusions:** We report for the first time the identification of multidrug-resistant *A. baumannii* producing KPC-3 carbapenemase in Portugal. The report of KPC-3-producing *A. baumannii* isolates is very worrisome, since these strains are resistant to all beta-lactam agents and to other antimicrobials, including colistin and tigecycline.

**P1207** Isolation of anaerobic bacteria and susceptibility pattern to antimicrobial agents from purulent specimens in a Greek hospital, 2008–2010

M. Orfanidou\*, T. Pitsia, T. Christacopoulou, G. Ganteris, E. Vagiakou, E. Malamou-Lada (Athens, GR)

**Objectives:** To study the occurrence of anaerobic bacteria isolated from purulent samples and their susceptibility to antibiotics, during a 3 year period, in a tertiary hospital of Athens

**Methods:** During the study period 499 anaerobic strains were isolated from 6313 purulent samples. The samples were cultured on appropriate media. The identification was performed by Gram staining, colony morphology, hemolysis, fluorescence, special potency antimicrobial agent disks and by the use of API rapid 32 A and Crystal ANA. MICs were determined by E-test method, according to CLSI instructions

**Results:** Out of 6313 purulent samples, submitted to the laboratory, bacteria were isolated in 4414 (70%) ones. Anaerobic bacteria were isolated from 419 purulent samples, which consisted a 6.6% of the total cultures and 9.5% of the positive cultures. Polymicrobial cultures were 355/419 (85%) and monomicrobial were 64/419 (15%). The infection origins of the samples were: soft tissue and extremities (181/419, 43%), head and neck (97/419, 23%), abdomen (65/419, 16%), surgical wound (63/419, 15%), genital track (13/419, 3%)

Out of 499 anaerobic isolated strains *Bacteroides fragilis* group was 34% (169/499), *Prevotella* spp. 33% (166/499), *Peptostreptococcus* spp. 11% (37/499), *Bacteroides* spp. non fragilis group 6% (30/499), *Fusobacterium* spp. 5% (27/499), *Clostridium* spp. 4% (18/499), *Propionibacterium acnes* 3% (17/499), *Porphyromonas* spp. 2% (10/499), others 1% (5/499)

*B. fragilis* group revealed resistance 13% to moxifloxacin, 12% to co-amoxiclav and 1% to meropenem. *Prevotella* spp. was 45% resistant to ampicillin, 33% to clindamycin, 3% to moxifloxacin, 0.7% to meropenem and 0.6% to co-amoxiclav. *Peptostreptococcus* spp. was resistant to clindamycin (16%) and penicillin (6%), while it was fully susceptible to moxifloxacin. None of the above strains appeared resistant to metronidazole.

**Conclusions:** Anaerobic infections are ~10% of positive purulent cultures. More than half of the anaerobes were isolated from soft tissue and head and neck infections. The most prevalent anaerobic species isolated were *B. fragilis* group and *Prevotella* spp. All strains were susceptible to metronidazole and with low resistance ratio to meropenem, which suggests a good alternative for empirical therapy in our hospital, in addition with co-amoxiclav and moxifloxacin for head and neck infections, where *Prevotella* spp. and *Peptostreptococcus* spp. were predominant.

**P1208** Reduced carbapenem susceptibility in the *Bacteroides fragilis* group – findings from the TEST programme 2007–2010

M. Hackel\*, S. Bouchillon, R. Badal, S. Hawser, D. Hoban, M. Dowzicky (Schaumburg, US; Epalinges, CH; Collegette, US)

**Background:** *Bacteroides fragilis* group organisms are important anaerobic co-pathogens in many polymicrobial infections. Reduced susceptibility to carbapenems in *B. fragilis* group is due primarily to the metallo-beta-lactamase CfiA gene (meropenem MICs 1–4) with high-level resistance secondary to acquired upstream insertion sequences (IS) causing expression of CfiA (MICs > 16).

**Methods:** The Tigecycline European Surveillance Trial (TEST) evaluated 164/1842 (8.9%) *B. fragilis* group organisms with reduced susceptibility to carbapenems (meropenem MIC  $\geq$  1 mg/L) from a collection of anaerobes spanning 4 years, 2007–2010. The isolates were identified to the species level at the participating sites and confirmed by a central laboratory. MICs were determined by the central laboratory using agar dilution according to CLSI guidelines.

**Results:** MIC<sub>90</sub> (mg/L)/% susceptible\* of *B. fragilis* group with meropenem MICs of  $\geq$ 1 mg/L by year (n/n total *B. fragilis* group isolates): \*EUCAST breakpoints used where available; CLSI breakpoint used for cefoxitin; FDA breakpoint used for tigecycline (Tygacil<sup>®</sup>, 2009).

	2007(40/506)	2008(33/430)	2009(45/541)	2010(46/365)
Tigecycline	2/100	4/97.0	2/97.8	4/93.5
Metronidazole	2/100	2/100	1/100	1/97.8
Pip-Tazo	16/80.0	32/84.9	64/77.8	32/78.3
Meropenem	8/70.0	8/54.6	>8/42.2	>8/52.2
Clindamycin	>8/65.0	>8/60.6	>8/64.4	>8/56.5
Cefoxitin	>32/52.5	>32/57.6	>32/55.6	>32/43.5

**Conclusions:** *B. fragilis* group isolates with reduced susceptibility to meropenem increased significantly between 2007 and 2010 ( $p < 0.05$ , Fisher's exact test). Greater than 93% of these isolates were susceptible to tigecycline and metronidazole, with no significant reduction in susceptibility for any of the compounds tested over the 4 years of analysis.

**P1209** An age analysis of the susceptibility of Gram-positive and Gram-negative pathogens from centres in the USA: TEST programme 2008–2010

D. Hoban\*, S. Hawser, M. Hackel, S. Bouchillon, B. Johnson, J. Johnson, M. Dowzicky (Schaumburg, US; Epalinges, CH; Collegette, US)

**Background:** Tigecycline has been shown to have potent broad spectrum activity against most commonly encountered species

responsible for community and hospital acquired infections. The Tigecycline Evaluation and Surveillance Trial (TEST) program determined the in vitro activity of tigecycline compared to most commonly prescribed broad spectrum antimicrobials against gram-negative and gram-positive species collected in the United States during 2008 to 2010. This report analyzes differences in susceptibilities in different age groups from this study.

**Methods:** A total of 12 852 clinical isolates from 131 cumulative United States testing sites were identified to the species level. Age groups are defined as Pediatrics: 0–17; Young Adult: 18–34; Adult: 35–64; and >65; Geriatrics. Minimum Inhibitory Concentrations (MICs) were determined by each site using supplied broth microdilution panels and interpreted according to CLSI/FDA guidelines.

**Results:** In general, tigecycline %S was very similar in the four age groups (pediatric, young adult, adult, and geriatric). Levofloxacin showed reduced activity against adult vs. pediatric isolates of *Acinetobacter* spp., Enterobacteriaceae, ESBLs, *Enterococcus* spp. and MRSA. *S. pneumoniae* penicillin susceptibility ranged from 63% for geriatric isolates to 60% for adults. All agents were active against *H. influenzae* and *S. agalactiae*.

**Conclusion:** Although many drugs showed little difference in activity among patient age groups, overall susceptibility levels were generally higher in the pediatric and young adult groups than in adults and geriatrics. Some of the problematic therapy issues seen in older patients (VRE, reduced fluoroquinolone efficacy) are not as prevalent in younger patients, while others (ESBLs, MRSA) can be found across all age groups. Although the effectiveness of tigecycline in patients <18 years of age has not been established, tigecycline in vitro activity in this study demonstrates potent activity against ESBL, MRSA and enterococci often associated with serious infections of the various age groups evaluated.

**P1210** Resistance phenotypes observed from amongst *Staphylococcus aureus* and pneumococci isolated from USA paediatric patients during the 2009 AWARE surveillance programme

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**Objectives:** Ceftaroline (CPT), the active form of CPT fosamil, is a new broad-spectrum cephalosporin with bactericidal activity against Gram-positive pathogens, including MRSA and penicillin-resistant pneumococci, as well as common Gram-negative pathogens. CPT fosamil is approved in the USA for treatment of adults with acute bacterial skin and skin structure infections or community-acquired bacterial pneumonia. The AWARE surveillance program is a global initiative that monitors susceptibility of key target pathogens to CPT and other clinically important antibacterials. This study analysed 2009 data for resistance (R) patterns among *S. aureus* (SA) and *S. pneumoniae* (SPN) by age.

**Methods:** Consecutive SA (n = 2311) and SPN (n = 1222) were collected from 52 sites from all nine USA census regions. Isolates were grouped by patient age (SA, SPN): ≤2 years (y) (n = 195, 170), 3–5 (n = 52, 82), 6–14 (n = 122, 94), 15–17 (n = 47, 14), 18–64 (n = 1232, 578), ≥65 (n = 663, 284). MICs were determined centrally by CLSI methods.

**Results:** MRSA rates were 42.3% (3–5 years) to 53.2% (15–17 years). Multidrug resistance (MDR; resistant to ≥2 classes) was most common in the very young and adults (50.4%) and least common in patients aged 3–17 years (38.5%). Nosocomial or community-acquired source was available for 1511/2311 SA. Within this subset, patients ≤2 (19%) and ≥18 (19.3%) y were ~3× more likely to have nosocomial infection than children and adolescents (7.2%). Levofloxacin R increased with age. CPT was consistently active (MIC90, 1 mg/L) against isolates from all age groups and resistant phenotypes.

PRSP rates were 0% in patients aged 15–17 years to 4.3% in 6–14 year-olds. MDR was most common in patients ≤14 years (50%), decreased to 21.4% in 15–17 year-olds, and was 26% in

adults. Amoxicillin-clavulanate R was most common in patients ≤2 years (30%) and decreased to 8.8% in patients ≥65 years. Ceftriaxone-R occurred in 5.9% of paediatric patients. Inverse correlations between age and resistance to clindamycin, tetracycline, and trimethoprim-sulfamethoxazole were noted. The CPT MIC90 was 0.25 mg/L against isolates across all age groups.

**Conclusion:** Observed R patterns suggest differences in profiles of SA and SPN isolated from US adult and paediatric patients during the 2009 AWARE Program. These differences possibly reflect antibiotic prescribing habits, frequency of hospital stay, and the use of the pneumococcal conjugate vaccine in the USA. CPT was consistently active against all isolates recovered in this study.

**P1211** Emergence of high-level mupirocin resistance in non-aureus staphylococci associated with increased mupirocin use

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**Background:** In our hospital, mupirocin has increasingly been used for eradication of nasal *S. aureus* carriage in patients scheduled for certain surgical procedures over the past 6 years. The target for mupirocin is isoleucyl transfer RNA synthetase (ileS). High-level resistance to mupirocin is conferred by acquisition of plasmids expressing a distinct ileS-gene (ileS-2).

**Objectives:** To assess the frequency of occurrence of high-level mupirocin resistance and look for a putative association with mupirocin use.

**Methods:** We assessed mupirocin resistance in Staphylococcal bloodstream isolates from 2006–2011, that had routinely been tested by Phoenix automated testing (PAT). In a sample survey, we evaluated reliability of PAT results using e-test in the first 40 consecutive non-aureus blood isolates of each year. Species determination was performed by maldi-TOF. We tested for presence of ileS-2 in the first 100 consecutive non-aureus bloodstream isolates of each year using RT-PCR. Data on mupirocin use was acquired from Utrecht Patient Oriented Database.

**Results:** High-level mupirocin resistance of non-aureus blood isolates increased from 13% in 2006 to 19% in 2011 (n = 2149, PAT results). Only two high-level mupirocin resistant *S. aureus* isolates were found. Sample survey (n = 237): Sensitivity and specificity of PAT to detect high-level mupirocin resistance was 0.97 and 0.97, respectively. Two isolates did not grow and PAT testing could not provide a result for one isolate. IleS-2 RT-PCR was performed on 598 isolates. In four phenotypically high-resistant ileS-2 RT-PCR was negative; three of these were *Rothia mucilaginosa*, *Kuceria* species, and *Micrococcus* species, known to be intrinsically resistant to mupirocin by other mechanisms than ileS-2. In three isolates ileS-2 was detected (all with C<sub>t</sub>-values <30), but were tested sensitive to mupirocin.

The yearly amount of mupirocin prescribed in our hospital increased from 3.6 kg in 2006 to 13.3 kg in 2010, and correlates with the increase in % non-aureus Staphylococci carrying ileS-2 (8% in 2006 to 22% in 2011; Spearman's rho 0.135, p = 0.01).

**Conclusion:** We observed a significant increase of high-level mupirocin resistant non-aureus Staphylococci, all linked to presence of ileS-2. This increase coincides with an increased use of mupirocin.

**P1212** A 5-year study of antimicrobial resistance of enterococcal isolates in a tertiary hospital

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**Objectives:** The aim of the present study was to assess resistance rates to various antimicrobials of *Enterococcus* sp. clinical isolates for a period of 5 years, and to characterize the mechanism of resistance to glycopeptides.

**Methods:** During a 5-year period, from 2006 to 2010, 846 *Enterococcus* isolates, one per patient, were collected from patients

hospitalized in different wards of our hospital. Enterococci were isolated from 185 blood, 340 pus, and 321 urine cultures. Identification and susceptibility testing were performed using the Vitek 2 automated system (bioMérieux, France). The antimicrobials tested were ampicillin, penicillin, ciprofloxacin, levofloxacin, high-level gentamicin, linezolid, tigecyclin, vancomycin and teicoplanin. MICs for vancomycin and teicoplanin were confirmed by E-test (AB Biodisk, Sweden), according to CLSI guidelines. Further analysis of the vancomycin resistance genes (vanA, and vanB) was performed by sandwich hybridization (EVIGENE VRE detection kit, Statens Serum Institut, Denmark).

**Results:** Identification yielded 646 *E. faecalis*, 183 *E. faecium*, nine *E. casseliflavus*, five *E. avium*, and three *E. gallinarum*. *E. faecium* isolates showed the highest level of resistance to ampicillin (77%), penicillin (78%), ciprofloxacin (72%), levofloxacin (78%) and high-level gentamicin (38%), while rates for *E. faecalis* isolates were 12%, 22%, 41%, 39% and 47% for ampicillin, penicillin, ciprofloxacin and levofloxacin and high-level gentamicin respectively. Twelve *E. faecalis* isolates and three *E. faecium* isolates were resistant to linezolid (2%). Tigecycline was the most active antimicrobial, all isolates being susceptible. Fifty-seven (6.7%) enterococcal isolates were glycopeptide-resistant (GRE), including 11 of 646 (1.7%) *E. faecalis* isolates, and 46 of 183 (25%) *E. faecium* isolates, half of which derived from blood cultures. MIC values for vancomycin were 64 mg/L or higher and 32 mg/L or higher for teicoplanin. Genotypic analysis of the isolates yielded that all glycopeptide resistant enterococci possessed the vanA gene.

**Conclusion:** High levels of resistance were observed among *E. faecium* isolates that also exhibited significant resistance to glycopeptides. Both *E. faecalis* and *E. faecium* isolates showed low level resistance to linezolid. Tigecycline was the most active agent against *E. faecalis* and *E. faecium* including GRE. Antimicrobial resistance surveillance and prudent use of antibiotics is mandatory for the management of life-threatening infections.

#### PI213 Susceptibility of *Enterococcus* species including vancomycin-resistant phenotypes: Asia/Pacific, 2006–2010

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**Background:** *Enterococcus faecium* and *E. faecalis* are significant pathogens both in community and hospital patients. The increasing prevalence of vancomycin-resistant *Enterococcus* spp. (VRE) worldwide dictates the continued monitoring of these phenotypes. The Tigecycline Evaluation and Surveillance Trial (TEST) has monitored the activity of tigecycline and comparators globally since 2004.

**Methods:** Forty-three sites in 10 Asia/Pacific countries collected 1004 *E. faecalis* and *E. faecium* isolates between 2006 and 2010. MICs were performed as specified by CLSI at each site using prepared broth microdilution panels and interpreted according to CLSI/FDA guidelines. Linear trends over time in % susceptible/resistant were assessed with the Cochran-Armitage test.

**Results:** In 2006–2010, two of 613 *E. faecalis* (0.3%) and 86 of 391 *E. faecium* (22%) were vancomycin-resistant. The overall VRE rate increased from 7% to 9% between 2006 and 2010, however this trend was not statistically significant ( $p > 0.05$ ). The in vitro activity of five agents are shown in the following table for 2006 and 2010 only, although the statistical test was applied to all 5 years studied.

Note: No statistically significant trends in % susceptible were found for any agents studied ( $p > 0.05$ ).

Drug	MIC <sub>50</sub> (mg/L) / % Susceptible					
	<i>E. faecalis</i>		<i>E. faecium</i>		VRE	
	2006	2010	2006	2010	2006	2010
Ampicillin	1/100	2/100	>16/11.5	>16/10.7	>16/0	>16/0
Levofloxacin	>32/62.5	32/72.0	>32/10.3	>32/5.4	>32/0	>32/0
Penicillin	4/100	4/98.8	>8/14.9	>8/10.7	>8/5.9	>8/0
Tigecycline	0.25/100	0.25/100	0.12/100	0.25/100	0.12/100	0.12/100
Vancomycin	2/98.6	2/100	>32/80.5	>32/78.6	>32/0	>32/0
n	144	82	87	56	17	12

**Conclusions:** Tigecycline demonstrated potent in vitro activity against *E. faecalis* and *E. faecium* isolates including vancomycin-resistant phenotypes with 100% of isolates remaining susceptible in 2010 and an MIC<sub>90</sub> of 0.12–0.25 mg/L that was unchanged between 2006 and 2010.

#### PI214 Primary investigation of the virulence factors of vancomycin-resistant *Enterococcus faecium* in a tertiary-care hospital of Sichuan, China

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**Objective:** To analyze the phenotypic and molecular characterization of vancomycin-resistant *Enterococcus faecium* (VREFm) isolates, and figure out the potential association of the three virulence factors: enterococcal surface protein (esp); hyaluronidase (hyl); adhesin of collagen from *E. faecium* (acm) with the colonization or infection of *E. faecium*.

**Methods:** VREFm isolates (n = 45) from intestinal colonization of patients in Intensive Care Unit (ICU) had been screened by VRE chromogenic agar and identified by multiplex polymerase chain reaction (PCR) for the D-Ala-D-Ala ligase gene (ddl gene). Infective ampicillin-resistant vancomycin-susceptible *E. faecium* (VSEFm) isolates (n = 60) and infective VREFm isolates (n = 8) had been identified by Vitek II compact. Use E-test to detect the minimum inhibitory concentrations (MICs) of VREFm. The vancomycin-resistant genes and the virulence factors (hyl, esp, acm) were detected by PCR.

**Results:** Fifty-three VREFm strains (eight infective strains and 45 intestinal colonization strains) and 60 VSEFm strains over 1 year period were collected. Thirty-four VSEFm strains from blood, other 26 VSEFm strains from pleural fluid and CSF. All the 53 VREFm isolates harbored the vanA gene and exhibited the VanA phenotype. Thirty-seven of 45 (82.22%) intestinal colonization VREFm strains, all the eight clinical infective VREFm strains and 32 of 60 (53.33%) clinical infective VSEFm strains carried the esp gene. Twenty-one of 45 (46.67%) intestinal colonization VREFm strains, three of eight (37.5%) clinical infective VREFm strains and 17 of 60 (28.3%) clinical infective VSEFm strains carried the hyl gene. Forty-one of 45 (91.11%) intestinal colonization VREFm strains, six of eight (75%) clinical infective VREFm strains carried the acm gene, compared with 55% acm-positive in clinical infective VSEFm strains. In VREFm group, 21 of 24 (87.5%) hyl-positive strains carried the esp gene, however, in VSE group, only five of 17 (29.41%) hyl-positive strains carried the esp gene. In addition, the positive rate of acm gene in VSEFm strains from blood (32.35%) is much lower than that from the pleural fluid and CSF (84.62%).

**Conclusion:** Esp, hyl and acm are highly related to the infection of *E. faecium*. Especially for VREFm, the esp and acm gene are more related to its infection or colonization than hyl gene. A further research will be carry out to confirm whether esp can help the *E. faecium* resistant to antibiotic in hyl-positive strains and whether acm can help the *E. faecium* invading the serous cavity.

#### PI215 Vancomycin MICs compared between MRSA and MSSA in nine countries

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**Objective:** Raised vancomycin (VAN) MICs in nominally susceptible *S. aureus* are suspected of contributing to less successful clinical outcomes. We reported previously that MICs were slightly, but significantly and consistently, lower for MRSA than MSSA in the UK and Ireland. We extended our focus to eight other countries to test the generality of this surprising result.

**Methods:** The SENTRY, CANWARD and BSAC resistance surveillance programmes contributed data on 33 978 *Staphylococcus aureus* (31 181 from blood) collected between 2001 and 2010. VAN

MICs were measured by the CLSI broth microdilution or BSAC agar dilution method. Data for the UK and Ireland were combined and counted as one country. Comparison of unimodal MIC distributions was by interval regression of  $\log_2$  MIC.

**Results:** SENTRY data for the UK and Ireland confirmed the BSAC observation of slightly but significantly lower VAN MICs for MRSA, but this was unique among the countries studied. Seven showed higher MICs for MRSA, statistically significant in six, while Germany showed no difference. These differences in MIC were small (up to 1.15-fold increase in geometric mean), but were generally consistent across years. The US results were replicated consistently in each of four census regions (West, MidWest, NorthEast and South). In all countries except the UK and Ireland, VAN MICs of 2 mg/L ("raised but susceptible") were significantly more common in MRSA than MSSA, generally seen for 3–10% of isolates vs. 1–4%. Only 16 of 33 978 isolates (0.05%) were nominally non-susceptible to vancomycin, all with MIC of 4 mg/L.

Country	No. of isolates		Geometric mean MIC, mg/L			% with MIC $\geq$ 2mg/L		
	MSSA	MRSA	MSSA	MRSA	P	MSSA	MRSA	P
Australia <sup>1</sup>	739	206	1.00	1.12	0.002	3	22	<0.001
Brazil <sup>1</sup>	1354	730	0.93	1.00	<0.001	4	8	<0.001
Canada <sup>2</sup>	3518	1110	0.87	0.94	<0.001	1	3	<0.001
France <sup>1</sup>	1797	658	0.89	0.99	<0.001	1	7	<0.001
Germany <sup>1</sup>	1390	273	0.86	0.85	0.88	1	5	<0.001
Italy <sup>1</sup>	556	343	0.87	0.94	0.16	1	10	<0.001
Spain <sup>1</sup>	675	170	0.88	0.98	0.011	1	5	<0.001
UK & Ireland <sup>1</sup>	1263	866	0.88	0.66	<0.001	2	3	0.17
UK & Ireland <sup>2</sup>	2094	1027	1.32	1.13	<0.001	42	23	<0.001
USA <sup>1</sup>	8086	7123	0.89	0.96	<0.001	2	6	<0.001

<sup>1</sup>SENTRY (2001–2010, blood, CLSI broth, <sup>†</sup>not 2005). <sup>2</sup>CANWARD (2007–2010, blood & other sources, CLSI broth). <sup>3</sup>BSAC (2001–2010, blood, BSAC agar dilution).  
Note: MICs for UK/Ireland are 1.6-fold higher in BSAC (agar) study than SENTRY (broth)

**Conclusion:** Frank vancomycin non-susceptibility (MIC  $\geq$  4 mg/L) is extremely rare in these nine countries (0.05%). Subtle differences in MICs between MRSA and MSSA exist and differ between countries; they may depend on clonal composition of local *S. aureus* populations. Their clinical significance remains uncertain.

#### P1216 Susceptibility of routine MRSA isolates to a series of antibiotics in the Netherlands

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**Objective:** Methicillin-resistant *Staphylococcus aureus* (MRSA) is a pathogen that is associated with serious infections. In The Netherlands, the incidence of MRSA infections remains low due to the restricted use of antibiotics. Although The Netherlands has a successful surveillance system for typing MRSA, very little is known about the incidence of resistance to antibiotics other than methicillin in these isolates. Therefore, susceptibility to a series of antibiotics was tested for randomly chosen MRSA isolates to investigate the susceptibility of these isolates and compare these results with our typing data.

**Methods:** A total of 249 *S. aureus* isolates from our national MRSA surveillance were used in this study. All isolates were typed with multiple-locus variable number of tandem repeat analysis (MLVA). Susceptibility was tested for; cefuroxime, ciprofloxacin, clindamycin, erythromycin, gentamicin, meropenem, mupirocin, oxacillin, rifampicin, tetracycline, tigecycline and vancomycin. The isolates were tested according to the EUCAST criteria.

**Results:** All 249 isolates were resistant against to at least one antibiotic and resistance was found against all antibiotics except for tigecycline and vancomycin. Most isolates (86, 35%) were resistant to four antibiotics. Six isolates (2%) showed the broadest resistance (eight antibiotics). Most resistance was found against gentamicin (243 isolates, 98%) and oxacillin (222, 89%). Resistance was also found against cefuroxime (222, 89%), erythromycin (121, 49%), tetracycline (112, 45%), ciprofloxacin (96, 39%), clindamycin (67, 27%), meropenem (27, 11%), mupirocin (18, 7%) and rifampicin (2, 1%). MLVA-typing resulted in 225 complete MLVA-profiles. MLVA complex (MC) 398 was predominant with 76 (34%) isolates, followed by MC5 (37, 16%) and MC8 (28, 12%). No correlations were found

between MLVA-typing results and the presence of antibiotic resistance, except for the presence of tetracycline resistance among MC398 isolates.

**Conclusion:** Our results show that most *S. aureus* isolates are resistant to four or more antibiotics and resistance levels to some antibiotics were 89% and higher. Only tigecycline and vancomycin had a 100% sensitivity rate. These results show that, despite the restricted use of antibiotics, antibiotic resistance among *S. aureus* isolates is widely present in The Netherlands and that a good surveillance system is necessary to monitor possible changes in antibiotic resistance in the future.

#### P1217 Activity of vancomycin, linezolid and daptomycin against staphylococci and enterococci isolated in Greek hospitals, 2008–2010

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**Objectives:** Evaluation of the activity of vancomycin, linezolid and daptomycin against staphylococci and enterococci collected from three tertiary Greek hospitals (Central and Southwestern Greece) from 2008 to 2010.

**Methods:** A total of 1052 staphylococci (831 *Staphylococcus aureus* and 221 Coagulase Negative Staphylococci, CNS) and 553 enterococci (353 *Enterococcus faecalis* and 200 *E. faecium*) recovered from clinically significant specimens (blood, urine, pus, etc) were included. Identification and susceptibility testing was performed using the automated Vitek 2 System (bioMérieux). Determination of MICs to linezolid, vancomycin and daptomycin was performed by Etest according to CLSI guidelines. Methicillin resistance among staphylococci and vancomycin resistance among enterococci were detected by PCR for *mecA* and *vanA/vanB* respectively.

**Results:** The majority of vancomycin-resistant enterococci (VRE: 287 isolates) were *E. faecium* (196 isolates, carrying *vanA* gene). Among *S. aureus* and CNS 512 and 166 were *mecA*-positive respectively (MRSA and MR-CNS). Table 1 represents the mean MIC values among the tested isolates. The mean MIC values did not differ significantly during the study period. Elevated MIC mean values of vancomycin were determined among MRSA and MR-CNS. The average values of linezolid MICs among MR-CNS differed among the participating hospitals (14.38, 7.95 and 1.5) corresponding to a variable number of linezolid-resistant isolates recovered from hospitalized patients.

Table 1. Mean values of MICs of vancomycin, linezolid and daptomycin among enterococci (VRE: vancomycin-resistant enterococci), staphylococci (MSSA, methicillin-sensitive *S. aureus*; MRSA, methicillin-resistant *S. aureus*; MR-CNS: methicillin-resistant coagulase-negative staphylococci; MR-CNS: methicillin-resistant coagulase-negative staphylococci).

Year	Vancomycin						Linezolid						Daptomycin					
	VRE	VRE	MSSA	MRSA	MR-CNS	MR-CNS	VRE	VRE	MSSA	MRSA	MR-CNS	MR-CNS	VRE	VRE	MSSA	MRSA	MR-CNS	MR-CNS
2008	2.00	2.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
2009	2.00	2.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
2010	2.00	2.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

**Conclusions:** Daptomycin remains active against the majority of Gram-positive cocci isolated in Greek clinical settings. The elevated levels of MICs to vancomycin among staphylococci above the therapeutic limit of the antibiotic, renders often this drug inactive. Additionally the detection of linezolid-resistant isolates, reflecting the differences in antibiotic usage in the participating hospitals, emphasizes the need of the development a drugs' restriction policy.

#### P1218 Continued decline of macrolide-resistant *Streptococcus pyogenes* in Portugal

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**Objectives:** In Portugal, we noted a steady decrease in macrolide resistance among *Streptococcus pyogenes* (GAS) causing pharyngitis in 1999–2006, accompanied by large fluctuations of the macrolide



resistance phenotypes, and changes in the clonal composition of the population. To establish if these have continued, we characterized the isolates recovered in 2007–2009.

**Methods:** Antimicrobial susceptibility testing and macrolide resistance phenotype were determined by disk diffusion. All macrolide-resistant isolates were further characterized. Macrolide resistance genotype was determined by PCR. A combination of T typing, emm typing and pulsed-field gel electrophoresis profiling (PFGE) was used to identify clones.

**Results:** During the 3 years of the study, the overall rate of erythromycin resistance was 6.8% (n = 106), much lower than the one reported in the previous period. A continuous decline in macrolide resistance was noted (12.4% in 2006 to 3.3% in 2009,  $p < 0.001$ , Cochran-Armitage test) and this was accompanied by fluctuations in the prevalence of the macrolide resistance phenotypes. PFGE identified seven major lineages, containing 79 isolates (78.2%), each exclusively associated with a single macrolide resistance phenotype. Two major clones were found among MLSB isolates characterized by T11/emm11 and T12/emm22 whereas among M isolates three clones were identified, characterized by T1/emm1, T4/emm4 and T25/emm75, including each approximately the same number of isolates. All clones identified had already been found in Portugal, but these changed in prevalence during the study period.

**Conclusion:** The decline in macrolide resistance among GAS causing pharyngitis in Portugal continued in 2007–2009, despite high macrolide use. Although the reasons behind these changes remain unidentified, they were accompanied by alterations in the prevalence of the resistance phenotypes and of the clonal composition of the population.

#### **P1219** Antimicrobial resistance prevalence at the United Kingdom's first academic health science centre

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**Objective:** To examine current bacterial resistance rates from two central London hospitals, a multisite renal unit and surrounding community 4 years after becoming part of the first UK AHSC.

**Method:** Imperial College Healthcare NHS Trust supplies microbiology services to five central London hospitals, a multisite renal unit in North West London and additionally to primary care practices across this area. Infection advice is provided by an integrated team of infection specialists with an established antimicrobial policy. Laboratory operating procedures are uniform for samples received from two of these hospitals and from the renal sites and community samples. The laboratory information management system was interrogated for these four subsets of the AHSC for all susceptibilities performed in a 1 year period from March 2010 to February 2011 providing 502 315 antibiograms. Sub group analysis for resistance rates in high intensity (level 2 and level 3 care) vs. low intensity areas (level 0 and level 1 care) were calculated for each hospital and the renal unit and compared to that in the community. Resistance rates were identified for AmpC and ESBL producing Enterobacteriaceae, glycopeptide resistant enterococci and *Pseudomonas aeruginosa*.

**Results:** We find that in North West London there are significant variations in resistance rates between geographically close units. Of 20 142 Enterobacteriaceae isolates we find 9.4% of isolates from the community display ESBL/AmpC phenotypes, rising to 22.4–25.6% ( $p = 0.173$ ) in low intensity inpatient areas and 28.1–56.5% ( $p < 0.001$ ) in high intensity areas. Of 5277 enterococci isolates we find community GRE rates of 0.5%, with 7.7–17.3% 9 ( $p = 0.005$ ) in low intensity inpatient areas and 34.5–59.5% ( $p < 0.001$ ) in high intensity areas. Of 3777 *Pseudomonas aeruginosa* isolates we find carbapenem resistance in the community of 3.2%, with rates from 7.1% to 9.8% ( $p = 0.370$ ) in low intensity inpatient areas and 19.7–34.7% ( $p = 0.005$ ) in high intensity areas.

**Conclusion:** Despite unified antimicrobial and infection control policies, standardised laboratory practice and integrated infection specialists there is significant variation in antimicrobial resistance between high intensity areas within the AHSC. Variations in antimicrobial use and specific clinical drivers of second and third line antimicrobial therapies must be considered as causative factors and may warrant unit specific policies allowing for these variabilities.

#### **P1220** National MRSA rates run along with fair play – European championship 2008

*E. Meyer\*, P. Gastmeier, F. Schwab (Berlin, DE)*

**Objective:** To assess the relationship between national MRSA rates and indicators for fair play in the European Football Championship 2008.

**Methods:** National MRSA rates were retrieved from the European Resistance Surveillance System (EARSS) in 2008. All teams which qualified for the final tournament and had reported data to EARSS were included in the analysis. Hosting countries were excluded. The number of yellow and red cards was calculated per 100 min as an indicator for fair play. Red cards were weighted like yellow cards. MRSA rates and the fair play indicator were calculated by Spearman correlation index and p-value was set  $< 0.05$ .

**Results:** In 2008, 16 countries qualified for the European Football Championship. Five countries (Turkey, Switzerland, Croatia, Russia and Austria) did not contribute MRSA rates to EARSS and/or were hosting countries. MRSA rates, entire playing time and number of cards are listed in the Table 1.

Correlation was highly significant ( $p = 0.038$ ) with a correlation coefficient of 0.632.

	MRSA%	entire playing time	red cards	yellow cards	cards/ 100 min
Czech Republic	14.6	270		4	1.48
France	22.8	270	1	7	2.96
Germany	18.4	540	1	7	1.48
Greece	40.4	270		8	2.96
Italy	37.4	390		9	2.31
Netherlands	1.0	300		5	1.67
Poland	20.2	270		7	2.59
Portugal	49.1	360		8	2.22
Romania	34.4	270		7	2.59
Spain	26.0	480		8	1.67
Sweden	1.0	270		3	1.11

**Conclusion:** Demographic and socioeconomic factors as well as safety culture are known to influence the MRSA situation. Other socio-psychological factors used to measure non-adherence with MRSA guidelines are scarce. Interestingly, indicators for fair play in the European national football correlated highly with national MRSA rates: Sweden and the Nederland played the fairest and had the lowest MRSA rates. It remains to be proven (e.g. in the Championship 2012) whether this factor indeed can serve as an indicator for adherence with MRSA guidelines or whether this correlation cannot be substantiated.

#### **P1221** ABSTRACT WITHDRAWN

## Highlights of the molecular bases of antimicrobial resistance in Enterobacteriaceae

### P1222 Efflux inhibitors induce ramA expression

A.J. Lawler\*, V. Ricci, L.J.V. Piddock (Birmingham, UK)

**Objectives:** The aim of this work was to identify compounds that influenced the expression of ramA, which encodes RamA, a regulator of the AcrAB-TolC efflux pump in *Salmonella enterica* serovar Typhimurium (SL1344). AcrAB-TolC is the major efflux pump in Enterobacteriaceae, and exports a wide range of substrates. One of the physiological functions of this pump is export of chemicals which are toxic to the bacterium. It was hypothesised that increased expression of acrAB and/or tolC could be via induction of ramA in response to hostile environments. As over-expression of AcrAB-TolC can lead to multidrug resistance (MDR), increased production, via the induction of ramA, could also lead to transient MDR.

**Methods:** The promoter of ramA was fused to gfp on the reporter plasmid pMW82. The GFP is unstable and so allows changes in expression to be measured via fluorescence. The ramA reporter plasmid was transformed into SL1344 and assays were carried out using a 96 well plate format. The response of ramA to sub-MIC of different antibiotic classes, biocides and efflux inhibitors (EIs) was determined.

**Results:** The efflux inhibitor (EI) PAbetaN, a competitive inhibitor of AcrB, caused a two fold increase in fluorescence. A similar increase was also observed with another EI, CCCP; which disrupts the proton motive force required for functional efflux. The greatest increase in fluorescence achieved was in response to chlorpromazine (CPZ), a compound with EI-like activity. At 50 µg/mL of CPZ a 6.3 fold increase in GFP fluorescence was observed, however, of the six antibiotics tested, all of which are substrates of acrB, four caused no significant increase in expression. Chloramphenicol and ciprofloxacin caused a modest increase in fluorescence of 1.48 and 1.18 fold, respectively.

**Conclusions:** Our data demonstrated that in this system compounds which act as EIs increased ramA expression. Antibiotics belonging to a number of classes had minimal or no influence on the level of ramA. This work was funded by MRC grant No G0801977.

### P1223 MDR efflux pumps have overlapping and distinct roles in antibiotic resistance and virulence

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**Objectives:** Antibiotic efflux is responsible for the intrinsic resistance of Gram-negative bacteria to many classes of antibiotics and biocides. *Salmonella* have several efflux pumps system of which AcrB mediates clinically relevant levels of resistance. The aim of this study was to investigate whether the roles of these efflux pumps overlapped or were distinct.

**Methods:** *S. enterica* mutants lacking combinations of efflux genes were constructed. The transcriptome of mutants lacking AcrB, AcrD or AcrF were studied using the Pan-Salmonella Generation IV array (Wellcome Trust Sanger Institute). The phenotype of all single, double and triple mutants was determined by MIC testing, efflux assays and infection assays. Real time RT-PCR was used to study the transcription levels of the pump genes after inactivation of one or more homologous system.

**Results:** Inactivation of single RND pump genes, acrB, acrD or acrF, caused multiple changes in the transcriptome. As shown previously, genes required for virulence had decreased expression after acrB inactivation. Expression of virulence genes from SPI-1, 2 and 3 was also decreased in an acrD mutant. Strains lacking AcrB, AcrD, AcrF, or combinations thereof, all invaded human intestinal cells poorly showing that these efflux systems each have a distinct role in virulence. As previously shown, inactivation of

acrB caused multi-drug hypersusceptibility while inactivation of AcrD or AcrF alone had no effect on susceptibility to the drugs tested. However, strains lacking two or three efflux pump components became increasingly susceptible to antimicrobials and effluxed less Hoescht dye than strains with intact efflux systems. Real time RT-PCR showed that transcription levels of efflux pump genes were adapted to compensate for loss of other efflux pumps.

**Conclusion:** AcrB, AcrD and AcrF have some overlapping functions because an increase in the transcription level of intact efflux pumps was able to partially compensate for loss of homologous systems. However, loss of any of the pumps inhibited the ability to invade host cells showing that up-regulation of other pumps cannot compensate for all the functions normally performed by other systems. These data are critical for the design of effective efflux pump inhibitors.

### P1224 Stress response and resistance of *Salmonella enterica* serotype Enteritidis to the efflux pump inhibitor neuroleptic drug thioridazine

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**Introduction:** The main reason for problematic therapy lies in the variety of responses that *Salmonella* activates when in a noxious environment, rendering the organism quite resistant to most antibiotics. Multidrug resistant (MDR) phenotype of most clinical bacterial isolates is due to the over-expression of multidrug efflux pumps. Compounds that are efflux pump inhibitors (EPIs) reduce or reverse resistance to antibiotics to which the bacterial strain is initially resistant.

**Objectives:** In the present study, thioridazine (TZ)-induced accumulation of the universal efflux pump substrate ethidium bromide and its subsequent efflux by *Salmonella enterica* serotype Enteritidis strains was investigated under different physiological conditions.

**Methods:** Concentrations of TZ were evaluated for activity against over-expressed MDR efflux pumps of *Salmonella* strains with the aid of the automated ethidium bromide (EB) real-time fluorimetric method. The activity of genes that regulate and code for the AcrB transporter, was demonstrated by real-time reverse transcription quantitative polymerase chain reaction (RT-qPCR).

**Results:** *Salmonella enterica* serotype Enteritidis cultured in medium containing increasing concentrations of TZ does not grow during the first 6–8 h, after which time its growth is similar to unexposed controls. At the end of a 16-hour exposure period, the organism is resistant to >250 mg/L TZ. The TZ promoted increase of accumulation of EB that is followed by efflux may be the reason for the resistance of Salmonellae to this phenothiazine.

The genetic response against TZ treatment was assessed by real-time reverse transcription quantitative polymerase chain reaction (RT-qPCR) at periodic intervals. It is demonstrated that a sequence of activation beginning with the stress gene soxS, followed by the global regulator ramA, then by the local regulator marA and then by the transporter acrB which remains over-expressed by the end of the 16 h culturing period.

**Conclusions:** TZ seems to present an environmental challenge to the organism, namely TZ induces resistance to the agent as a consequence of the activation of genes that regulate and code for the main efflux pump AcrAB. Furthermore, TZ also activates the two-component regulon PmrA/B and because the activation of pmrA/B also activates acrB, the development of high resistance to TZ during a 16-hour culture period is in part due to activation of the two-component regulon.

### P1225 Genomic evolution of antibiotic resistance in *Salmonella enterica* serovar Typhimurium following biocide challenge

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**Objectives:** Biocides are essential in preventing infection or microbial contamination in a range of environments. There are concerns that

biocide exposure is helping drive selection of antibiotic resistant bacteria due to common mechanisms of resistance. The aim of this study was to identify the genetic basis of antibiotic resistance in mutants selected after biocide exposure.

**Methods:** *S. Typhimurium* was challenged continuously for 5 days with four separate biocides with differing modes of action. Throughout the challenges, each population was sampled and screened for the emergence of antibiotic resistant mutants. Resistant mutants were characterised phenotypically and the genomes of four mutants from each biocide challenge were sequenced, mutations identified and investigated.

**Results:** The earliest mutants had appeared by the second sub-culture in some biocides but not until the fifth in others. The biocides Superkill and AQAS selected mutants that exhibited a low level multi-drug resistant phenotype consistent with de-repression of efflux pumps. Trigen selected mutants that were specifically resistant to high-levels of quinolones and triclosan.

Genome sequencing identified a ramR mutation in the mutants isolated from Superkill and AQAS, consistent with the low-level MDR phenotype. Mutations within both *gyrA* and *fabI* were present in mutants isolated after exposure to Trigen; these mutations have previously been associated with quinolone/triclosan resistance respectively. Additional mutations were also consistently identified in *rpoA* from mutants isolated after exposure to AQAS and in *zur* in mutants recovered from the trigen challenge. These mutations have not previously been linked to drug resistance and suggest new roles for these genes in the regulation of multidrug resistance.

**Conclusions:** All biocides tested could select antibiotic resistant mutants however their propensity to do so differed. Some biocides selected resistance to specific antibiotics rather than generic multi-drug resistance. Next generation sequencing allowed us to identify genes involved in biocide-antibiotic cross resistance, some of which have been previously linked to drug resistance and some of which are novel. This knowledge will provide useful insights when designing new biocides or dictating policies to minimise selection of antibiotic resistant mutants.

**P1226** Characterisation of mechanisms of resistance and determination of plasmid incompatibility group of *E. coli* and *Salmonella* strains isolated from healthy food animals with decreased susceptibility to cefotaxime

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**Objectives:** In a trans-European surveillance programme (European Antimicrobial Susceptibility Surveillance in Animals; EASSA), 4501 *Escherichia coli* isolates and 659 *Salmonella* spp. isolates have been recovered between 2002 and 2006 from cattle, pigs and chickens. Screening of this collection resulted in 116 *E. coli* isolates (MICs 0.5–>64 mg/L) and 19 *Salmonella* isolates (MICs 1–8 mg/L) showing decreased susceptibility or resistance to cefotaxime (CTX). The purpose of this study was to characterize the  $\beta$ -lactamases and to determine the plasmid incompatibility group (Inc) of plasmids encoding those resistance genes.

**Methods:** Whole-cell DNA of each isolate was used in standard PCR experiments with sets of primers designed for detection of class A or D  $\beta$ -lactamases and plasmid-mediated class C (AmpC) cephalosporinases. PCR products were sequenced for obtaining the entire sequence of each  $\beta$ -lactamase gene. Sequencing of the AmpC-type  $\beta$ -lactamase gene was also performed for the *E. coli* isolates without transferable  $\beta$ -lactamase genes. Plasmid identification was performed by extraction of plasmid DNA from transconjugants and amplification by PCR. Amplicons were subsequently sequenced and typing was confirmed by Southern blot hybridization.

**Results:** Overall, 90 of the 116 *E. coli* and three of the 19 *Salmonella* isolates investigated, exhibited plasmid-mediated ESBLs or AmpC (Table 1). For 26 strains (MICs 0.5–1 mg/L) penicillinases-, narrow-spectrum oxacillinase or outer-membrane permeability defect were

found. The vast majority of the ESBL producers were from chicken (90%) and from Spain and the Netherlands. All ESBLs, with the exception of few SHV enzymes, and plasmid mediated AmpC conferred resistance to CTX with MICs  $\geq$  4 mg/L. In most cases, ESBL genes were harboured on IncI1 plasmids and AmpC on IncA/C plasmids.

Bacterial species	Resistance mechanism	Animal species (number of isolates)			CTX MIC range (mg/L)	Plasmid Inc group
		Chicken	Pigs	Cattle		
<i>E. coli</i>	<b>ESBLs</b>					
	SHV-12	10	1	0	2-16	I1
	SHV-2a	2	0	0	1	nd
	TEM-52	12	0	0	4-32	I1 or neg
	CTX-M-1	9	0	0	16-64	I1
	CTX-M-2	9	0	0	$\geq$ 64	P
	CTX-M-14	8	0	0	4-32	neg
	CTX-M-9	2	0	0	4-8	F/P
	CTX-M-32	2	0	0	32	neg
	CTX-M-3, -51	2	0	0	4	neg
	<b>AmpC</b>					
	Plasmid-mediated	8	0	0	4-16	A/C
Non plasmid-mediated	14	6	5	1-8	nd	
<i>Salmonella</i> spp.	<b>ESBLs</b>					
	CTX-M-9	2	0	0	16	nd
	SHV-12	0	1	0	32	N

nd: not determined ; neg: no plasmid incompatibility group identified

**Conclusions:** Enterobacterial isolates with reduced susceptibility to cefotaxime were mainly recovered from poultry across the EU. High MIC values to cefotaxime were associated with ESBLs or AmpC cephalosporinases whereas low MICs values were associated penicillinases, oxacillinases or permeability defect. None of the identified ESBLs were of the CTX-M-15 type widely identified among human isolates.

**P1227** A mutation upstream of a chromosomal parMR locus is responsible for antibiotic resistance gene silencing in *Escherichia coli* 345-2

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**Objectives:** We previously reported the naturally occurring silencing of antibiotic resistance genes encoded on the IncN plasmid pVE46 in *Escherichia coli* 345-2RifC, a wild-type strain of porcine origin. In affected isolates transcription of *aadA1*, *blaOXA-2*, *su11* and *tet(A)* was absent but intact wild-type genes were retained, following passage through the pig gut. The phenomenon was reversible at frequencies of approximately  $10^7$  and likely due to a mutation elsewhere on the genome of 345-2RifC. Here, we aim to identify the mutation responsible for transcriptional silencing.

**Methods:** The complete genome sequences of the wild-type *E. coli* 345-2RifC/pVE46, a strain with a silent phenotype, L5, and a revertant of L5 back to resistance were obtained by 454 sequencing. Sequence analysis was carried out using Artemis. Mutations were confirmed by PCR amplification followed by Sanger sequencing. Transcription was assessed by RT-PCR. The role of the parMR mutation in silencing was investigated by deleting the locus using a lambda red recombinase system.

**Results:** Whole genome coverage was obtained and revealed the genome of *E. coli* 345-2RifC to be 5.2 megabases in size. There were just two polymorphisms between 345-2RifC/pVE46 and the sequenced silent isolate, L5; an ACCA nucleotide substitution in the *tuf* gene and a TA mutation in the upstream region of a chromosomal parMR locus. The parMR mutation returned to the wild-type T in the revertant isolate. PCR amplification and

independent re-sequencing by Sanger sequencing was unable to reproduce the mutation in *tuf* whilst the mutation upstream of *parMR* was confirmed. *parMR* is believed to encode a type II plasmid partitioning system which has become embedded in the chromosome of *E. coli* 345-2RifC through insertion by the CP-933T phage. It is not related to the pVE46 plasmid. The expression of the *parR* and *parM* genes was approximately 10-fold higher in the silent isolate L5 than in 345-2RifC/pVE46. Deletion of the *parMR* locus in L5 obliterated the silencing phenotypes and restored the antibiotic resistance phenotype conferred by pVE46.

**Conclusion:** Up-regulation of a chromosomal *parMR* plasmid-partitioning locus, likely due to a point mutation in its upstream region, appears responsible for the silencing of antibiotic resistance genes on plasmid pVE46. The mechanism by which this occurs is as yet unknown, but other plasmid-partitioning genes have previously been implicated in transcriptional gene silencing.

**P1228** **In vivo selection of a complex mutant TEM allele (TEM-158) from an inhibitor-resistant TEM allele (TEM-35) producing *E. coli* strain**

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**Objectives:** Many alleles of the penicillinase TEM-1 or TEM-2 were described so far in clinical isolates. Their spectrum of resistance varies with regard to point mutations in the *bla* sequence, with some mutations leading to extended spectrum beta-lactamase (ESBL) and others to inhibitor-resistant TEM (IRT). These IRT enzymes can broaden their spectrum to third generation cephalosporins by the occurrence of additional mutations: they are commonly referred to as “complex mutant TEM” or CMT. If many studies have shown that modifications of the spectrum of TEM-type enzymes is driven by antibiotic selection pressure, no study has ever described a CMT derived from an IRT by in vivo selection.

**Methods:** We describe the case of a patient hospitalized for an acute myeloid leukemia in the Haematology ward at Saint-Louis University Hospital in Paris. During hospitalisation, the patient was treated with ceftazidime for a bacteremia due to an *Escherichia coli* strain expressing an IRT phenotype (strain EC1). After 8 days of treatment, there was no clinical improvement and new blood cultures yielded a strain of *E. coli* with an IRT phenotype but with resistance extended to ceftazidime (strain EC2).

**Results:** ERIC-PCR analysis showed the clonality of the two strains. PCR-sequencing of the TEM allele from EC1 identified the mutations M69L and N276D, corresponding to the TEM-35 allele also known as IRT-4. TEM gene sequencing from EC2 identified the additional mutation R164S, which was previously reported for the TEM-158 allele already described as a CMT allele (CMT-9).

**Conclusions:** to our knowledge, this is the first report of in vivo selection of a complex mutant TEM allele from an inhibitor resistant TEM allele producing *E. coli* strain. This event occurred during a treatment with ceftazidime.

**P1229** **Carbapenem-hydrolysing GES-5-encoding gene on different plasmid types from a sewage treatment plant**

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**Objectives:** The aquatic environment may be considered as a reservoir for dissemination of antibiotic resistance determinants. Two plasmids, pRSB113 and pRSB115, conferring decreased susceptibility to carbapenems in *Pseudomonas* sp. were isolated from bacterial communities residing in the activated sludge compartment of a wastewater treatment plant in Germany. The aim of this work was to characterize the beta-lactamase resistance genes involved and to identify the genetic structures involved in their mobilization and expression.

**Methods:** *Pseudomonas* sp. B13 GFP1 was used as host for electroporation of plasmids recovered from bacteria of activated sludge. Plasmid DNA was extracted from two carbapenem-non-susceptible *Pseudomonas* sp. B13 GFP1 (pRSB113/pRSB115) transformants. A PCR-based approach was used with primers specific to seek for the most common carbapenemase genes. The genetic environment of the *bla*GES-5 gene was determined by using a primer-walking approach. Plasmid size was determined after extraction by the Kieser method. Direct transfer of the *bla*GES-5 determinant was attempted by conjugation and electroporation experiments in *Escherichia coli*.

**Results:** The *bla*GES-5 gene was identified on both pRSB113 and pRSB115 plasmids, in a form of a gene cassette of a class 1 integron. Beta-lactamase GES-5 is a point mutant derivative of the extended-spectrum beta-lactamase GES-1. In addition, to broad-spectrum cephalosporins, it hydrolyses cephamycins and carbapenems. The class 1 integrons were inserted in two distinct structures comprising transposase genes (IS1326 and a novel insertion sequence, named ISUnCu16), a mercuric operon, and a truncated gene of restriction/modification system (*pacR7IR*). The 30-, and 40-kb plasmids both replicated in *E. coli*, but were not self-transferable. Pairwise comparison of the amino acid sequence of RepA and MobA showed that pRSB113 and pRSB115 belonged to two distinct clades of the MobP plasmid family.

**Conclusion:** The *bla*GES-5 gene was identified on two different plasmids belonging to distinct incompatibility groups, that could replicate in both *Pseudomonas* sp. and *E. coli*, and that are mobilizable. This study emphasizes that aquatic environment may be the reservoir of clinically-significant carbapenemase genes.

**P1230** **Multidrug resistance IncHII plasmids carrying blaCTX-M-1 from equine *Escherichia coli* from the Czech Republic**

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**Objective:** Plasmids of incompatibility group HII are important vectors of antibiotic resistance in *Salmonella Typhi* and *S. Paratyphi* A, the major causal agents of enteric fever.

It has been demonstrated that IncHII plasmids allow these pathogens to acquire genes available in the environment. We have previously described IncHII plasmids carrying *bla*CTX-M-1 gene in *Escherichia coli* isolates disseminated in an equine clinic in the Czech Republic (Dolejska et al. 2011, JAC 66:757–64). The aim of this study was to compare, by plasmid MLST (pMLST), four representative CTX-M-1-positive IncHII plasmids from *E. coli* isolated between 2008 and 2010 in the clinic with those found in *S. Typhi* and *S. Paratyphi* A and to perform complete sequencing of selected IncHII plasmids.

**Methods:** Three conjugative IncHII and one IncHII/X1 plasmids ranging 220–285 kb in size, harbouring *bla*CTX-M-1 were purified from *E. coli* transconjugants and compared by restriction fragment length polymorphism (RFLP) and pMLST. Complete nucleotide sequencing of one 220 kb IncHII and a fused 285 kb IncHII/X1 plasmid was performed by the 454-Genome Sequencer FLX procedure on a library constructed on plasmid DNA purified from the *E. coli* transconjugants.

**Results:** All plasmids showed closely related RFLP, identical pMLST profiles and nucleotide sequence homology. One plasmid showed a higher molecular weight because of the fusion with an IncX1 plasmid carrying the *qnrS1* plasmid mediated quinolone resistance (PMQR) gene. Five loci HCM1.043, HCM1.099, HCM1.116, HCM1.178ac, and HCM1.259 showed the allele 1 as also seen in IncHII plasmids of sequence types (ST) 1 and 2 found in *S. Typhi* and *S. Paratyphi*. The gene HCM1.064 coding RepHIIA replication initiation protein in equine isolates showed a new allele with 99.8% nucleotide similarity to previously described allele 2, establishing a new ST for these plasmids.

**Conclusion:** Our data show successful dissemination of *S. Typhi* and *S. Paratyphi* A IncHII plasmid variants to *E. coli* from animals. Up to date the IncHII plasmid type was only identified in *Salmonella* sp. and

lacked ESBL and PMQR genes. Our results show that these plasmids are rapidly evolving toward a larger antimicrobial gene content by mutation, acquisition of resistance determinants and plasmid fusion with other resistance plasmids.

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**P1231** Development during therapy of carbapenem resistance in *Salmonella enterica* serotype Typhimurium

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**Objectives:** Multidrug-resistant *Salmonella* infection is a global problem and carbapenems may serve as the last therapeutic choice. The emergence of carbapenem resistance in *Salmonella*, therefore, represents a serious problem due to the lack of other therapeutic choices. Here we report the identification and characterization of a clinical isolate of carbapenem-resistant *Salmonella enterica* serotype Typhimurium.

**Methods:** Genetic relatedness of the isolates was analyzed by pulsed-field gel electrophoresis. PCR and sequencing were used to detect the presence of specific resistance genes. The expression and deficiency of outer membrane proteins (Omps) were studied by reverse-transcription (RT) PCR and DNA sequencing. Replicon and sequence types of the resistance plasmids were characterized by published methods.

**Results:** A patient with urinary tract infection was hospitalized for treatment. The urine culture yielded multi-resistant *S. Typhimurium* (U1) susceptible only to carbapenem. The patient was discharged after ertapenem therapy. Two months later, the patient was hospitalized again due to leg injury. The initial wound culture yielded ceftriaxone-resistant *Proteus mirabilis* and ertapenem was prescribed. Wound debridement was performed and the wound pus yielded *S. Typhimurium* (W1) showing the same antibiogram as those of the U1 strain. The patient developed diarrhoea during the therapy. The stool culture grew *S. Typhimurium* (S1) multiply resistant to all antibiotics including carbapenem. The patient was still on ertapenem therapy and was discharged after appropriate wound debridement. All isolates were found to have the same PFGE patterns and carried a blaCMY-2-containing, self-transferable IncII plasmid that belonged to the sequence type ST54. OmpD deficiency due to IS1 insertion at 88 bp upstream of the ompD gene was found among the isolates. In the carbapenem-resistant strain S1, OmpC deficiency through a point mutation, leading to amino acid change from tryptophan (TGG) to a stop codon (TGA) at codon 77, was further identified. No other known carbapenemases, including IMPs, KPCs, VIMs, and OXAs, could be identified from the carbapenem-resistant strain.

**Conclusion:** Development of OmpC deficiency in the OmpD-deficient, CMY-2-producing *S. Typhimurium* resulted in the carbapenem resistance. *Salmonella* appears very adaptive to antimicrobial selection pressure. Caution must be taken by physicians when treating multidrug-resistant *Salmonella* infection.

**P1232** ABSTRACT WITHDRAWN

**P1233** Occurrence of carbapenemase-producing Enterobacteriaceae in Sultanate of Oman

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**Background:** Carbapenemase-producing Enterobacteriaceae are increasingly identified worldwide and represent a major public health

concern. The most clinically significant carbapenemases are KPC, IMP, VIM and more recently NDM and OXA-48.

**Methods:** Our study focused on a retrospective collection of enterobacterial isolates recovered at the Royal Hospital, Muscat, Sultanate of Oman. Twenty-two multidrug resistance Enterobacteriaceae (15 *Klebsiella pneumoniae*, five *Escherichia coli*, one *Citrobacter freundii*, one *Serratia marcescens*) isolated over a 5-month period (November 2010 to March 2011) were selected, all showing decreased susceptibility to carbapenems. The MICs were determined by Etest and PCR assays followed by sequencing were carried out for detection and identification of carbapenemase genes. Multi locus sequence typing (MLST) and pulsed-field electrophoresis (PGFE) were performed to evaluate the clonal relationship. Plasmids were isolated by mating-out assays and typed by PCR-based replicon typing (PBRT).

**Results:** Twelve non-clonally related carbapenemase-producing isolates were identified. Seven produced NDM-1 and four produced OXA-48. One *K. pneumoniae* isolate co-produced NDM-1 and OXA-181, which is a point-mutant derivative of OXA-48. With the exception of a single *K. pneumoniae* isolate that only expressed OXA-48, all other isolates co-produced an extended-spectrum beta-lactamase.

In all NDM-1 producers, the blaNDM-1 gene was plasmid-located and bracketed upstream by insertion sequence ISAbA125 (entire or partial) and downstream by the bleMBL gene encoding resistance to bleomycin.

In OXA-48 producers, the blaOXA-48 gene was always associated to insertion sequence IS1999. A single IncLM plasmid of ca. 62-kb was responsible for the acquisition of the blaOXA-48 gene among all OXA-48 producers, whereas the blaNDM-1 gene was identified on different plasmid backbones.

**Conclusions:** This study emphasizes the dissemination of carbapenemase-producing enterobacterial isolates in the Middle East and especially in Gulf countries where populations from the Indian subcontinent and North African countries (respectively important reservoirs of NDM-1 and OXA-48 producers) are important.

**P1234** Importation of OXA-48-producing and carbapenem-resistant *Klebsiella pneumoniae* from Kuwait to France

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**Objectives:** The aim of this study was to investigate the mechanisms responsible for carbapenem resistance in one *Klebsiella pneumoniae* isolate recovered from Kuwait.

**Methods:** The strain was first screened using primers designed to PCR amplify known carbapenemase genes. The genetic environment of the blaOXA-48 gene was studied by PCR combination using specific primers of IS1999 followed by sequencing. The plasmid scaffolds were typed by using primers specific for the blaOXA-48-bearing plasmid known to circulate in different countries.

**Results:** *K. pneumoniae* ALI was recovered from rectal swabs performed at the hospital entrance of a diabetic woman transferred from Kuwait to France. She had been amputated in Kuwait City from her left leg and was hospitalized in France for a prosthesis surgery. *K. pneumoniae* ALI was resistant in particular to piperacillin-tazobactam and carbapenems, with MICs of IMP, MER, ERT being at 32, 24, and >32 mg/L, respectively. It produced the class D carbapenemase OXA-48, together with a naturally-occurring narrow-spectrum class A  $\beta$ -lactamase SHV-28. Noteworthy, *K. pneumoniae* ALI did not produce any extended-spectrum beta-lactamase, and MICs of CAZ and CTX were at 2 and 4 mg/L, respectively. Plasmid analysis identified a ca. 62-kb plasmid, that does not harbor other resistance determinants and possessed the same genetic profile than blaOXA-48-positive plasmids identified in most OXA-48 producers.

**Discussion:** This study identified the first OXA-48 producer in the Gulf region, whereas carbapenemases of the VIM, NDM, and OXA-181 types had been already described. These findings extend the knowledge on the current diffusion of OXA-48 producers that now also includes the Arabic peninsula.

**P1235** Detection of *Acinetobacter baumannii* derived oxa-51 and oxa-58 carbapenemase genes in enteric bacteria: a new dimension on carbapenemase resistance

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**Objectives:** We investigated the occurrence of genes associated with the production of carbapenem hydrolysing carbapenemases in *Escherichia coli* and *Klebsiella pneumoniae* isolates recovered from two ICU patients.

**Methods:** Antimicrobial susceptibility testing was performed and resistance genes were characterized by PCR amplification and sequencing. The Modified Hodge Test (MHT), MBL E-test, EDTA and boronic acid combined disk diffusion method, and a disk enzymatic assay were performed for the screening of carbapenemases.

**Results:** For *K. pneumoniae*, MICs of carbapenems were as follows (mg/L) ertapenem 8, meropenem 1, imipenem 0.25 and doripenem 0.5. This isolate demonstrated positive results in the ESBL, EDTA and APBA combine tests, and disk enzymatic assay. PCR and sequencing revealed the presence of, blaOXA-51 and blaCTX-M-15 beta-lactamase genes. For *E. coli*, MICs of carbapenems were as follows (mg/L) ertapenem and meropenem 0.06, imipenem 2 and doripenem 0.25. *E. coli* strain showed positive results in the ESBL, MHT and disk enzymatic assay tests. PCR and sequencing revealed the presence of, blaOXA-58 and blaCTX-M-15 beta-lactamase genes. Plasmids from both strains were not transferred by conjugation to recipient *E. coli*. In conclusion, we report on the first detection of the OXA-51 and OXA-58 harboring *K. pneumoniae* and *E. coli* isolates and co-produced a CTX-M-15 beta-lactamase.

**P1236** Naturally – occurring metallo-beta-lactamases in *Erythrobacter* sp.

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**Objectives:** In-silico analysis identified a metallo-beta-lactamase (MBL) in *Erythrobacter litoralis* HTCC2594, sharing 55% amino acid identity with NDM-1. *Erythrobacter* sp. are strict aerobic bacteria, most frequently found in nutrient-rich coastal seawaters. The aim of this work was to characterize the chromosomally-encoded MBL from several *Erythrobacter* species, that may represent potential reservoirs of acquired MBLs.

**Methods:** *Erythrobacter citreus*, *E. flavus*, *E. longus*, *E. aquimaris*, and *E. vulgaris* were from the collection strains. DNA was extracted and used as template for shotgun cloning experiments. Corresponding beta-lactamase genes were expressed in *E. coli*. Resulting recombinant strains *E. coli* (pCIT-1), *E. coli* (pFLA-1), *E. coli* (pLON-1), *E. coli* (pAQI-1), and *E. coli* (pVUL-1) were selected onto amoxicillin (50 µg/mL) and kanamycin (30 µg/mL) agar plates. MICs were determined by E-test. The deduced amino acid sequences were analyzed and compared with the BLASTP database. Enzymatic activity of bacterial extracts from recombinant *E. coli* strains was determined by UV spectrophotometry with imipenem (100 µM) as substrate.

**Results:** Sequencing of the inserts of the *E. coli* recombinant strains identified hypothetical MBL-encoding genes. MICs of beta-lactams showed a decreased susceptibility to carbapenems only for *E. coli* (pFLA-1) and *E. coli* (pLON-1), expressing the MBL from *E. flavus* and from *E. longus*, respectively. MBLs from other *Erythrobacter* species conferred resistance or reduced susceptibility to amino- and carboxypenicillins, to narrow- and broad-spectrum cephalosporins, but not to carbapenems. MBLs from these different *Erythrobacter* species shared a weak amino acid identity ranging from 45% to 75%. MBLs from these *Erythrobacter* species differed significantly from acquired MBLs, including NDM-1, VIM-1, IMP-1, sharing only 11–23% amino acid identity. Enzymatic activity against imipenem was detectable but weak in all these recombinant *E. coli* strains, except for *E. coli* (pFLA-1), in which the specific activity against imipenem was higher (90 nmol of imipenem hydrolyzed/min/mg of protein).

**Conclusion:** *Erythrobacter* species harbour chromosomally-encoded MBLs showing a wide diversity of amino acid sequences and surprisingly, only few of them exhibited significant carbapenemase activity. None of these species could be considered as a progenitor of any known plasmid-mediated carbapenemase disseminating worldwide.

**P1237** Description of IMP-31, a novel metallo-beta-lactamase very divergent from other known IMP carbapenemases

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**Introduction:** The worldwide increase of multidrug-resistance in gram-negative bacteria has become an important clinical challenge. Carbapenem resistance can be caused by a variety of mechanisms, however the worldwide spread of carbapenemases is especially important. A worrying trend is the dissemination of Ambler class B metallo-beta-lactamases (MBL). Here we describe a novel IMP-type MBL, IMP-31, that was found in an outbreak of multidrug resistant *P. aeruginosa* in Germany.

**Methods:** Susceptibility to antibiotics was determined by disk diffusion and Etest. The presence of a MBL was determined by EDTA combined-disc-tests, MBL Etest and by a bioassay based on cell-free extracts. A modified Hodge-Test was performed. PCRs and subsequent sequencing were performed for VIM, IMP, NDM, GIM, SIM, SPM, AIM, DIM and KHM. Clonal relatedness of the isolates was determined by RAPD-PCR and PFGE. Integron structures were analysed by PCR and sequencing. The IMP-31 encoding sequence was cloned into the pBK-CMV vector and transformed into *E. coli* DH5alpha for activity analysis. Localisation of the gene was determined by PFGE and Southern blotting.

**Results:** Clonally related strains from an outbreak of *P. aeruginosa* in Germany were sent to the National Reference Laboratory for Multidrug-resistant Bacteria for further characterisation. The isolates were resistant to piperacillin, piperacillin/tazobactam, ceftazidime, cefepime, aztreonam, imipenem, meropenem and doripenem as well as to gentamicin, tobramycin, amikacin, ciprofloxacin and levofloxacin. The modified Hodge-test was positive for imipenem, meropenem and ertapenem. The isolates showed synergy with EDTA in the combined disk test and the MBL Etest. A chromosomally integrated class I integron was identified, harbouring the IMP-31 gene, a OXA-10 gene and the gene for an aminoglycoside adenylyltransferase (aadA6). The sequence of IMP-31 showed only 85% homology with its closest relatives IMP-8 and IMP-24. It mediates resistance to all carbapenems and most other beta-lactams.

**Conclusion:** The strains harbour a novel IMP-type metallo-beta-lactamase very divergent to any other known IMP variant. Its closest relatives are IMP-8 and IMP-24 with 39 and 40 amino acid substitutions, respectively. Regarding the amount of mutations, it is likely that the source of IMP-31 is a so far unknown environmental bacterium and that it was mobilised quite recently as IMP-31 was only found in German isolates up to date.

**P1238** Characterisation of carbapenemase IMI-2 in *Enterobacter* spp. clinical isolates from France

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**Objectives:** The Ambler class A IMI carbapenemase was first described in 1996 in *E. cloacae* isolates. Three variants have been reported from clinical isolates but also from environment (US rivers) and are either chromosome- or plasmid-encoded. IMI enzymes remain rare in clinical practice. Here, we describe four clinical imipenem-resistant strains of *Enterobacter* spp. isolated between 2007 and 2011, in France.

**Methods:** Carbapenem-resistant *Enterobacter* spp. isolates were characterized by standard biochemical methods, RpoB sequencing,

disc diffusion susceptibility testing, and E-tests. Genes coding for beta-lactamases and the genetic structure surrounding bla<sub>IMI</sub> were sought by PCR and sequencing. Isolates were compared by PFGE, RAPD, Rep-PCR and by their plasmid content.

**Results:** Imipenem-resistant *E. asburiae* BRE-1, PAR-1 and ROC-1 were isolated from patients hospitalized for bone infection and for pneumoniae. Two of these patients had in common a recent contact with an aquatic source. A fourth imipenem-resistant strain of *Enterobacter cloacae*, VAL-1, was isolated from blood culture. These four isolates were identified in four different hospitals located in different French cities (400–1000 km apart from each other). All isolates were resistant to carbapenems but remained susceptible to extended-spectrum cephalosporins. Imipenem hydrolysis was detected in all isolates. A clavulanic acid-inhibited beta-lactamase IMI-1 was identified in VAL-1 and its variant IMI-2 was found in BRE-1, PAR-1 and ROC-1. The latest were genetically indistinguishable by RAPD and Rep-PCR analysis, but different from IMI-2-isolates of US rivers. The bla<sub>IMI-2</sub> gene was located on a self-transferable 90-kb plasmid, whereas bla<sub>IMI-1</sub> was chromosome-encoded. Plasmid migration profiles of BRE-1, PAR-1 and ROC-1 after enzymatic restriction were similar. A LysR-type regulator gene, IMIR-2, involved in inducible expression of IMI-2 was identified upstream of bla<sub>IMI-2</sub>, along with an IS2-like element and a transposase gene tnpA from Tn2501 (Tn3 family). The immediate genetic environment was similar to that described in IMI-2-isolates of US-rivers.

**Conclusion:** This study highlights that IMI carbapenemase-producers may be responsible of human infections in Europe. Furthermore, we have shown that a single *E. asburiae* clone producing IMI-2 may be responsible of infections, and these isolates likely originate from an aquatic source.

#### **P1239** Detection and characterisation of a new VIM-31 variant in *Enterobacter cloacae*

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**Objectives:** To characterize a new VIM-2-derived variant, VIM-31, detected in carbapenem-non susceptible (CNSE) *Enterobacter cloacae* (Eclo11236) isolated in a Belgian hospital.

**Methods:** Antimicrobial susceptibility testing was performed by Etest and microdilution according to CLSI guidelines. Resistance genes and their genetic environment were characterized by Check-points CT102 array and by PCR-sequencing. Plasmids were extracted by Kieser method, typed by PCR-based replicon typing and transferred by mating-out assays and transformation. VIM-31 was cloned in pET-28a and purified by ion-exchange chromatography and gel filtration. Kinetic parameters of VIM-31 were determined against various beta-lactams and a 3D homology model was constructed using EsyPred3D server.

**Results:** A CNSE isolate (Eclo11236) was recovered from several blood cultures of an 87-year old female who underwent colectomy for intestinal adenocarcinoma and who subsequently developed a recto-vaginal fistula and secondary peritonitis. Eclo11236 was intermediately susceptible to imipenem (IMI) (MIC = 3 µg/mL) and resistant to ertapenem (MIC = 6 µg/mL) while remaining susceptible to meropenem (MIC = 0.5 µg/mL), aztreonam, tigecycline, amikacin and ciprofloxacin. The presence of an MBL in this isolate was evidenced by a positive double disc synergy test with imipenem (IMI) and EDTA; the patient gradually improved following treatment with ciprofloxacin, but she remained persistently colonized with the same Eclo11236 in urine. PCR-sequencing revealed a new bla<sub>VIM-31</sub> allele coding two Tyr224His and His252Arg mutations in comparison to VIM-2 and located upstream to aacA4' gene in a class 1 integron (In669) carried on a 42-kb non-typeable but transferable plasmid.

VIM-31 presents a broad-spectrum activity, affecting all beta-lactam compounds except aztreonam. Comparatively to VIM-2, VIM-31 exhibits a lower catalytic activity (lower  $k_{cat}$  and higher  $K_m$  values)

against beta-lactams with  $k_{cat}/K_m$  ratios ranging from 4.5 (ceftazidime), 900 (ampicillin), 1900 (cefotaxime) to 2400/mM/s (imipenem). **Conclusion:** We described here a new VIM-31 variant with 2 point mutations leading to modifications of its catalytic properties in comparison to VIM-2. The fact that this VIM-31-expressing *E. cloacae* isolate was detected in a patient who had not traveled abroad and who remained colonized after hospital discharge underlines the risk of silent spread of CNSE in the community.

#### **P1240** Retrospective search for NDM-1 reveals Indian origin of DIM-1 metallo-beta-lactamase

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**Objectives:** To assess the early occurrence of NDM-1 and other carbapenemases in a collection of Gram-negative bacilli (GNB) isolates collected in India during 2000. We previously demonstrated that NDM-1-producing isolates were present in India as early as 2006, but no data is available for prior sample years.

**Methods:** Among 220 GNB isolates collected in India during 2000, 22 strains showing elevated imipenem MIC values ( $\geq 0.5$  mg/L) were further evaluated for the presence of carbapenemases. Modified Hodge test (MHT) was performed. Isolates were tested by PCR for genes encoding KPC, IMP, VIM, NDM, SPM, SIM, KHM, DIM, BIC, GIM, SME, IMI, NMC-A, GES and OXA-48. DIM-1-producer was compared to index strain (kindly supplied by L. Poirel, Bicetre Hospital, France) by PFGE and integron structures were amplified using primers located in the conserved sequences (CS).

**Results:** Twenty-two GNB tested belonged to eight bacterial species, including five *E. cloacae*, four *P. aeruginosa*, four *P. fluorescens*, two of each *K. pneumoniae*, *A. baumannii*, *C. freundii*, *P. stutzeri* and one *P. vulgaris*. These strains were collected in five cities: Mumbai, Vellore, New Delhi, Lucknow and Indore. Only one strain yielded positive PCR results for bla<sub>DIM</sub> primers. No isolates were positive for NDM-1 or other carbapenemase-encoding genes. The *P. stutzeri* strain carrying bla<sub>DIM-1</sub> was genetically distinct from the index *P. stutzeri* strain carrying this gene previously described in The Netherlands. Integron structure showed that bla<sub>DIM-1</sub> was located in the second position of a class I integron downstream of aadB and an intact 3'-CS structure (qacEdelta1/sul1). In contrast the index strain carried bla<sub>DIM-1</sub> in the first position followed by aadB and qacH, but no 3'-CS.

**Conclusions:** NDM-producing strains were not detected in this bacterial collection from five Indian cities in 2000, narrowing the interval for the emergence of NDM-producing strains in India. On the other hand, the detection of a DIM-1-producing *P. stutzeri* from India collected many years prior to the finding of this gene in the Dutch strain, suggests that the Indian subcontinent could be the source of another metallo-beta-lactamase gene. Further studies should be performed to investigate the origin of DIM-1 and its prevalence in India.

#### **P1241** Biochemical characterisation of GES-18 carbapenemase with an additional Val80Ile substitution compared to GES-5

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**Objectives:** GES-1 is a class A beta-lactamase conferring resistance to penicillins, first-, second-generation cephalosporins and to ceftazidime. However, GES-1 does not hydrolyze aztreonam, cephamycins or carbapenems. Until now, 17 GES variants differing by 1–3 amino acid residues and with differences in the substrate spectra have been reported. A carbapenemase activity has been demonstrated only in some variants, including GES-5, with a substitution at the Gly170 residue. Here, we investigated the biochemical and structural characteristics of a new GES-18 variant.

**Methods:** Susceptibility profile was determined by disc diffusion and microbroth dilution MIC method according to CLSI guidelines. GES-18 was identified by PCR-sequencing. GES-18 was purified from *Escherichia coli* BL21 (DE3) (pET28a/blaGES-18) by ion-exchange and gel filtration chromatography. Steady-state kinetic parameters for the hydrolysis of beta-lactam antibiotics were determined by measuring spectrophotometrically the initial reaction rates. Inhibition by tazobactam and clavulanic acid was investigated using nitrocefin as reporter substrate. Crystallographic study was also performed.

**Results:** blaGES-18 is an integron-borne allele found in a multidrug-resistant *Pseudomonas aeruginosa* clinical isolate. GES-18 has an additional Val80Ile change compared to GES-5. Compared to GES-1, GES-18 shows an increased activity against carbapenems and cephamycins but presents a reduced activity against ceftazidime and cefotaxime. Aztreonam is a poor substrate of GES-18 with very low *k<sub>cat</sub>* and relatively high *K<sub>m</sub>* values. GES-18 was less susceptible than GES-1 to inhibition by tazobactam and clavulanic acid. The crystal structure of GES-18 was solved by molecular replacement using the structure of GES-1 (PDB code 2QPN) as model.

**Conclusion:** GES-18 is a new GES variant which displays a carbapenemase activity very similar to that of GES-5. We obtained the first crystallographic structure of a Ser170-possessing GES-type carbapenemase.

#### P1242 Identifying *terIS* of ISCR1 and new ISCR elements

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**Objectives:** ISCR elements are responsible for capturing and mobilising certain antibiotic resistance genes. They are unusual insertion sequences, related to the IS91 family, that have *oriIS* and *terIS* motifs at their outer ends and move by rolling circle replication catalysed by the Rcr protein encoded within the element. Continuation of replication beyond the *terIS* end allows capture of adjacent DNA segments. ISCR1 is always found adjacent to the same position of the 3'-conserved segment of class 1 integrons, possibly explained by a deletion encompassing part of an ancestral ISCR element and part of the 3'-CS. However, at 2154 bp ISCR1 appears longer than related elements and could contain captured segment(s) adjacent to *terIS*. Here, searches with the Rcr1 protein of ISCR1 were used to identify related elements to try and shed light on this. Searches with the sequences of selected ISCR and Rcr proteins were also carried out to identify new ISCR-like elements.

**Methods:** The sequences of selected, known ISCR1 elements and their Rcr proteins were used in BLASTn, BLASTp and tBLASTn searches (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). The nucleotide sequences of these putative elements and/or regions containing putative *rcr* genes were analysed to try and identify element boundaries.

**Results:** Searches identified a protein 87% identical to Rcr1 from ISCR1 in pHH1107, from an uncultured bacterium isolated from manure in Germany. An alignment of the nucleotide sequences revealed 79% identity over 1811 bp and short inverted repeats, a characteristic of *terIS* of IS91-like elements, were identified near to the end of this match in ISCR1. An element encoding a protein 84% identical to Rcr20 in a region 85% identical to ISCR20 is associated with a cat-like gene and inserted in a class 1 integron. Part of the same region is also present upstream of the *armA* gene in the composite transposon Tn1548, found on a number of plasmids, and upstream of the *sul3* gene found beyond the cassette array in some class 1 integrons.

**Conclusions:** Identification of elements related to the region currently defined as ISCR1 appears to support the hypothesis that this element may contain an ancestral ISCR plus additional segments adjacent to *terIS*. Identification of fragments of a previously unrecognised ISCR-like element adjacent to known antibiotic resistance genes suggests they may have had a role in the original capture of these genes.

#### P1243 Discovery of a new 16S rRNA methylase, RmtF, conferring high-level aminoglycoside resistance in human Enterobacteriaceae isolates from India

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**Objectives:** 16S rRNA methyltransferases (ArmA, RmtA-E, NpmA) have been described in recent years as an emerging mechanism conferring high-level resistance to clinically relevant aminoglycosides. These genes are usually borne by mobile genetic elements and they have been associated with several important resistance mechanisms such as Qnr, ESBLs or more recently the newly discovered carbapenemase NDM-1. A total of 35 Enterobacteriaceae strains (three *Citrobacter freundii*, three *Enterobacter cloacae*, one *Enterobacter aerogenes*, 11 *Escherichia coli* and 17 *Klebsiella pneumoniae*) isolated from human specimens from an Indian hospital were analysed for their high level resistance (MIC  $\geq$  200 mg/L) to gentamicin and amikacin.

**Methods:** PCR screening for the known methylase genes was performed on the 35 isolates. Cloning experiments and subsequent sequencing were performed using plasmid extractions from some of the strains negative for the all the known methylase genes. NDM-1 gene was sought by PCR in strains highly resistant to carbapenems. Plasmids with the new methylase gene were classified according to a PCR-based replication typing and pMLST, as well as by determination of their size by S1-PFGE technique.

**Results:** All the 35 Enterobacteriaceae isolates carry a new 16S methylase gene, named *rmtF*, which is able to confer by itself high-level resistance (MIC  $\geq$  200 mg/L) to the 4,6-disubstituted aminoglycosides (such as gentamicin, amikacin or tobramycin) and shared the highest amino acid identity (46%) with RmtD. In some cases, this new methylase has been found in the same strain (but not on the same plasmid) with another 16S rRNA methylase: ArmA (29% of the strains), RmtB (6%) or RmtC (9%). NDM-1 has been detected in 57% of the strains. Replicon typing revealed that in many strains *rmtF* is borne by an IncN plasmid of approximately 400 kb in size, whereas in others the plasmid is non-typable.

**Conclusions:** This study reports the discovery of a new 16S rRNA methylase, RmtF, which confers high-level resistance to most clinically relevant aminoglycosides. This resistance determinant seems to be spreading quickly among enterobacteria associated with other emerging resistance mechanisms, including NDM-1 carbapenemase. A detailed characterization must be done in order to elucidate the basis of this new resistance determinant. Further surveillance of RmtF among human, animal and food isolates will be needed to track its path worldwide.

#### P1244 Co-linkage of novel extended spectrum beta-lactamase VEB-5 and 16S rRNA methyltransferase ArmA in *Salmonella enterica* from the United Kingdom

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**Objectives:** Methylation of the aminoacyl site of bacterial 16S rRNA confers high-level resistance to clinically important aminoglycosides. Seven 16S rRNA methyltransferase genes, *armA*, *rmtA*, *rmtB*, *rmtC*, *rmtD*, *rmtE* and *npmA*, have been identified to date. The aims of this study were to investigate the occurrence of 16S rRNA methyltransferases in *Salmonella enterica* isolates selected from the Health Protection Agency (HPA) Laboratory of Gastrointestinal Pathogens culture collection expressing high-level resistance to aminoglycosides.



**Methods:** One *S. enterica* serovar Thompson and three *S. enterica* serovar Worthington isolates were selected based on ability to grow on 500 mg/L of amikacin. PCR screening of the four isolates for the known methylase genes was performed. A series of multiplex PCRs was used to screen for the presence of genes encoding TEM, SHV, OXA 1/4/30/48, CTX-M-1,3,9,8/25, ACC, FOX, MOX, DHA, CIT, EBC, GES, PER, VEB, IMP, VIM and KPC beta-lactamases. Conjugation was attempted in broth culture and transconjugants were selected on Brain Heart Infusion agar plates containing nalidixic acid (50 mg/L) and gentamicin (50 mg/L). Plasmid profiles of the wild-type strains and the transconjugants was analysed by S1-PFGE method. PCR mapping for Tn1548 was performed using plasmid extractions as templates.

**Results:** armA was identified in the four isolates. Multiplex PCRs for the presence of the main beta-lactam resistance genes resulted in identification of blaVEB and blaCMY in *S. Worthington*. The nucleotide sequence of the blaVEB gene shared 100% sequence identity with blaVEB-5 originally identified in *Escherichia coli* in the United States. Furthermore, blaVEB-5 alone was confirmed to confer high-level resistance to aztreonam. The CMY gene was identified as blaCMY-2. Conjugation assays showed the association of armA, blaVEB-5 and blaCMY-2 on the same plasmid in *S. Worthington*. A genetic structure related to Tn1548 was found in both *S. Thompson* and *S. Worthington* to be the mobile element responsible for armA spread.

**Conclusion:** Here we describe for the first time the co-linkage of armA with a VEB beta-lactamase. This is also the first report of blaVEB-5 in *Salmonella enterica*. These findings are relevant due to the combined presence of resistance to aminoglycosides and monobactams. Ongoing surveillance of these genes in bacteria will help to slow down resistance to these clinically relevant antibiotics.

#### P1245 Characterisation of two new variants of 16S rRNA methylase encoding genes, rmtB2 and rmtB3

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**Objectives:** To characterize two G1405 16S rRNA methylase encoding genes showing 3–4 amino acid changes compared to rmtB from Enterobacteriaceae isolates collected in 2005 and 2006.

**Methods:** Genes encoding RmtB-like were sequenced on both strands. Clinical strains carrying these genes were analyzed. Primers comprising the open reading frame of the rmtB-like genes were used to amplify the entire gene and amplicons were cloned into PCRScript/XL1 Blue *E. coli* kanR. *E. coli* DH5alpha was used as a secondary host and transformation plated onto selective media containing 30 mg/L of chloramphenicol. Plasmid preparations of clinical strains were transformed into *E. coli* DH5alpha by electroporation and selected in media containing 4 mg/L of kanamycin. Susceptibility testing was performed according to CLSI reference broth microdilution methods using extended MIC dilution ranges for amikacin, tobramycin, gentamicin, arbekacin, apramycin, kanamycin, neomycin and streptomycin.

**Results:** rmtB was sequenced in nine strains initially positive by PCR using primers targeting this gene. Six strains carried variants of the rmtB gene: rmtB2 showing three amino acid changes A41T, I124V and I132V and rmtB3 showing one additional alteration at position 82 (A–V). rmtB2 was detected in three isolates from Mexico (two *E. cloacae* strains; two hospitals) and one *E. coli* from Brazil. rmtB3 was detected among three strains from USA (Texas; *E. coli*) and Mexico (one *E. cloacae* and one *K. pneumoniae*). Susceptibility testing demonstrated that isolates carrying rmtB, rmtB2 and rmtB3 had elevated MIC values for amikacin (32–256 mg/L), tobramycin (16–64 mg/L), gentamicin (4–64 mg/L), arbekacin (16–64 mg/L) and kanamycin (64–256 mg/L) when compared to the *E. coli* host carrying PCRScript plasmid without insert. RmtB-variant produced MIC values for apramycin, neomycin and streptomycin modestly higher (4–8 mg/L) when compared to rmtB (0.25–2 mg/L) expressed in the same genetic background. Plasmids from three of the six clinical strains were transferred to *E. coli* and

MICs were elevated for aminoglycosides (8–256-fold) that are susceptible to G1405 methylation.

**Conclusions:** Two G1405 16S rRNA methylase genes similar to rmtB were detected among several Enterobacteriaceae isolates collected during 2005–2006 from different countries in Latin and North America, suggesting that these variants could be widespread in this geographic region.

Epidemiology and molecular antimicrobial resistance of *Acinetobacter* sp.

#### P1246 Carbapenem and fluoroquinolone resistance in *Acinetobacter radioresistens*

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**Objectives:** *Acinetobacter radioresistens* is increasingly found in hospitalised patients where it may be cause of catheter-related bloodstream infections. Although in possession of an intrinsic blaOXA-23, *A. radioresistens* is usually carbapenem-susceptible. However, blaOXA-23 is the commonest acquired carbapenem-resistance determinant in *Acinetobacter baumannii* and is associated with insertion elements (IS) that provide strong promoters leading to overexpression. We recovered two *A. radioresistens* isolates from a patient 17 days apart. Ciprofloxacin (CIP) therapy was administered over this time. Both isolates were carbapenem-resistant. The pre-CIP therapy isolate was fluoroquinolone- (FQ) susceptible and the post therapy isolate was FQ-resistant (Table 1). We investigated the epidemiology and mechanisms of carbapenem- and FQ-resistance in these isolates.

**Methods:** Strain identity was confirmed using rep-PCR (DiversiLab). Carbapenem and FQ MICs were determined by E-test. Carbapenem-resistance was investigated by sequencing blaOXA-23 and its genetic environment. To investigate expression of blaOXA-23, qRT-PCR was performed and compared to the carbapenem-susceptible *A. radioresistens* SH164 isolate. The blaOXA-23 environment including the novel ISAcra1 (see Results) was cloned into shuttle plasmid pWH1266 and transformed into carbapenem-susceptible *A. baumannii* ATCC 17978. FQ-resistance was investigated by sequencing gyrA and parC.

**Results:** The isolates were found to be identical by rep-PCR. A novel IS element was found upstream of blaOXA-23 and was termed ISAcra1 by the IS Database (<http://www-is.biotoul.fr/>). qRT-PCR showed that blaOXA-23 was overexpressed in both isolates compared to control strain SH164. *A. baumannii* ATCC 17978 transformed with ISAcra1-blaOXA-23 had imipenem and meropenem MICs of >32 mg/L (Table 1). Sequencing of gyrA revealed a mutation in the post-CIP therapy isolate, leading to a Ser83-Phe substitution, and was associated with FQ resistance (Table 1). No changes were found in parC.

Table showing carbapenem and fluoroquinolone MICs in *A. radioresistens* strains 1 and 2 and the amino acid substitution in GyrA. Carbapenem MICs of *A. baumannii* ATCC 17978 and *M. luteus* transformant are shown.

Strain	MIC (mg/L)				GyrA
	IPM	MEM	CIP	MOX	
<i>A. radioresistens</i> 1	>32	>32	0.25	<0.094	Ser-83
<i>A. radioresistens</i> 2	>32	>32	>32	2	Phe-83
<i>A. baumannii</i> ATCC 17978	0.25	0.25	at	at	at
<i>A. baumannii</i> ATCC 17978 (pOXA-23)	>32	>32	at	at	at

IPM, imipenem; MEM, meropenem; CIP, ciprofloxacin; MOX, moxifloxacin; at, not tested

**Conclusion:** This study shows that carbapenem-resistance was mediated through overexpression of the intrinsic blaOXA-23 and was associated with the novel ISAcra1. FQ resistance developed during CIP-therapy and was associated with a gyrA mutation. These data highlight the ability of *A. radioresistens* to develop fluoroquinolone resistance during therapy. ISAcra1 has the potential to spread OXA-23 mediated carbapenem-resistance in *A. radioresistens* and *A. baumannii*.

**P1247** Epidemiology of multidrug-resistant *Acinetobacter baumannii*/hemolytic complex in the largest Russian cancer hospital

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Multidrug-resistant (MDR) *A. baumannii* is widely spread in nature and in the hospital environment and often causes a variety of difficult-to-treat hospital infections.

**Objective:** To identify clones of *A. baumannii* in the hospital environment and to trace the relationship with the nosocomial infections in cancer patients.

**Methods:** Between March and September 2011, 123 strains of *A. baumannii*/hemolytic complex (ABHC) were isolated, including 101 strains from 54 cancer patients (48% from bronchoscopic swabs, 41% from wounds, 11% from other biomaterials [67% of all strains were from ICU patients]) and 26 strains from hospital environment objects. Identification, antimicrobial susceptibility testing and biotypes were determined with automated system MicroScan (WalkAway, Siemens) with 8-digit level of expertise.

**Results:** Seventeen different biotypes of ABHC were revealed in patients. Five biotypes were presented as “wild” strains and 12 biotypes – as MDR strains. Fifteen biotypes occurred once or twice and two biotypes occurred frequently: 57 of 101 (56%) strains had biotype 00062730 and 26 of 101 (26%) strains had biotype 00062720. According to identification code, the two biotypes differed only in susceptibility to tobramycin 4 mg/mL concentration.

Environmental strains of ABHC (22 strains) were presented with nine biotypes. Two biotypes (one strain each) were “wild” strains and seven biotypes (20 strains) were MDR. The most frequent biotypes were 00062720 (eight strains) and 00062730 (seven strains), the same as biotypes most frequently encountered in patients.

Thus total 34 strains of 00062720 biotype and 64 strains of 00062730 biotype of ABHC with different antimicrobial susceptibility were isolated. 00062720 biotype was more sensitive to antibiotics than 00062730 biotype. Proportion of susceptible strains to cefepime was 50% vs. 0%, ceftazidime – 15% vs. 0%, gentamicin – 53% vs. 0%, levofloxacin – 94% vs. 48%, moxifloxacin 88% vs. 51%, tetracycline 100% vs. 23%, tobramycin – 100% vs. 0%, trimethoprim/sulfamethoxazole – 94% vs. 0%, ampicillin/sulbactam – 0% vs. 8%, respectively. All strains of both biotypes were susceptible to colistin ( $p < 0.05$ –0.0001).

**Conclusion:** Two biotypes of MDR *A. baumannii*/hemolytic complex (00062730 and 00062720) are nosocomial and are identified from the objects of hospital environment and cancer patients with infectious complications. Interruption of transmission is the main task and will be an effective method for preventing nosocomial infections.

**P1248** Diversity in *Acinetobacter baumannii* isolates from paediatric cancer patients in Egypt

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**Objectives:** Cancer patients are at a higher risk from serious infections because they are immuno-compromised and many causative organisms are multi-drug resistant. In this study we report the genetic and epidemiological diversity of *Acinetobacter baumannii* isolated from paediatric cancer patients in Egypt, an emerging problem in cancer centres.

**Materials and methods:** Thirty-four *Acinetobacter baumannii* strains were collected from the Children’s Cancer Hospital (CCH) 57357 and the National Cancer Institute (NCI) in Cairo from March 2010–June 2011. They were initially identified phenotypically and then genotypically by PCR amplification and sequencing of blaOXA-51-like, and restriction analysis of 16s–23s rRNA spacer sequences using AluI and NdeII. Minimum inhibitory concentrations (MIC) of imipenem and meropenem was performed and interpreted according to BSAC guidelines. Isolates were also screened for the presence of

class D carbapenamases: blaOXA-23, blaOXA-24 and blaOXA-58 by PCR amplification and sequencing.

**Results:** Sequencing of blaOXA-51-like gene revealed a large diversity among the strains with eight different genes identified: blaOXA-64, blaOXA-65, blaOXA-66, blaOXA-69, blaOXA-71, blaOXA-78, blaOXA-94 and blaOXA-100. This large diversity showed the presence of the three major sequence groups (blaOXA-66, blaOXA-69, and blaOXA-71) in addition to other unrelated clones. Overall carbapenem resistance was 47% in all isolates (MIC  $\geq$  8 mg/L), with resistance to meropenem being slightly higher than Imipenem in most strains. All three class D carbapenamases were detected in the isolates, with blaOXA-23 being most common (18 isolates), whereas seven isolates harboured the blaOXA-58 and two isolates had blaOXA-40. Seven blaOXA-23 isolates had ISAbal inserted upstream and this correlated with higher resistance to carbapenems. Two isolates had the unusual combination of both blaOXA-23 and blaOXA-58.

**Conclusion:** The two Egyptian hospitals are tertiary referral centres and these results show that the *A. baumannii* isolated from their patients had diverse origins although they included the three major European clones. The data strongly suggest that many of the strains have been brought to the hospital by the patients themselves rather than acquired by the more usual method of transmission through patient-to-patient cross infection. The results also show how these patients can serve as reservoirs for the survival and eventual dissemination of *A. baumannii*.

**P1249** Emergence of multi-resistant *Acinetobacter baumannii* among Libyan military personnel during the recent Libyan conflict

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Multidrug-resistant (MDR) *Acinetobacter* infections are occurring at alarming rates in traumatic war injuries. The recent Libyan crises have resulted in heavy war wound infection caused by highly-resistant pathogens that Libyan hospitals are not familiar with; among them MDR *Acinetobacter baumannii*. The objectives of this study were to determine (i) Epidemiological manifestations of *A. baumannii* among injured military personnel admitted to Tripoli Medical Centre (ii) Antimicrobial resistance patterns of this pathogen (iii) Phenotypes and molecular characteristics of such pathogen.

**Methods:** A total of 498 patients with wounds classified as war wound associated infections (WWAI) showed positive bacterial cultures isolated from the swabs or the wound debridement were studied. Off these 144 culture showed *A. baumannii* according the standard microbiological identification methods antimicrobial susceptibility tests were carried out using, ceftazidime, gentamicin, Amikacin, ciprofloxacin and Imipenem. Pulsed-field gel electrophoresis (PFGE) analysis were carried to determine clonality.

**Results:** *A. baumannii* infection were more likely to be associated with gunshot wounds (70%), external fixators (55%), Blast (30%), blunt (20%), fragmentation (15%). Antibiotic resistance rates vary according to the antibiotic used, ceftazidime (90%), gentamicin (95%), amikacin (85%), ciprofloxacin (95%) and Imipenem (45%). PFGE analysis showed four different heterogeneous patterns with no occupational transmission was proofed.

**Conclusion:** *A. baumannii* was found to be an emerging problem among Libyan patients particularly those associated with Trauma. This is an alarming issue as preventive measures and specific guide lines should be implemented both for infection control and empiric therapy for suspected infected patients with *A. baumannii*

**P1250** Epidemiology of *Acinetobacter* species infection in a gastroenterology and liver transplant ICU in a multi speciality hospital in India

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**Introduction:** Infections influence the outcome of the critically ill patients and are more common in immunosuppressed individuals. Multi

drug resistant *Acinetobacter* species are emerging as one of the most dreaded bacteria and leads to increase in mortality.

**Hypothesis:** Microbiological data of 759 patients admitted from June 2010 to May 2011 in a Gastroenterology and Liver transplant ICU was analysed.

**Methods:** Results of all the culture samples were retrieved. Blood, urine, Endotracheal/Tracheal secretion and body fluid cultures which are positive for *Acinetobacter* species were analysed. Antibiotic sensitivity pattern were also documented.

**Results:** Fifty eight samples from forty two patients were positive for *Acinetobacter* species.

Thirty two samples (55.2%) were positive from respiratory secretions with radiological confirmation of consolidation, fifteen samples (25.8%) were positive from body fluids (Ascitic, Pleural fluid, Pus) and eleven blood culture samples (18.9%) were detected positive for *Acinetobacter* species. Out of 42, 12 patients had positive cultures from two sites, two patients from three sites and rest 28 patients had culture positivity from single site. Out of 58 *Acinetobacter* isolates, 54 (93.1%) were identified as of *A. baumannii*, two (3.4%) as of *A. hemolyticus* and one (1.7%) each as of *A. lwoffii* and *A. junii*. All these *Acinetobacter* isolates were showing high resistant to carbapenems, cephalosporins, aminoglycosides and fluoroquinolones. All the isolates (100%) were sensitive to Colistimethate sodium with MIC < 0.5. Tigecycline was sensitive in 53 isolates (91.0%) and MIC range was <0.5–4. Out of 58 only eight isolates (13.7%) were sensitive to carbapenams, six (10%) to cefepime-sulbactam combination and aminoglycosides, and three (5%) to fluoroquinolones. Twenty-one (48.8%) out of 43 culture positive patients died within 28 days of detection of positive cultures. Incidence of *Acinetobacter* species infection is 5.6% in our ICU.

**Conclusions:** Despite a reputation of being relatively low virulence *Acinetobacter* infections pose a formidable threat especially to compromised patients. *A. baumannii* is a highly resistant pathogen and carries a high mortality. In our ICU Colistimethate sodium shows excellent sensitivity followed by Tigecycline.

#### **P1251** Surveillance of *Acinetobacter baumannii* isolates resistant to carbapenems during 10-year period (2001–2010) in Aveiro, Portugal

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**Objectives:** *Acinetobacter* spp. (Aba) is an important nosocomial pathogen worldwide and exhibits high rates of acquired resistance to many antimicrobial agents. It is also responsible for a high number of reported outbreaks worldwide. The aim of this work was to evaluate the temporal evolution of antimicrobial resistance among *Acinetobacter* spp. isolated in a central region of Portugal (Hospital Infante D. Pedro, Aveiro).

**Methods:** Consecutive non-duplicate bacterial pathogens were collected in the last 10 years from hospitalised patients in the Hospital Infante D. Pedro. Sites of isolation included: Skin and soft tissue, bloodstream, urinary tract and lower respiratory tract. The isolates were identified by the automatic VITEK 2 system and Advanced Expert System (VITEK 2 AES) (BioMérieux, Marcy L'Étoile, France). The antimicrobial susceptibility profile to >20 antimicrobial agents was evaluated by CLSI broth microdilution methods and interpreted by CLSI M100-S20 (2010).

**Results:** A total of 440 isolates were collected during the 10 years period. The highest number of isolates was collected in 2004 (11.6%), 2006 (12.5%), 2009 (13.6%), 2010 (28%). Most of the isolates were collected from respiratory tract followed by urinary tract and bloodstream infections. From November 2009 to February 2010, 36 Aba isolates were isolated from samples collected from patients hospitalized in the ICU, causing an outbreak. This Aba clone was endemic in 2006, and had also produced an outbreak in 2007, in the same ward.

**Conclusions:** Polymyxin B remains the most active drug against *Acinetobacter* spp. in the isolates collected in our hospital and

tigecycline was also active. An important reduction in the susceptibility rates to some broad-spectrum antimicrobials was observed, limiting therapeutic options available. Proper control of this multidrug-resistant Aba will require a multidisciplinary approach, including identification of resistance mechanisms, optimizing antibiotic use based on local epidemiology and vigorous implementation of infection control procedures.

#### **P1252** Use of DiversiLab repetitive-sequence-based PCR for epidemiologic analysis of *A. baumannii* from Australia and Asia

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**Objectives:** A commercial repetitive-sequence-based PCR (rep-PCR) technique (DiversiLab system, bioMérieux, Marcy l'Etoile, France) was used to assess the molecular epidemiology of *A. baumannii* from Australia and Asia. The aim of this study was to assess whether DiversiLab was a reliable diagnostic tool for clonal analysis.

**Methods:** A total of 140 non-repetitive *A. baumannii* isolates (46 from Australia, 54 from Thailand, 25 from Singapore, nine from Malaysia and six from Japan) were analysed by the rep-PCR method using the DiversiLab system. Representative isolates determined by DiversiLab patterns from the five countries were also analysed by multilocus sequence typing (MLST).

**Results:** *A. baumannii* clonal complex (CC) 92 was the predominant clone in all five countries. The DiversiLab patterns of CC92 were relatively specific for each country. *A. baumannii* CC92 was the only clone present amongst carbapenem resistant *A. baumannii* from Thailand (similarities  $\geq 95\%$ ). Two isolates from Thailand had only 90% similarities to the major clone, one belonged to a novel ST and another isolate belonged to ST195. *A. baumannii* from Queensland, Australia, were also predominantly CC92 (78%); and all except one were carbapenem resistant. In contrast, the isolates from Western Australia were diverse and with variability in carbapenem resistance. ST73, 125, 126, 127, 128 and ST129 were non-CC92 of Queensland *A. baumannii*. ST130 belonged to CC92; however, it was susceptible to carbapenems. All but one *A. baumannii* from Japan were closely related by  $\geq 95\%$  and clustered together. Interestingly, the DiversiLab patterns of CC92 from Malaysia were closely related to Thailand *A. baumannii*; whereas, the CC92 from Singapore were closely related to Australian *A. baumannii*.

**Conclusion:** The rep-PCR assay with the DiversiLab system used for *A. baumannii* proved to be a rapid and reliable method for molecular analysis of nosocomial outbreaks as well as for epidemiologic analysis for routine purpose. The rep-PCR assay could also differentiate the country of origin of *A. baumannii* based on their typical DiversiLab patterns. It is likely that evolution of common global clonal clusters has occurred in each country.

#### **P1253** Comparison of carbapenem-resistant *Acinetobacter baumannii* isolates from various body sites of colonised patients

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**Objectives:** There is no standard surveillance culture method for carbapenem-resistant *Acinetobacter baumannii* (*A. baumannii*). Before selection of culture site and method for surveillance culture, it should be confirmed that the strains obtained from all the body sites are identical. Therefore, we took cultures from multiple body sites of patients with carbapenem-resistant *A. baumannii* colonization and performed pulsed-field gel electrophoresis (PFGE).

**Methods:** From 1 September to 14 September 2011, five patients colonized with carbapenem-resistant *A. baumannii* in intensive care unit (ICU) were included. The strains from their sputum cultures were regarded as results of colonization because there was no clinical or

radiological evidences of pneumonia. We took cultures from six different body sites for each patient, which were buccal mucosa, nostril, axilla, upper arm, groin, thigh and rectum. All the specimens were enriched in nutrient broth in 37°C for 1 hour. Next, 100 µL of the broth was taken from each specimen and inoculated onto CHROMagar™ *Acinetobacter* (CHROMagar, Paris, France). The plates were then incubated at 37°C for 24 hours before reading. Antimicrobial susceptibility testing was performed by disk diffusion and interpreted in accordance with the Clinical and Laboratory Standards Institute guidelines. PFGE was performed on all the strains obtained from sputum and seven body sites.

**Results:** Carbapenem-resistant *A. baumannii* was found in specimens from following sites: buccal mucosa, three specimens, nostril, 5, axilla, 2, upper arm, 5, thigh, 1, and rectum, 2. However, it was not obtained from the groin specimens. For four patients, all the strains in each patient were identical and for one patient, one isolate were classified as closely related with the others (one band difference). The PFGE data are summarized in Figure 1.

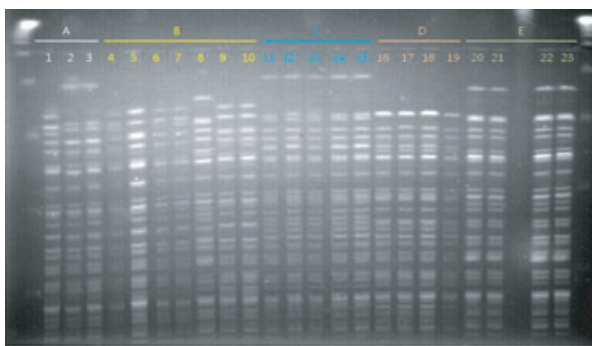


Figure 1. Pulsed-field gel electrophoresis of carbapenem-resistant *Acinetobacter baumannii* strains from various body sites. Capital letters indicate case patients and numbers indicate strains obtained from each site of body.  
A-1: sputum, A-2: nostril, A-3: upper arm.  
B-4: sputum, B-5: nostril, B-6: buccal mucosa, B-7: axilla, B-8: rectum, B-9: upper arm, B-10: thigh.  
C-11: sputum, C-12: nostril, C-13: buccal mucosa, C-14: axilla, C-15: upper arm.  
D-16: sputum, D-17: nostril, D-18: buccal mucosa, D-19: upper arm.  
E-20: sputum, E-21: nostril, E-22: rectum, E-23: upper arm.

**Conclusion:** Our results showed that *A. baumannii* isolated from any sites of body in carbapenem-resistant *A. baumannii* colonized patients were identical. Based on this result, we can select candidate site for surveillance culture by higher culture sensitivity. Larger studies would be needed to determine most pertinent surveillance culture site.

#### P1254 Preceding colonisation of carbapenem-resistant *Acinetobacter baumannii* in respiratory tract of sepsis patients

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**Objectives:** We compared the genetic relations of carbapenem-resistant *Acinetobacter baumannii* (*A. baumannii*) isolated from respiratory specimen and blood to determine whether colonization on respiratory tract precedes bacteremia.

**Methods:** We reviewed 141 carbapenem-resistant *A. baumannii* bacteremia patients who had prior respiratory cultures (e.g., sputum, transtendotracheal aspirates, bronchial washing and bronchoalveolar lavage) with the same organism from 1 January 2009 to 31 November 2010, at Severance hospital. Among them, we excluded cases with blood and respiratory culture in the same day or cases with pneumonia. Finally 32 cases which had carbapenem-resistant *A. baumannii* respiratory tract colonization prior to bacteremia were included. We reviewed their medical records and laboratory findings. Pulsed-field gel electrophoresis (PFGE) was performed on the blood isolates and corresponding respiratory ones.

**Results:** The average interval between the first isolation from respiratory specimens and blood culture with carbapenem-resistant *A. baumannii* was 11.5 days (Range: 1–51 days). Twenty-four of 32 patients had identical PFGE band pattern between isolates from blood

and respiratory specimens. Carbapenem-resistant *A. baumannii* strains from six patients were determined as closely related. The strains from two patients showed different PFGE pattern. The PFGE data are summarized in Figure 1.

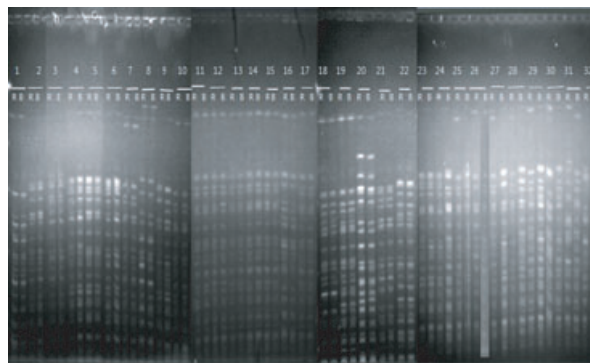


Figure 1. Pulsed-field gel electrophoresis of carbapenem-resistant *Acinetobacter baumannii*. Numbers indicate case patients. Each case patient has 2 PFGE results, R and B. "R" is a PFGE result from respiratory specimen and "B" is from blood. Twenty six patients (except 3,25,26,27,28,29,30 and 31) had identical PFGE band pattern between R and B. PFGE patterns of R and B are similar in 9,25,26,27,28 and 29 and different in 30 and 31.

**Conclusion:** This study suggests that colonization of carbapenem-resistant *A. baumannii* in respiratory tract may precede bacteremia. Further investigation about the frequency of colonization prior to infection, the most common site of colonization and the outcome benefits related to early detection would be needed to make more efficient carbapenem-resistant *A. baumannii* infection control strategy.

#### P1255 Identification of a clinical isolate of *Acinetobacter* genomic species 10 with an integron-borne blaVIM-2 gene flanked by miniature inverted-repeat transposable elements

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**Objective:** Reports on carbapenem-resistance in species other than *Acinetobacter baumannii* are rare. As part of our ongoing study of integrons, we have examined a Portuguese collection of *Acinetobacter* spp. isolates. We found a *A. junii* (Api20NE) strain with reduced susceptibility to imipenem. We report the molecular identification and characterization of the mechanism underlying this unusual phenotype in a rarely found clinical species, describing also the genetic basis and flanking regions of an integron.

**Methods:** The clinical strain 118FFC was isolated in March 1998 from the blood of an inpatient of a Surgery ward of the University Hospital of Coimbra. Re-identification was performed by ARDRA and AFLP. Antimicrobial susceptibility was determined by the disc diffusion and by E-test. Results were interpreted according to the CLSI guidelines. The beta-lactamase activity of bacterial extracts was determined by spectrophotometry, using imipenem and nitrocephin as substrates in the presence and absence of EDTA and zinc. Metallo-beta-lactamase production was detected by a microbiological method with EDTA. Screening for class 1 integron and Miniature Inverted-Repeat Transposable Elements (MITEs) were done by PCR, followed by sequencing of amplicons.

**Results:** Strain 118FFC was identified as *Acinetobacter* genomic species 10 (proposed *A. berezinae*). It showed resistance to ampicillin, cefotaxime, ceftazidime, sulphonamides, netilmicin and tobramycin, and reduced susceptibility to imipenem (MIC = 8 mg/L). Beta-lactamase activity and phenotypic test suggested the production of a metallo-beta-lactamase. PCR and sequencing revealed the presence of a class 1 integron (2.2 kb) with the resistance genes cassette aacA7-blaVIM2-aacC1. MITE-like structures (439 bp) flanked the integron on both sides. These were 100% identical to the only two previously identified MITEs, found in *A. baumannii* 65FFC isolated from the same hospital, and in *A. johnsonii* recovered from a prawn in Australia.

**Discussion:** The results showed that an uncommon clinical species of *Acinetobacter* carries a carbapenemase gene, blaVIM-2, without showing resistance to carbapenems. There may be an underestimated prevalence of metallo-beta-lactamases in *Acinetobacter*. The reported resistance genes are embedded in an integron, and the newest reported MITE-like structures, which may facilitate their dissemination. This is the first report of an integron with this gene cassette array, and flanking MITE-like sequences.

**P1256** **Molecular epidemiology and mechanisms of carbapenem-resistant *Acinetobacter baumannii* in a Saudi Arabia hospital**

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**Objectives:** *Acinetobacter baumannii* (AB) may become highly resistant to carbapenems and almost all of the  $\beta$ -lactams by production of carbapenemases. This study was set up to investigate the molecular epidemiology of carbapenem-resistant *A. baumannii* isolates (CRAB) collected at the Prince Suleiman hospital in Riyadh (Saudi Arabia) from January through December 2010.

**Methods:** The resistance levels to antibiotics were determined in a collection of 27 CRAB by the E-Test method and interpreted according to the EUCAST breakpoints. Acquired beta-lactamases were identified using phenotypic (Double Disk Synergy Tests) and genetic (PCR amplification followed with DNA sequencing) methods according to standard protocols. The clonal relatedness of the strains was investigated by multilocus sequence typing (MLST) according to the Pasteur Institute scheme.

**Results:** The whole collection was highly resistant (MIC<sub>90</sub> > 256  $\mu$ g/mL) to ceftazidime (CAZ), cefepime, and piperacillin-tazobactam. Although all the strains were intermediate or resistant to meropenem (4 to >32  $\mu$ g/mL), two of them appeared susceptible to imipenem (2 to >32  $\mu$ g/mL). DDST using CAZ and clavulanic acid indicated production of a class A extended-spectrum beta-lactamase (ESBL) in 13 isolates contrasting with the detection of genes encoding enzymes PER-1 (n = 13), GES-1 (n = 6), GES-5 (n = 1), and GES-11 (n = 3) in 23 strains. Carbapenemases OXA-51, OXA-23 and OXA-40 were produced by 27, 16 and one isolates, respectively; the ISAbal element was systematically found upstream of the corresponding genes blaOXA-51, blaOXA-23, and blaOXA-40. Of note, 13/27 CRAB coproduced an ESBL and a carbapenemase. As shown by MLST analysis, the 27 strains distributed in eight sequence types (STs). Only 11 isolates belonged to the two most prevalent Clonal Complex in the world, CC1 (ST1, ST7) and CC2 (ST2). Five singleton STs were identified including ST15 and ST113 to ST116, which were new ST identified in this study.

**Conclusion:** The emergence of CRAB is becoming a major concern in Saudi Arabia. Unlike in many other countries, the spread of the OXA-23 enzyme in Saudi strains occurs across different STs.

**P1257** **Molecular analysis of *A. baumannii* isolated from invasive infections in 2009 in Poland**

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**Objectives:** To characterize *Acinetobacter baumannii* strains causing invasive infections in Polish hospitals.

**Methods:** Thirty clinical isolates of *A. baumannii* from bloodstream infections and meningitis were collected in 2009, during the national surveillance program. They were recovered in 16 hospitals in 13 towns all over Poland. Identification to the *A. calcoaceticus*-*A. baumannii* complex was done by API32GN tests or Vitek2 system (bioMérieux). Species identification was performed by sequencing the 16S-23S rRNA intergenic spacer. Susceptibility to expanded-spectrum cephalosporins and carbapenems was evaluated by disc-diffusion (according to EUCAST); the presence of ESBLs, MBLs and AmpC overexpression was assessed by

specific phenotypic tests. Typing was performed by PFGE and MLST. beta-Lactamases were profiled by isoelectric focusing; genes coding for OXA-23-, OXA-40-, OXA-51- and OXA-58-like CHDLs, PER-1 ESBL and TEM-1-like enzymes were identified by PCR and sequencing. Presence of the ISAbal element upstream of the blaOXA-51-like and blaADC-like natural beta-lactamase genes was analyzed by PCR.

**Results:** The majority of isolates (90%) belonged to two STs: ST2 (European clone II; n = 21) and ST1 (European clone I; n = 6); the remaining clones were ST5 and ST12. PFGE revealed 24 patterns, grouped into six types, and correlating well with STs. All but one of the isolates tested positive for the ADC cephalosporinase overexpression which correlated with the presence of ISAbal upstream of the blaADC genes. Among the 21 ST2 isolates, a diversity of beta-lactamase profiles was observed, with 14 isolates carrying blaPER-1 and 16 having blaTEM-1. Carbapenem non-susceptibility in these isolates correlated either with blaOXA-23- (n = 6), blaOXA-40- (n = 1) or blaOXA-58- (n = 1) like genes; in one OXA-23 producer ISAbal was inserted upstream of the blaOXA-51-like gene blaOXA-66. Among six ST1 isolates, the five carbapenem-non-susceptible isolates carried ISAbal integrated upstream of blaOXA-51-like genes (blaOXA-69) and had no acquired CHDL genes. No MBLs were observed.

**Conclusions:** This is the first detailed molecular analysis of a bigger collection of *A. baumannii* isolates in Poland, and one of the first in Central/Eastern Europe. Similar to observations from other countries, a high representation of the international clones was revealed, as well as the high heterogeneity of beta-lactamase-associated mechanisms of resistance to newer beta lactams.

**P1258** **A novel and hybrid composite transposon at the origin of acquisition of the blaCARB-14 gene in *Acinetobacter baumannii***

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**Background:** Carbenicillin-hydrolyzing beta-lactamases (also named CARB enzymes) are narrow-spectrum class A penicillinases sharing <50% amino acid identity with SHV and TEM. CARB enzymes are known in *Salmonella* sp. and *Pseudomonas* sp. and more rarely in *Acinetobacter* sp.

**Methods:** *A. baumannii* RAB was identified by using the API20NE system (bioMérieux) and confirmed by 16S rDNA gene sequencing and culture at 44°C. MICs were determined by using Etest strips. PCR approaches with standard conditions were used to search for blaTEM, blaSHV, blaCARB genes, blaAmpC, and blaOXA-51. Whole-cell DNA of *A. baumannii* RAB was used to perform shotgun cloning. To compare the hydrolytic spectrum of CARB-14 in comparison to narrow-spectrum CARB-8 and extended-spectrum CARB-10, the corresponding genes were cloned and expressed in an isogenic *E. coli* background under the control of the same promoter.

**Results:** *A. baumannii* RAB was recovered from urine in a 55-year-old patient hospitalized in a French University hospital in 2008. It was resistant to penicillins, cefotaxime, cefepime, of intermediate susceptibility to ceftazidime, and was susceptible to imipenem and piperacillin/tazobactam. It harboured a blaCARB-like gene encoding CARB-14 which is a novel variant exhibiting two amino acid substitutions compared to the closest CARB-5 enzyme. CARB-14 possessed a narrow spectrum hydrolysis profile. Attempts to transfer this resistance determinant by electroporation and by mating out assays failed, suggesting a chromosomal location of the blaCARB-14 gene. Sequence analysis of the region flanking the 5'-end of blaCARB-14 identified a novel insertion sequence ISAb21 belonging to the IS3 family. Downstream of blaCARB-14, another IS belonging to IS3 family named ISAb14 was identified, being in the same orientation as compared to ISAb21. Its inverted repeats shared significant homologies with those of ISAb21. Analysis of the left-end extremity of ISAb21 and the right-end extremity of ISAb14 identified direct repeat (DR) sequences of 3 bp, being the likely signature of a transposition process. Both IS were therefore forming a composite transposon named Tn2114 likely at the origin of the blaCARB gene acquisition.

**Conclusions:** We report here a very peculiar composite transposon made of different IS bracketing a gene coding for a novel carbencillinase, CARB-14. This transposon was made of one copy of ISAb21 and one of ISAb14 belonging to the same IS family.

**P1259** Conversion of OXA-66 into OXA-82 in clinical *Acinetobacter baumannii* isolates and association with altered carbapenem susceptibility

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**Objectives:** Carbapenem resistance in *Acinetobacter baumannii* is most frequently conferred by carbapenem-hydrolysing OXA enzymes. *A. baumannii* possess an intrinsic blaOXA-51-like, with 68 variants identified. Over-expression of blaOXA-51-like is associated with carbapenem resistance which is commonly mediated by an upstream located insertion element (ISAb1). Reduced expression of the porins CarO, OprD-like and 33–36 kDa Omp is also associated with carbapenem resistance. The aim of this study was to investigate altered carbapenem susceptibility in three clinical *A. baumannii* isolates which were part of an outbreak.

**Methods:** Three *A. baumannii* isolates were recovered from three separate patients (Table 1). Carbapenem susceptibility was determined by Etest. Molecular relatedness was investigated by rep-PCR (DiversiLab) and blaOXA-51-like typing. Presence of acquired OXAs was tested by multiplex PCR. Expression of blaOXA-51-like, carO, oprD-like and 33–36 kDa omp was investigated using qRT-PCR. rpoB was used as a housekeeping control gene.

**Results:** Results are summarised in the Table 1. Clinical isolates A, B and C were clonally related (>98% similarity) as assessed by rep-PCR. blaOXA-51-like typing confirmed affiliation to worldwide clonal lineage WW2. Isolates B and C showed reduced carbapenem susceptibility compared to susceptible isolate A (Table 1). Isolate C was resistant to both carbapenems, whereas isolate B was intermediate to imipenem but resistant to meropenem. Isolate A had OXA-66 while isolates B and C had OXA-82 (L167V) which was also associated with ISAb1. qRT-PCR revealed blaOXA-82 genes were >40-fold over-expressed compared to blaOXA-66. Comparison of porin expression revealed that isolates B and C had reduced expression of carO and oprD-like compared to isolate A (Table 1). Expression of 33–36 kDa omp was reduced in carbapenem-resistant isolate C and was increased in isolate B. Therefore the major difference between isolates B and C was expression of 33–36 kDa omp.

Table: phenotypical and genotypical characterisation of clinical isolates

Isolate	DOI	OXA-51 variant and presence of ISAb1	MIC [mg/L]		Difference in gene expression* [x-fold]			
			IPM	MEM	blaOXA-51-like	carO	oprD-like	33-36 kDa omp
A	30/01/09	OXA-66	4	2	1	1	1	1
B	15/09/10	ISAb1-OXA-82	8	16	16.5	0.79	0.61	2.05
C	09/09/10	ISAb1-OXA-82	>32	>32	46.5	0.22	0.23	0.14

\*compared to gene expression in isolate A; DOI, date of isolation; IPM, imipenem; MEM, meropenem

**Conclusions:** Decreased carbapenem susceptibility in two outbreak-related isolates was associated with conversion of OXA-66 into OXA-82 and its over-expression mediated by ISAb1. However, carbapenem resistance was only found in the blaOXA-82 over-expresser with reduced expression of the three outer-membrane proteins. Therefore carbapenem resistance was not solely associated with an enzymatic mechanism but with a combination of reduced permeability and over-expression of blaOXA-82.

**P1260** Investigation of repetitive PCR results and diversity of oxacillinases among invasive *Acinetobacter baumannii* isolates through 6 years (2004–2010) in a Turkish university hospital

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**Objectives:** *Acinetobacter baumannii* is a nosocomial pathogen which needs to be investigated by molecular epidemiologic tools for determining appropriate therapeutic regimen especially when multidrug resistance exists. The aim of the study was to evaluate molecular relationship of blood culture isolates of *A. baumannii* with repetitive PCR and search diversity of oxacillinases (OXA) through 2004–2010.

**Methods:** A total of 100 non-duplicate *A. baumannii* blood culture isolates diagnosed with conventional methods and BD Phoenix automated system between 2004 and 2010 in Hacettepe University, Ankara were evaluated. Of these, 87 *A. baumannii* isolates were analyzed using rep-PCR (DiversiLab, bioMerieux, FR). Antimicrobial susceptibility for piperacillin, amikacin, gentamicin, imipenem, meropenem, cefotaxime, ceftazidime, cefepim, ciprofloxacin and levofloxacin was determined by broth microdilution according to CLSI. Colistin, doripenem and tigecycline susceptibilities were performed by E-test. Presence of OXAs, OXA23-like, OXA24-like, OXA51-like and OXA58-like was investigated by PCR.

**Results:** Forty-four (44%) of the isolates were from intensive care units. Nineteen major rep-PCR clusters (A–T) were defined: A (11 isolates), B (nine isolates), C (eight isolates) and D (six isolates) being the most prevalent groups. Overall, 100% of the isolates were resistant to at least three classes of antibiotics. Colistin (98% susceptible) and tigecycline (94% susceptible) were the most susceptible antibiotics tested. Among 100 isolates, there was no significant difference between imipenem, meropenem (17%, both) and doripenem (18%) susceptibility. Carbapenem resistance was mostly associated to the presence of OXAs; OXA23-like (31%) and OXA58-like (23%). All the isolates harboured OXA51-like and none yielded OXA24-like. OXA23-like positive isolates had higher MIC90 levels, 64 mg/L for both imipenem, meropenem and 32 mg/L for doripenem. The occurrence of OXA58-like has increased through 2004–2009 (n:23) until 2010 (n:0), in contrast OXA23-like increased in 2008–2010 (n:31) period. Occurrence of OXA58-like and OXA23-like were more frequent among isolates of cluster A and D, respectively.

**Conclusion:** Analysis of cluster patterns and oxacillinases during 6 years demonstrated a high carbapenem resistance coherent with the replacement of isolates positive for OXA58-like to OXA23-like carbapenemase which highlights the monitoring of epidemic resistance control against *A. baumannii* invasive isolates.

**P1261** blaOXA-24/40-carrying plasmids have an important role in the dissemination process of carbapenem-resistant *Acinetobacter* spp.

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**Objectives:** The spread of OXA-24/40-producing *Acinetobacter* spp. has been strongly influenced by clonal expansion, but the role of horizontal gene transfer (HGT) has scarcely been explored. We studied the diversity of OXA-24/40 encoding plasmids and genetic environment of the blaOXA-24/40 genes among *Acinetobacter* spp. clinical isolates from two Portuguese hospitals.

**Methods:** blaOXA-24/40-carrying plasmids and genetic environments were characterized in representative *A. baumannii* (AB, n = 13) belonging to ST2/ST98 and *A. haemolyticus* (AH, n = 2) (2001–2007), selected from a collection of 157 carbapenem-resistant *Acinetobacter* spp. clinical isolates. AB were chosen on the basis of their ApaI-PFGE patterns (A, A1, A3 and A4). Electroporation was conducted using *A. baylyi* ADP1 as a recipient strain. blaOXA-24/40 genetic location was assessed by hybridization of I-CeuI and S1 gels with specific probes. Plasmid characterization was further accomplished by AB-PBRT, sequencing, and RFLP. The obtained sequences were compared with blaOXA-24/40-harbouring plasmids deposited in GenBank database, following a systematic in silico analysis.

**Results:** Transformants of two AB (PFGE A and A4) and one AH were resistant to all b-lactams except to ceftazidime, aztreonam and cefepime. Highly related 30 kb blaOXA-24/40-carrying plasmids (RFLP pattern A) identified in nine AB (PFGE A, A3 and A4) and

two AH, presented the replicase gene repA\_AB (GR12) and the sequences surrounding blaOXA-24/40 gene were identical to those of the blaOXA-24/40-carrying pAB02 plasmid. An OXA-24/40-encoding plasmid with 10 kb (RFLP pattern B) harbouring the replicase gene aci2 (GR2) was identified in five AB (PFGE A1, A3 and A4) and the blaOXA-24/40 genetic environment was closely related with that from pABVA01 and pMMC3 blaOXA-24/40 carrying plasmids. The two types of plasmids were observed in one AB (PFGE A4). Both blaOXA-24/40 and larger contiguous modules were flanked by XerC/XerD-like binding sites which might be responsible for their mobilization and identification of blaOXA-24/40 and/or common modules in either different plasmid types or in the chromosome.

**Conclusion:** The circulation of common genetic backbones and plasmids (30 kb-repA\_AB; 10 kb-aci2) harbouring blaOXA-24/40 among different AB PFGE-types and species of *Acinetobacter*, along with some previous evidences for HGT of blaOXA-24/40-carrying modules, strongly supports the role of particular platforms and plasmids in the dissemination process of OXA-24/40.

**P1262 Comparison of blaVIM, blaIMP, OXA-58, and OXA-23 real-time PCR results with various phenotypic methods in carbapenem-resistant *Acinetobacter baumannii* and *Pseudomonas* spp.**

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**Objectives:** The aim of this study was to determine the prevalence of carbapenemase production among *Acinetobacter baumannii* and *Pseudomonas* spp. with modified Hodge test, combined disk, and double disk synergy tests, and compare the results with real time PCR. **Methods:** The study was conducted at our hospital between November 2008 and May 2009. Non-repeat 100 carbapenem resistant *A. baumannii* and *Pseudomonas* spp. isolated from clinical specimens were included in the study.

Strains were tested for carbapenemase production with modified Hodge test, imipenem, imipenem-EDTA combined disk, and ceftazidime-2MPA double disk synergy tests. blaVIM, blaIMP, OXA-58, and OXA-23 genes were investigated with real time PCR.

**Results:** Eighty-two of all carbapenem resistant strains were also resistant to ceftazidime. Either one of the genes were detected in 95 of the strains. BlaOXA23 was the most common type of carbapenemases detected. The most sensitive phenotypic method was IMP-IMP + EDTA, on the other hand the most specific phenotypic method was CAZ-2MPA.

The overall results of MHT, IMP-EDTA, CAZ-2MPA disk tests, real time PCR results, and sensitivity, specificity, positive and negative predictive values of phenotypic methods are presented in Tables 1, 2 and 3.

Table 1. Results of MHT,IMP-EDTA,CAZ-2MPA disk tests.

	IMP-EDTA positive	IMP-EDTA negative	CAZ-2MPA Positive	CAZ-2MPA negative	MHT positive	MHT negative
<i>A.baumannii</i> (n:66)	-	100%	10.6%	89.4%	81.8%	18.2%
<i>P.aeruginosa</i> (n:33)	45.5%	54.5%	-	100%	9.1%	90.9%
<i>P.flourescens</i> (n:1)	100%	-	-	100%	-	100%

Table 2. Real time PCR results.

	IMP	OXA-23	OXA-58	OXA-23 IMP	OXA-23 OXA-58	OXA-58 IMP	OXA-23 VIM	OXA-23 IMP VIM
<i>A.baumannii</i>	-	40	1	4	18	-	1	1
<i>P.aeruginosa</i>	3	21	-	-	4	1	-	-
<i>P.flourescens</i>	-	1	-	-	-	-	-	-

Table 3. Sensitivity,speciicity,PPV,and NPV of phenotypic methods

	Sensitivity	Specificity	PPV	NPV
MHT	57.8%	60.0%	96.5%	6.9%
IMP-IMP+EDTA	93.6%	20.0%	95.6%	14.2%
CAZ-2MPA	7.4%	100.0%	100.0%	5.3%

**Conclusion:** Rapid detection of carbapenemase-producing *A. baumannii* and *Pseudomonas* spp. is critical to prevent their widespread dissemination in intensive care units. Thus far, no standardized phenotyping method is available, and sensitivities and specificities of them are variable. According to results of this study high PPVs of all three phenotypic methods make them adequate for detection of carbapenemases in *A. baumannii* and *Pseudomonas* spp.

**P1263 A new method for studying efflux and membrane permeability in *Acinetobacter* species by measuring accumulation of Hoechst 33342 (bis-benzamide)**

G.E. Richmond\*, L.J.V. Piddock (Birmingham, UK)

**Objectives:** Multidrug resistant *Acinetobacter* species are an increasing problem in the hospital setting and nosocomial infection with these pathogens is becoming more and more common. Increase in efflux and loss of outer membrane porins in these bacteria can contribute to resistance to a wide range of clinically relevant antibiotics. The objective of this study was to develop an assay to measure accumulation of Hoechst (H) 33342 to study active efflux and membrane permeability in *Acinetobacter*.

**Methods:** H33342 is a fluorescent probe which permeates the cell membrane and fluoresces when intercalated with DNA or bound to a lipid membrane. The assay was carried out using various clinical and non-clinical and resistant and susceptible isolates of *Acinetobacter baumannii* inoculated into a black 96 well microtitre tray, and intracellular accumulation of the probe was measured in the FLUOstar using excitation and emission filters of 355 and 460 nm respectively. Concentrations of various efflux pump inhibitors (EPIs) non-inhibitory to growth of all isolates tested were determined by growth kinetics and the effect of addition of these EPIs on the accumulation rate of H33342 was studied.

**Results:** Addition of H33342 to *Acinetobacter* cells grown to mid-log phase produced a rapid increase in fluorescence when measured in the FLUOstar. This increase varied between strains, reflecting differing levels of efflux activity and membrane permeability. Addition of efflux inhibitors such as CCCP, a disruptor of the proton motive force, and PABN, a competitive inhibitor of RND pumps, gave rise to increased fluorescence levels reflecting higher levels of accumulation in cells due to reduced efflux activity, confirming H33342 as a substrate of *Acinetobacter* efflux pumps.

**Discussion:** By studying the intracellular accumulation of H33342 in real time, this assay allows the levels of efflux activity to be inferred. It provides a simple and efficient method for comparing efflux and membrane permeability in clinical isolates of *Acinetobacter*, a species in which efflux is a major contributing factor to multidrug resistance. This work was supported by MRC grant G0801977.

**P1264 Deletion of AdeFGH pump increases susceptibility of multidrug-resistant *Acinetobacter baumannii* to carbapenems**

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**Objectives:** *Acinetobacter baumannii* is a major etiological agent of nosocomial infection worldwide and is becoming increasingly resistant to multiple antibiotics, including carbapenems. The objective of this study is two-fold: to develop a genetic tool for creating gene deletions in multidrug-resistant (MDR) *A. baumannii* and to identify an efflux pump that confers resistance to carbapenems.

**Methods:** *A. baumannii* R2 (TTS6013 654325/03) is a MDR isolate from a collection by the Network for Antimicrobial Resistance Surveillance (Singapore). The adeFGH operon was deleted using a modified markerless gene replacement approach. DNA fragments flanking the deletion was cloned into a mobilizable suicide plasmid, pMo130TelR, and introduced into *A. baumannii* R2 by biparental conjugation. Transconjugants were selected on LB agar containing

tellurite and R2 that had undergone allelic exchange to produce the adeFGH deletion was selected by passaging in LB containing sucrose. The adeFGH deletion in R2 was confirmed by PCR. Antimicrobial susceptibility of the mutant and parental strain was determined using BSAC standard agar dilution and NCCLS broth microdilution methods.

**Results:** The R2Delta adeFGH mutant was created using a modified markerless gene replacement methodology. Incorporation of a tellurite-resistance cassette into pMo130 facilitated selection of MDR *A. baumannii* harboring the plasmid construct. Subsequent passages in LB containing 10% sucrose yielded R2 mutants with deletion of adeFGH. Deletion of adeFGH was confirmed by PCR. Antimicrobial susceptibility testing revealed at least 64-fold increase in susceptibility to meropenem and imipenem in R2Delta adeFGH compared to the parental R2 strain. Susceptibility to aminoglycosides, tetracyclines, chloramphenicol, fluoroquinolone, macrolide, beta-lactams and trimethoprim were unaffected by deletion of adeFGH in MDR *A. baumannii* R2.

**Conclusion:** We have successfully modified a markerless gene replacement strategy and used it to generate efflux pump deletion in MDR *A. baumannii* R2. Deletion of the AdeFGH efflux pump specifically increases susceptibility of MDR *A. baumannii* R2 to carbapenems but not other classes of antibiotics.

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#### **P1265** Evaluation of colistin-resistant *Acinetobacter* strains

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**Objectives:** *Acinetobacter baumannii* is an important pathogen in ICU patients. In nosocomial *A. baumannii* isolates resistance to extended spectrum beta-lactams, carbapenems, aminoglycosides and fluoroquinolones are widespread. Recently, colistin seems last resort for therapy. The first colistin resistant *A. baumannii* isolate was reported in March 2011 in our hospital and followed by repeated isolation of other resistant strains. In this study phenotypic and genotypic properties of colistin resistant *A. baumannii* strains was investigated.

**Methods:** Nine colistin resistant *A. baumannii* strains isolated from seven patients were included the study. We included more than one isolates some of the patients because they were from different body sites with different MIC concentrations. Hospital Infection Control Committee explained two of this isolates were colonization and seven were pathogens. Identification and antimicrobial susceptibility of isolates were determined by VITEK2 (bioMerieux/France) system. Confirmation was done by E-test. Clonal analysis were evaluated by PFGE. Environmental culture specimens from related clinics were investigated.

**Results:** Three different groups were identified by PFGE as group A, B and C. There was blood and tracheal aspirate specimens of the first patient in group A. MICs of this strains were found to be 4 and 32 µg/mL respectively. There was seven specimens of six patients from same ICU in group B. MIC concentrations were 64 µg/mL of all strains. One patient from burn unit was in group C. MIC of this isolate was 7 µg/mL. Isolates in group B and C was only susceptible to trimethoprim-sulphametaxazole. Five of this patients were dead before their therapy were changed. One patient was not given therapy because the strain was determined as colonisation. The other patient was the first resistance case and cured by colistin therapy. No *A. baumannii* was isolated from environmental cultures.

**Conclusion:** Six patients in group B was considered as a cluster formation. After taking proper precautions no other cases has been seen. It was interesting that all strains in this group were susceptible to trimethoprim-sulphametaxazole and it is needed to investigate in vivo study. Resistance to colistin for multidrug resistant *Acinetobacter* strains is a very important problem. In order to prevent spread of this resistance health workers should strictly abide infection control precautions and rational antimicrobial usage should be applied.

#### **P1266** Involvement of the outer membrane protein OmpW in colistin resistance of *Acinetobacter baumannii*

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**Objectives:** The increasing number of *Acinetobacter baumannii* multi-drug resistant isolates, especially the carbapenem-resistant ones, has converted colistin in a drug of last resort to treat severe infections caused by this emerging pathogen. The objectives of this study were to decipher the mechanisms that could develop *A. baumannii* at the outer membrane (OM) level to resist to the activity of colistin.

**Methods:** Colistin mutant production was undertaken by consecutive passages on media with increasing concentrations of colistin sulphate salt. The comparison of OM protein profiles between the colistin-resistant mutant (MIC > 256 µg/mL) and the susceptible strain, was performed by SDS-PAGE and protein identification by mass spectrometry. Production of *A. baumannii* OmpW was achieved via overexpression in *Escherichia coli* BL21, purification by affinity chromatography and refolding by a dilution method. Functional analyses, single-channel conductance and colistin, Fe<sup>3+</sup> and LDAO specificity measurements, were performed by reconstitution in artificial planar lipid bilayers. The crystal structures of *Pseudomonas aeruginosa* OprG and *E. coli* OmpW were used as template structures for creating an homology model of the *A. baumannii* OmpW generated by the Modeler 8v1 program.

**Results:** Comparison of OM protein profiles allowed us to identify a loss of expression of the outer membrane OmpW in the colistin-resistant mutant. After purification of a recombinant *A. baumannii* OmpW, functional reconstitutions determined a single-channel conductance value of 23 pS (in 1 M KCl), and highlighted a marked specificity of OmpW towards Fe<sup>3+</sup>, LDAO and colistin. The predictive OmpW 3-D model envisaged an eight beta-stranded beta-barrel in which the structural features of *P. aeruginosa* OprG and *E. coli* OmpW were conserved, i.e., a hydrophobic entrance of the channel and a lateral opening that could allow the small hydrophobic compound diffusion.

**Conclusion:** Taken together, these results allowed to conclude that OmpW from *A. baumannii*, as do OmpW proteins from other bacteria, could participate to the assimilation of iron and hydrophobic compounds in *A. baumannii*. Moreover, the structural features of this hydrophobic channel might explain its capacity to bind the colistin. Its loss in the OM could be one of the means, together with lipopolysaccharide modifications, for *A. baumannii* to develop colistin resistance.

#### **P1267** High-throughput mass spectrometry analysis reveals alterations in iron acquisition systems in meropenem heteroresistant *Acinetobacter baumannii*

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**Objectives:** To further investigate the proteomic profile of a characterized meropenem heteroresistant *Acinetobacter baumannii* isolate using high-throughput mass spectrometry analysis.

**Methods:** Sodium-dodecyl-sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) of protein crude extract, in-gel protein digestion of bands of interest, nano-high performance liquid chromatography and high – throughput mass spectrometry (MS/MS) analysis for protein identification were performed. All techniques were applied to both the native and the meropenem heteroresistant populations of a previously characterized meropenem heteroresistant *A. baumannii*. The MS raw file was searched using the SEQUEST algorithm against ATCC 17978 *A. baumannii* protein database.

**Results:** A putative siderophore receptor (MW: 55.2; pI: 5.57), two ferric enterobactin receptor precursors (MW: 57.7; pI: 5.28 and MW: 19.4; pI: 8.78 respectively) as well as two putative ferric siderophore receptor proteins (MW: 81.2; pI: 6.05 and MW: 80.0; pI: 6.23 respectively) were not present in the meropenem heteroresistant



populations in contrast to the native population. On the other hand a putative ferric siderophore receptor protein (MW: 85.4; pI: 7.80) was present in the meropenem heteroresistant populations but not in the native population.

**Conclusion:** The siderophore mediated iron acquisition systems may play a role in the meropenem heteroresistant phenotype of *A. baumannii*.

**P1268** **Modelling in vitro time growth to predict the fitness cost of drug-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa***

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**Objectives:** It has been proposed that antimicrobial resistance is usually associated with a fitness cost. The fitness is often determined by competition experiments between isogenic strains (wild-type and resistant mutant). However, this approach is time-consuming and labor-intensive. An alternative method was developed to assess fitness cost in drug-resistant bacteria.

**Methods:** Time-growth studies were performed with 10<sup>5</sup> CFU/mL of *Acinetobacter baumannii* or *Pseudomonas aeruginosa* at baseline. Serial samples were obtained to quantify the bacterial burden over 24 hours. The in vitro growth rates (Kg) of isogenic strains were determined individually in full- and 0.1-strength broth. The relative abundance of the two strains in a co-culture over time was predicted based on the difference in Kg, and subsequently validated by in vitro growth competition experiments for up to 144 hours.

**Results:** The in vitro growth rates of *A. baumannii* were not significantly different in different strengths of broth; the Kg for the wild-type and resistant mutant was 2.38 and 1.23 per hour, respectively. In contrast, the difference in Kg for the wild-type and resistant mutant was not as great in *P. aeruginosa* (1.22 per hour vs. 1.07 per hour). For both bacteria, the proportion of the resistant mutant in the competition co-culture gradually diminished. These experimental observations were in general agreement with the model predictions, suggesting good predicting ability of the mathematical model.

**Conclusion:** The model was found to be reasonable in characterizing in vitro bacterial growth and predicting the fitness cost of resistance. The simple method appeared robust in the assessment of fitness cost associated with drug resistance.

## Epidemiology and molecular resistance of *Staphylococcus* sp. and *Enterococcus* sp.

**P1269** **Spread of methicillin-susceptible *Staphylococcus aureus* ST398 in patients, health care workers and environment in an intensive care unit**

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**Objectives:** An increasing number of human infections with livestock-associated *Staphylococcus aureus* sequence type (ST) 398 has been recently reported. Here, *S. aureus* ST398 isolated from patients, health care workers (HCW) and environmental samples in an Intensive Care Unit (ICU) of Montpellier Hospital were characterized with the aim to understand the circulation of this pathogen into the ICU.

**Methods:** One hundred and twenty-five *S. aureus* were isolated between February and June 2011 in the ICU from patients, HCWs and environment, and typed by MultiLocus Sequence typing (MLST). *S. aureus* ST398 strains were analyzed by double-locus sequence typing (DLST) and accessory gene regulation (agr) typing. Resistance to antibiotics was detected by disk-diffusion method. Macrolide-lincosamide-streptogramin type B (MLSB) resistant strains were screened by PCR for erm(A), erm(C), erm(T) and msr(A) genes. Virulence genes were detected by specific PCRs.

**Results:** Out of the 125 isolates, 29 methicillin-susceptible *S. aureus* (MSSA) ST398 or variant were isolated in nasal carriage and invasive

diseases in five patients (n = 12), nasal colonization of two HCWs (n = 2) and environmental samples (n = 15). For the first time, four isolates were identified as variant of ST 398 with a mutation in the pta gene. All isolates were agr1 and DLST-type 144–186. Erythromycin resistance and inducible MLSB phenotype were observed for 76% of the isolates. Seven environmental isolates showed additional resistance to Kanamycin, Tobramycin and Gentamicin. All strains harbored the erm(T) gene and different combinations of erm(A) and erm(C) and the absence of msr(A). No isolate contained the genes encoding the Pantone Valentine Leukocidin, TSST-1 and Staphylococcal Enterotoxin A. No history of contact with livestock was identified in patients and HCWs. Two patients presented nosocomial pneumonia after acquired-nasal colonization.

**Conclusion:** Isolation of MSSA ST398 strains in patients, HCWs and environment in an ICU during short time period underlines the capacity of this emerging pathogen to rapid person-to-person transmission and the role of the environment as potential reservoir. Despite the absence of large antibiotic resistance and virulence traits, MSSA ST398 can lead to severe infections in critically ill patients. Evolutionary capacity of ST398 genotype is underlined here by description of a mutated genotype, the impact of the mutation on the phenotypic and spreading properties of ST398 genotype has to be investigated.

**P1270** **Modulating activity of vancomycin and daptomycin on the expression of autolysis cell-wall turnover and membrane charge genes in hVISA and VISA strains**

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**Objectives:** Emerging resistance to glycopeptides in Methicillin-Resistant *Staphylococcus aureus* (MRSA) poses a great threat to antimicrobial chemotherapy worldwide. Recent publications have added to the knowledge of the complex changes taking place in *Staphylococci* evolving towards the reduced glycopeptide susceptibility phenomenon that involves different genetic loci (regulatory, autolytic, cell-wall turnover and cell-envelope positive charge genes). In addition, reduced susceptibility to vancomycin can influence the development of resistance to daptomycin.

**Methods:** The aim of our study was to investigate, by real time RT-PCR, the relative quantitative expression of genes involved in autolysis (atl-lytM), cell-wall turnover (sceD), cell-envelope charges (mprF-dltA) and regulatory mechanisms (agr-locus-graRS-walKR), in heterogeneous Vancomycin-Intermediate-*S. aureus* (hVISA), i.e. Mu3 and three clinical isolates, and Vancomycin-Intermediate-*S. aureus* (VISA) i.e. Mu50, cultured with or without vancomycin and daptomycin.

**Results:** Our results show that hVISA and VISA presented an up-regulation of sceD, and mprF or dltA (in Mu3 and Mu50, respectively) together with a progressive agr-locus down-regulation with respect to Vancomycin Susceptible *S. aureus* (VSSA). Indeed, VISA showed an atl/lytM down-regulation with respect to hVISA and obviously VSSA. The addition of vancomycin or daptomycin sub-inhibitory concentrations to the growth-medium with respect to the free-drug conditions, determined an atl/lytM down-regulation in hVISA, whereas only daptomycin up-regulated the mprF expression both in hVISA and VISA.

**Conclusions:** hVISA and VISA present common features that distinguish them from VSSA, responsible for the intermediate glycopeptide resistance i.e. an increased cell-wall turnover, an increased positive cell-envelope charge responsible for a repulsion mechanism towards vancomycin and daptomycin, and reduced agr-functionality. Vancomycin and daptomycin, acting in a similar manner in hVISA and VISA, can influence their cross-resistance mechanisms promoting VISA behavior in hVISA and enhancing the cell-wall pathways responsible for the intermediate vancomycin resistance in VISA. Daptomycin can also induce a charge repulsion mechanism both in hVISA and VISA increasing the mprF activity.

**P1271** Staphylococcal chromosome cassette mec stability in major endemic methicillin-resistant *Staphylococcus aureus* clones over 20 years.

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**Objectives:** Hospital acquired-Methicillin-Resistant *Staphylococcus aureus* (MRSA) clones are defined by the combination of genetic background and Staphylococcal chromosome cassette mec (SCCmec) type. However in some cases the same clone may be associated to different SCCmec types or variants of the initial type. The aim of this study was to analyze the stability of the SCCmec element among MRSA representative of major endemic clones in the Hospital de Bellvitge (HB) over the last 20 years (1990–2010).

**Methods:** A collection of 166 isolates from bacteremic patients and belonging to three major clones was selected for study: 100 isolates belonged to Clonal Complex (CC) 5 (ST5, ST125, ST146 and ST228); 58 to CC8 (ST8, ST247 and ST1819); and 8 to CC22 (ST22). Isolates were chosen to include the highest variability of PFGE subtypes and antibiotic resistance profiles (ARP) identified for each clone. All selected isolates were typed by SCCmec, agr, spa, ACME (detection of arc and opp3 genes), ccrB sequencing and MLST.

**Results:** The Iberian clone (ST247-SCCmecI-agr-I) was the most prevalent from 1990 to 2000 and no change was observed in its SCCmec structure. Forty-eight isolates from this pandemic clone, showed a single ARP and four different spa types, spa t051 being the most frequent (65%). Between 1996 and 2003, isolates of CC5 rapidly increased and have remained dominant through 2010. All 82 CC5 isolates analyzed showed SCCmec type IVc and were distributed in three clones: ST5-IVc-agr-II; ST125-IVc-agr-II and ST146-IVc-agr-II. CC5 isolates showed 11 ARP and 16 spa types: t002 (32%) and t067 (47%) accounting for 79% of the isolates. Other important endemic clones found in the 2004–2010 period were: ST8-IVc-agr-I, spa t008 (n = 10); ST228-I-agr-II, spa t041 (n = 18) and ST22-IVh-agr-I, spa t032 (n = 8). The ccrB sequencing confirmed the SCCmec type obtained by the multiplex strategy. Gene clusters arc and opp3 were not found in any of the studied isolates.

**Conclusion:** The SCCmec element has remained highly conserved in isolates of a given clone over extended time intervals. Isolates of CC5 in our setting have shown significant stability of SCCmec type IVc in spite of variable antibiotic resistance and spa types.

**P1272** MRSA harbouring mecA-LGA251, a new highly divergent mecA variant: performance of the methods used in routine labs to screen, detect and confirm methicillin resistance

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**Background:** A new mecA variant, named mecA-LGA251, showing <70% homology with the classical mecA gene, has recently been described in methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from human and animal. We investigated the performance of different phenotypic and genotypic methods routinely used in microbiological labs to screen, detect, and confirm the presence of isolates harboring such a methicillin-resistance (MR) mechanism.

**Methods:** A large collection including 111 mecA-LGA251-positive isolates were collected in UK, Scotland, Denmark and France. Four chromogenic MRSA selective media (MRSA Select (bR), ChromID MRSA (bM), BBL CHROMagar MRSA II (BD), Brilliance MRSA 2 (Oxoid)) were tested for screening. Antimicrobial susceptibility tests (AST) included MIC for FOX and OXA using BMD, AST-P581 (Vitek), PMIC/ID-60 (Phoenix), Pos MIC Panel Type 31 (Microscan). Immunological detection of additional PBP were performed using Clearview Exact PBP2a (Alere) and PBP2a agglutination (Oxoid).

Finally, molecular tests, including “homebrew” mecA PCR, BD GeneOhm StaphSR assay (BD), Xpert MRSA/MSSA SSTI and nasal (Cepheid), NucliSENS EasyQ MRSA (bM) and DNA microarray StaphyType (Alere), were performed.

**Results:** The 111 isolates belonged to CC130 (n = 92, agr 3, 16 spa-types), CC1943 (n = 14, agr 4, four spa-types) and CC425 (n = 5, agr 2, four spa-types). All were MR but susceptible to all the other antibiotics tested.

Data highlighted a highly variable sensitivity for the various selective media and AST tested (see Table 1). Clearview Exact PBP2a test, performed after cefoxitin induction (disc), were the only method allowing the confirmation of expression of additional PBP in all isolates. None of the homebrew mecA PCR or commercial molecular kits currently available was able to identify these isolates. Using DNA microarrays (n = 37), assignment to the specific clones known to be positive for mecA-LGA251 gene were achieved and data revealed the seldom presence of some toxins and virulence genes: tst (n = 7), egc (n = 9), edinB (n = 8), sec (n = 3), sel (n = 3).

CC	agr	n	Number of different spa	Antimicrobial susceptibility test						Immuno (chromatography)		Screening selective media (number of MRSA detected after 24h)				
				Vitek		Phoenix		Microscan		Clearview without induction	Clearview with induction	MRSA Select (bS)	Brilliance (bM)	ChromAgar (bR)	ChromID (bM)	
				MISA	MISA	MISA	MISA	MISA	MISA							
130	3	92	16	2	90	22	70	3	89	10	92	62	89	74	91	
1943	4	14	4	1	13	1	13	1	13	0	14	6	14	9	14	
425	2	5	5	0	5	3	2	0	5	0	5	2	5	5	5	
Total correctly identified (%)					97.3		76.6		96.4		9.1	100	63.6	97.3	80	99.1

Table 1: Data obtained for a collection of 111 European MRSA isolates harboring mecA-LGA251 gene.

**Conclusion:** The data presented demonstrates that (i) the ability of commercial methods used to screen, identify or confirm mecA-LGA251-positive isolates is highly variable, (ii) such isolates may be missed depending on the used algorithms. The only ways to definitively confirm the methicillin-resistance in such isolates are the use of specific mecA-LGA251 PCR or Clearview Exact PBP2a after cefoxitin induction.

**P1273** A new mechanism for the mobilisation of mecA independent of the action of cassette chromosome recombinase

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**Objectives:** To investigate the genetic context of mecA in a methicillin-resistant *Staphylococcus haemolyticus* isolate, WCSH1, in which no cassette chromosome recombinase (ccr) genes could be detected.

**Methods:** mecA and ccr genes were screened using primers published previously. The region between mecA and orfX, the insertion site of the staphylococcal cassette chromosome (SCC), was amplified using long-range PCR and then sequenced. The region downstream of mecA was obtained using a few inverse PCR with self-ligated enzyme-restricted WCSH1 genomic fragments as the template and PCR mapping. Linkages between different genetic components were confirmed using overlapping PCR and sequencing.

**Results:** A 46-kb region containing mecA was obtained from WCSH1, bounded by orfX at one end and several orfs that were present on the chromosome of the completely-sequenced *S. haemolyticus* JCSC1435 at the other (Figure 1). Of note, orfX was located at the left side of mecA rather than at the right side as seen in almost all contexts of mecA. This 46-kb region was very complex in structure with five copies of IS431 and contained multiple genetic components with different origins. For instance, the 3.7-kb structure adjacent to orfX was almost identical to that on the chromosome of *Staphylococcus epidermidis* RP62A but was absent from *S. haemolyticus* JCSC1435. Sequences resembling the terminal inverted repeats of SCC were found but no ccr genes could be detected. mecA was bracketed by two copies of IS431 and a 8-bp (CTTTTTGC) direct target repeat sequence (DR) was

identified flanking this IS431-mecA-IS431 structure. The 8-bp DR was part of the spacer sequence between *arsR* (encoding the arsenical resistance operon repressor) and *copA* (encoding a copper-exporting ATPase).



**Conclusion:** The presence of 8-bp DR indicates that the two copies of IS431 composed a composite transposon that mobilised *mecA* into the spacer between *arsR* and *copA*, which is independent of the action of cassette chromosome recombinase. This finding is of significance as multiple copies of IS431 are commonly present in the contexts of *mecA* and might form various composite transposons for mobilising *mecA*. The fact that genetic components with different origins were separated by multiple copies of IS431 suggests that IS431 served as the “adapter” facilitating the formation of large mosaic contexts containing *mecA* by promoting the joining of different regions.

**P1274** Emergence of high-level mupirocin resistance in hospital- and community-acquired methicillin-resistant *Staphylococcus aureus* in Pontevedra, Spain

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**Objectives:** Mupirocin is used to control the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA). High-level mupirocin resistance (Hi-MupR, MICs  $\geq 512$  mg/L) is of particular concern, having been frequently associated with treatment failure. The aim of this study was to determine the emergence and mode/s of spread of Hi-MupR in the MRSA population recovered from 2002 to 2009 in four medical centers in Pontevedra, Galicia, Spain.

**Methods:** All hospital- and community-acquired MRSA isolates were included and screened for mupirocin resistance. High-level mupirocin resistant MRSA were characterized by antibiotyping, genotyping (multilocus sequence typing, *spa* typing and pulsed-field gel electrophoresis), and plasmid analysis.

**Results:** Thirty-one single patient high-level mupirocin resistant (MICs  $\geq 1024$   $\mu$ g/mL) MRSA isolates were identified. Four main clonal types, as defined by molecular typing were recognized: ST8/t008/PFGE types H and I (four isolates), ST36/t018/PFGE types E and F (seven isolates), ST72/t148/PFGE type G (three isolates), and ST125/t067/PFGE types A to D (15 isolates). The high-level mupirocin resistance *ileS2* gene was harbored by nine plasmid types (i.e., pCPEP\_1 to pCPEP\_9) based on distinct IS257-*ileS2* spacer configurations that correlated with *ileS2*-locus hybridization polymorphs. Some plasmid types were present in different MRSA clonal types and were recovered from patients treated in distinct medical centers over several years. pCPEP\_1 (configuration UpR882-DnR267/hybridization polymorph I) and pCPEP\_4 (configuration UpR319-DnR267/hybridization polymorph IV) were the most prevalent and were dispersed among clonal complexes (CCs) 5, 8, and 30. Furthermore, diverse plasmid types were recovered from isolates of the same clonal type. Remarkably, seven plasmid types were recovered from ST125 MRSA isolates. Plasmid types pCPEP\_5, 7, 8, and 9 were associated with unique clonal types.

**Conclusions:** This study remark the utility of typing IS257-*ileS2* spacer regions for the rapid differentiation of *ileS2*-encoding plasmid types. Horizontal transmission of a diverse set of plasmids promoted the emergence of Hi-MupR. Clonal expansion of MRSA hardly contributed to the dispersion of the resistance. This valuable epidemiological information is of great importance for the design of appropriate containment epidemiology strategies to preserve the effectiveness of mupirocin.

**P1275** Molecular analysis of high-level mupirocin-resistant methicillin-resistant *Staphylococcus aureus* recovered in Ireland between 1998 and 2009

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**Introduction:** High-level mupirocin resistance (Hi-MupR) in methicillin-resistant *Staphylococcus aureus* (MRSA) is mediated by the plasmid-encoded *ileS2* (*mupA*) gene. In Ireland, the incidence of Hi-MupR MRSA from bloodstream infections increased from 1.4% to 3.1% between 2005 and 2009. Isolates exhibiting antibiogram-resistogram (AR) and pulsed-field gel electrophoresis (PFGE) types indicative of ST8-MRSA-IIA-E predominated among Hi-MupR MRSA between 1999 and 2005 and those indicative of the current endemic clone, ST22-MRSA-IV, predominated since 2006. The majority of Hi-MupR ST22-MRSA-IV isolates also exhibited aminoglycoside resistance.

**Objective:** To undertake comparative genetic analysis of Hi-MupR MRSA recovered from patients in Irish hospitals between 1998 and 2009.

**Materials and methods:** A total of 109 isolates, representative of the different AR-PFGE patterns of Hi-MupR MRSA recovered from patients in Irish hospitals between 1998 and 2009, were analysed by DNA array profiling (Alere, Germany). Hi-MupR-conferring conjugative plasmids from MRSA, representing the main genotypes prevalent among the Hi-MupR isolates, were subjected to fingerprinting with BglIII, EcoRI and HindIII, Southern hybridisation with an *ileS2* probe and sequence analysis of the *ileS2* flanking regions.

**Results:** The 109 Hi-MupR MRSA isolates were assigned to five genotypes, namely ST22-IV (53%), ST8-IIA-E (38%), ST36-II (7%), ST239-III (1%) and ST5-IV (1%). Plasmid analysis of the three main genotypes revealed a distinct ca. 40 kb *ileS2*-encoding conjugative plasmid and a second large plasmid harbouring *aacA*-aphD. The trimethoprim resistance gene *dhfr*S1 was detected on a Hi-MupR-encoding plasmid in one ST22-MRSA-IV isolate.

**Conclusion:** This study has so far revealed the presence of large distinct conjugative plasmids encoding Hi-MupR in Irish MRSA isolates representative of three distinct genotypes. Significant spread of these plasmids into hospital-associated and community-associated MRSA may compromise our ability to eradicate nasal colonisation of MRSA using mupirocin.

**P1276** Role of insertion sequence IS257 in the dissemination of high-level mupirocin resistance *ileS2* gene in staphylococci: a preliminary study

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**Objectives:** Our previous comparative analysis of four completely sequenced pSK41-like plasmids showed that the DNA segments containing the high-level mupirocin resistance *ileS2* gene flanked by copies of insertion sequence IS257 occupy a unique position within each plasmid backbone. These findings imply independent acquisition events through the activity of IS257 and the data suggested that transposition has contributed to the process. In this preliminary study the aim was to obtain further information on the role of IS257 to mediate the genetic capture and dissemination of the *ileS2* gene.

**Methods:** A total of 55 high-level mupirocin resistant (MICs  $\geq 1024$  mg/L) staphylococci containing non-sequenced *ileS2*-carrying plasmids were included. These plasmids were classified into distinct structural groups based on different IS257-*ileS2* spacer configurations. For each isolate the insertion site of segments containing the *ileS2* gene into the plasmid backbone was determined through PCR amplification and subsequent sequencing of amplicons using primers specific to the up- and downstream IS257-*ileS2* spacer regions. Additionally, comparative analyses of DNA sequences of

ileS2-flanking IS257s and adjacent regions were determined in order to obtain insights about the integration mechanisms.

**Results:** Our strategy allowed us to get the adjacent DNA sequences outside to flanking IS257 copies to precisely locate the insertion sites of ileS2-encoding segments within the pSK41-like conserved backbone without the need to conduct comprehensive plasmid sequencing. This analysis mapped several integration sites of IS257-ileS2-IS257 segments. As expected, an identical integration site was found for plasmids belonging to the same structural group. However, structurally different plasmids also shared the location of ileS2-encoding segments. As previously observed, for some plasmids, the existence of flanking 8-bp target duplications adjacent to flanking IS257s imply that transposition has contributed to the process and this was supported by the identity or near-identity of the IS257 sequences. In other plasmids, there was no evidence of a transposition event.

**Conclusions:** Results allowed to conclude that the number of insertion sites of ileS2-encoding segments is smaller than expected based on the IS257-ileS2 configurations. As noted previously, our data suggested that a transposition mechanisms mediated by IS257 participate in the movement of the ileS2 gene.

#### **P1277** Characterisation of a cfr-positive methicillin-resistant *Staphylococcus epidermidis* strain of the lineage ST22 implicated in a life-threatening human infection

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**Objectives:** The gene cfr encodes a methyltransferase which produces the methylation of the 23S rRNA leading to a multiresistant phenotype. This gene was identified in Methicillin resistant *Staphylococcus epidermidis* (MRSE) isolates from a very serious human case. The aim was to characterize the cfr positive MRSE isolates and to determine the localization and the genetic environment of this gene.

**Methods:** Three MRSE isolates were detected in samples from cerebrospinal fluid, pleural drainage and vascular catheter of the patient. Antibiotic susceptibility testing was performed by Wider system and for nine agents also by the agar dilution method (chloramphenicol, clindamycin, erythromycin, lincomycin, linezolid, tetracycline, tiamulina, trimethoprim and virginiamycin). The three MRSE isolates were typed by MLST, PFGE and SCCmec-typing. The presence of resistance genes was studied by PCR. The presence of mutations in 23S rRNA, L3, L4, L22, grlA, gyrA and fusA was investigated by PCR and sequencing. The three MRSE isolates were tested by PCR for the genes lukF/lukS-PV, icaA, icaB, icaC and for the IS256 element. Plasmid or chromosomal gene location was determined by S1-PFGE and I-CeuI-PFGE hybridization. Genetic environment was studied by PCR-mapping and sequencing. Conjugative transfer of cfr gene was performed.

**Results:** The three MRSE strains showed the same PFGE-pattern, belonged to ST22 and had SCCmec-type III. All of them were resistant to 10 antimicrobials groups. The presence of cfr, fexA, aac(6')-aph(2), dfrA, icaA, icaB and icaC genes was confirmed by PCR. Mutations mediating quinolone resistance revealed the S80F and D84N exchanges in GrlA and S84F in GyrA. MRSE isolates possessed L101V and A58T substitutions and 135QGRGPM136 insertion in L3 and N64K and N158S exchanges and 71G72 insertion in L4. The sequencing of 23S rRNA revealed the mutation C2534T. No mutations were identified in L22 and in fusA. Hybridization experiments revealed the presence of the cfr gene in a plasmid of 45-kb and in chromosomal DNA. One transconjugant was obtained and the genetic environment was similar to pSCFS7 (FR675942.1), including the gene fexA. PVL and the IS256 element were not detected.

**Conclusion:** We describe a fatal human case in which cfr-positive MRSE ST22 isolates were detected. The spread of this resistance mechanism is especially worrisome due to this gene lead to simultaneous resistance for several antimicrobials.

#### **P1278** New MLSB resistance gene in *Staphylococcus lentus*

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**Objectives:** One *S. lentus* isolated from a healthy dog displayed resistance to macrolides, lincosamides and streptogramins B (MLSB). Resistance to lincosamide was inducible. However, the underlying mechanism of resistance remains unknown. The aim of the study was to identify and characterize the MLSB resistance determinant in *S. lentus*.

**Methods:** Antibiotic susceptibility was determined by the measurement of the MIC by broth dilution. Inducible resistance was determined by D-test and in broth in the presence of 4 mg/L erythromycin. The genome of *S. lentus* SD952 was sequenced by 454 technology and analyzed for putative erm genes. An erm candidate gene was cloned into the *E. coli* – *S. aureus* shuttle vectors pBUS1 and pRB474 and electro-transformed into susceptible *S. aureus* RN4220.

**Results:** A new Erm determinant was identified by whole genome sequencing of *S. lentus* SD952. The new Erm determinant showed the closest identity to Erm(A) of *S. aureus* with 60% amino-acid identity and 40% DNA identity. Expression of the new erm gene in RN4220 showed an increase of resistance to the macrolide erythromycin (MIC > 64 mg/L) and an inducible increase of resistance to the lincosamide clindamycin (MIC > 128 mg/L) and to the streptogramin B pristinamycin IA (MIC > 64 mg/L). PCR analysis using primers specific for the new erm gene revealed that it was also present in methicillin-resistant *S. lentus* isolates from poultry and slaughterhouse employees.

**Conclusion:** This study identified and characterized a novel inducible resistance gene. Genetic relationship and phenotypic resistance showed that this new gene belongs to the Erm determinant conferring resistance to MLSB antibiotics. This study showed that this gene is also widespread in *S. lentus* isolates from healthy animals and humans working in animal environment.

#### **P1279** Fluoroquinolone recognition by prokaryotic *S. aureus* NorA and eukaryotic murine Mrp4 efflux transporters: a combined experimental and structural study

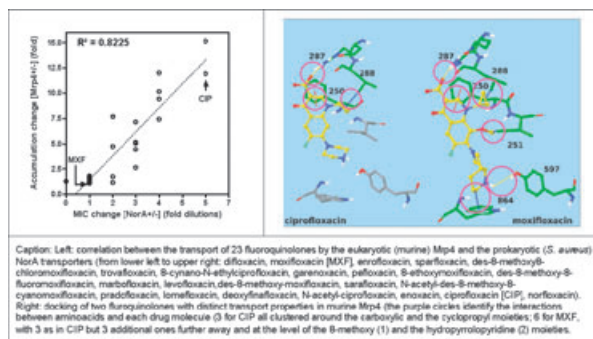
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**Objectives:** Efflux of antibiotics from prokaryotic cells is a well-known mechanism of resistance. Antibiotic transporters, however, are also found in eukaryotic cells where they modulate the distribution of these drugs. Our objective was to compare the *S. aureus* NorA and the mouse macrophage Mrp4 transporters with respect to recognition of fluoroquinolones (FQ).

**Methods:** A panel of 23 FQ (see Table and JAC 2011, 66:2801–8) with distinct logP and logD at pH 7 (calculated with the Reaxys web-based software [http://www.reaxys.com]) was tested (i) for NorA efflux: by the ratio of MIC observed with the SA-1 *S. aureus* strain overexpressing NorA (AAC 2006, 50:1931–6) to that of the reference strain ATCC 25923 (the latter in the presence of reserpine [10 mg/L] to fully inhibit the basal constitutive expression of NorA); (ii) for Mrp4 efflux: by the ratio of accumulation observed in J774 macrophages overexpressing this transporter (AAC 2009, 53:2410–6) to that observed in wild-type cells (the latter with of the Mrp4 inhibitor gemfibrozil [0.5 mM] to also fully inhibit the basal expression of Mrp4). In parallel, three-dimensional models were constructed by homology modelling using the crystal structures of the prokaryotic lactose permease (LacY) for NorA and of the eukaryotic P-glycoprotein for Mrp4. Docking was performed using an induced fit procedure in the 3D models. The putative binding site of NorA was defined as that of the lactose analog in LacY complex structure and that of Mrp4 identified using mutagenesis data (*Mol Pharmacol* 2008; 74: 964–971).

**Results:** The left panel of the Figure shows that there is a significant ( $p < 0.0001$ ) correlation ( $p < 0.0001$ ) between transport of the fluoroquinolones tested by NorA and Mrp4. However, no correlation

was evidenced between transport and global lipophilicity (log P or logD at pH 7). In the structural analysis, molecules poorly transported established more interactions with the binding sites of both NorA and Mrp4, as illustrated with CIP and MXF for Mrp4 (right panel of the Figure).



**Conclusions:** Although phylogenetically very different from each other, NorA and Mrp4 show similar recognition properties for fluoroquinolones, with those undergoing little efflux actually featuring more numerous interactions in the binding sites. This suggests that the lack of transport is not due to poor recognition but rather to the inability of tightly bound drugs to progress for efflux within the transporter.

#### P1280 The role of NorA in the adaptative response of *Staphylococcus aureus* to stress stimuli

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**Objectives:** Efflux of antimicrobial compounds is a first-line defence mechanism towards chemical stress. We have shown that adaptation to ethidium bromide (EtBr) of the pan-susceptible *S. aureus* ATCC25923 (ATCC) yielded the progeny strain, ATCC-EtBr, with increased resistance to EtBr, fluoroquinolones and biocides, associated with overexpression of the efflux pump gene *norA*. We now continued this study by reversing the EtBr adaptation process and comparing the parental and derivative strains, in order to understand the importance of the efflux pump NorA to the adaptative response of *S. aureus* to EtBr.

**Methods:** Reversion process of ATCC-EtBr was done by serial passages in EtBr-free media, resulting in ATCC-Rev. Susceptibility profile of the parental and derivative strains was assessed by minimum inhibitory concentration determination for several antimicrobial compounds. Efflux activity was evaluated by real-time fluorometry and RT-qPCR used to analyze gene expression of *norA* and other efflux pump genes (*norB*, *norC*, *mepA* and *mdeA*), as well as of the global regulator *mgrA*. The *norA* gene was sequenced and its transcription initiation site determined by 5'-RACE PCR. *norA* mRNA half-life was determined for ATCC and ATCC-EtBr.

**Results:** The ATCC-EtBr overexpressed *norA* presented a new transcription initiation site, but no alteration was detected on the *norA* mRNA stability, since both ATCC-EtBr *norA* mRNA and ATCC *norA* mRNA showed comparable half-lives. ATCC-EtBr *norA* also carried a mutation leading to the substitution Phe303Tyr. The global regulator *mgrA* was also found to be overexpressed. ATCC-Rev showed a complete reversion of the increased resistance profile presented by ATCC-EtBr, linked to the loss of efflux activity. This was accompanied by a decrease of both *norA* and *mgrA* expression levels and the introduction of a stop codon in *norA*, originating a NorA truncated at the aminoacid 274.

**Conclusion:** This work highlights the importance of efflux pumps in the response of *S. aureus* to stress stimuli. The presence/withdrawal of EtBr led to an increase/decrease in the expression of *norA* and of the regulator *mgrA*. However, we found that other factors, such as alterations at the transcription initiation site or in the aminoacid sequence are also to be considered when analyzing differences in NorA

expression and/or activity. All these factors interplay in an intricate network of cellular responses to chemical stress.

#### P1281 Resistance to biocides conveyed by efflux pumps in clinical isolates of MRSA

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**Objectives:** Biocides, compounds with antiseptic and disinfectant properties, have a central role in the prevention and control of nosocomial infections, being largely used in health-care settings and more recently, also in the community. Resistance to these compounds can be bestowed by chromosomal and plasmid-encoded efflux pumps (EPs). In this study, we evaluate the response of clinical isolates of *S. aureus* harbouring plasmid-encoded efflux pumps to the selective pressure of biocides.

**Methods:** The biocide resistance profile of two clinical methicillin-resistant *S. aureus* (MRSA) isolates carrying plasmids harbouring the efflux pumps *Smr* and *QacA* was assayed by minimum inhibitory concentration (MIC) determination for the quaternary ammonium compounds cetrimide (CET), cetylpyridinium chloride (CPC), benzalkonium chloride, chlorhexidine, ethidium bromide (EtBr), among others. The efflux activity was evaluated by real-time fluorometry. Expression analysis by RT-qPCR of the plasmid-encoded efflux pump genes *smr* and *qacA* and of the chromosomal efflux pump genes *norA*, *norB*, *norC*, *mepA* and *mdeA* was evaluated in the presence of sub-lethal concentrations of CET, CPC, EtBr and the fluoroquinolone ciprofloxacin. Two antibiotic pan-susceptible reference strains were used as controls.

**Results:** Clinical isolates carrying the *qacA* or the *smr* genes presented higher MIC values for the biocides tested than the reference susceptible strains. Real-time fluorometry also detected a pronounced efflux activity in those isolates in opposition to the reference strains. RT-qPCR assays revealed that the expression of efflux pump genes varied according to the agent tested. In particular, EtBr induced the overexpression of *smr* and *qacA* genes; whereas CET or CPC induced the joint overexpression of *smr* and other chromosomal efflux pump genes; ciprofloxacin, which is not a substrate for plasmid-encoded efflux pumps, triggered the expression of the chromosomally-encoded efflux pumps only.

**Conclusion:** The diversity in the efflux pump gene expression pattern observed might reflect the different mode of action of each agent tested. While EtBr binds to DNA, both CET and CPC are membrane-active detergents, with a more immediate effect on the *S. aureus* cell. Therefore, these two biocides may trigger a global stress response, in which chromosomal and plasmid-encoded efflux pumps may act together to detoxify the cell from these noxious compounds.

#### P1282 Role of resident and acquired multi-drug efflux pumps in reduced susceptibility to cationic biocides in *Staphylococcus aureus*

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**Objectives:** In view of the requirements posed by licensing process of disinfectants according to the EC biocide directive, we evaluated the possibility to devise an in vitro test for evaluation of resistance to cationic antibacterial compounds including quaternary ammonium compounds and bisbiguanides.

**Methods:** In three strains of *S. aureus* mutants were selected in vitro with benzalkonium chloride (BZC; quaternary ammonium compound), chlorhexidine (CHX; bisbiguanide), acriflavine (AF) and ethidium bromide (EB) and their phenotypes and *norA* promoter sequences were determined. Data on laboratory mutants was compared to molecular data from of 246 clinical *S. aureus* strains showing decreased susceptibility to BZC or CHX.

**Results:** A survey for susceptibility to EB, AF, BZC and CHX on 75 clinical *S. aureus* isolates showed a bimodal distribution of susceptibility profiles for EB and AF and a normal distribution of susceptibility profiles to CHX and BZC. Mutation frequency in vitro to these compounds was found to be around  $1E-10$  for EB and AF, while no mutants could be selected in a single step protocol for BZC and CHX. Multiple passages on selective plates allowed to select also mutants with BZC and CHX. Irrespective the selective agent all mutants showed important increases in MIC and MBC to norfloxacin, ciprofloxacin, EB and AF. For BZC and CHX the MIC and MBC did either not change or increased by a single dilution. All mutants showed mutations in the promoter region of the NorA MDR efflux pump. Upon the 246 clinical isolates with reduced susceptibility to BZC or CHX, 77 were positive for qacA, 13 for qacC and 1 for qacG and all of these had increased MIC for EB. For 35 clinical strains with reduced EB or BZC susceptibility the norA promoter was sequenced. Out of these nine had a short duplication, five a mutated and 21 a wt norA promoter region. In only one case a clinical strain matched to a mutation also generated in vitro.

**Conclusion:** Our data show (i) that EB and AF are suitable agents for monitoring efflux phenotypes and related genotypes, while BZC and CHX not, (ii) that standard mutation selection assays cannot be performed for BZC and CHX, (iii) and that the mutations selected in vitro by BZC and CHX do not match those detected in clinical isolates. Summarised these data indicate that for *S. aureus* an in vitro test for prediction of resistance development to BZC and CHX is not feasible and, in any case, would yield results of no clinical relevance.

**P1283** *Staphylococcus aureus* mutants and clinical isolates with reduced susceptibility to the biocide triclosan differ in phenotype and genotype

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**Objectives:** In view of the requirements posed by licensing process of disinfectants according to the EC biocide directive, we evaluated the possibility to devise an in vitro assay for testing the bacterial resistance to the biocide Triclosan. Triclosan is a synthetic bisphenol compound and active ingredient in many human hygiene biocidal products, targeting specifically the bacterial fabI gene encoding a enoyl-[acyl-carrier-protein] reductase.

**Methods:** Starting from five *S. aureus* reference strains 32 mutant were selected in vitro by culturing strains in medium containing triclosan. Mutants were both selected by single exposure on selective media or by cycling on increasing conditions of the biocide. The phenotypes and fabI sequences were determined in all mutants and compared to data from of 76 clinical *S. aureus* strains showing decreased susceptibility to Triclosan.

**Results:** The frequency of selection of *S. aureus* mutants in vitro for triclosan was between  $2 \times 10^9$  and  $3 \times 10^{10}$  depending on the strain. The single passage mutants showed an MIC of 4–8  $\mu\text{g}/\text{mL}$  and MBC of 8–16  $\mu\text{g}/\text{mL}$ , while the multi-passage mutants all showed a MIC of 16–32. In contrast the clinical isolates had a variable range of MIC values (0.125–4  $\mu\text{g}/\text{mL}$ ). All except three in vitro selected mutants showed mutations in the fabI coding region. In contrast only half of the clinical isolates showed mutations in fabI. Except for two mutations, none of the mutations selected in vitro matched to those found in clinical strains.

**Conclusion:** Both the MIC and MBC profiles and the mutations of in vitro selected triclosan resistant mutants differed from those detected in clinical isolates with reduced susceptibility to triclosan. In addition our data clearly indicate that there are additional and yet undescribed mechanisms of resistance to triclosan in clinical isolates of *S. aureus*. Summarised these data indicate that for *S. aureus* an in vitro test for prediction of resistance development to triclosan is not feasible and, in any case, would yield results of no clinical relevance.

**P1284** Genetic basis for in vitro and in vivo resistance to lincosamides and streptogramins A (LSA phenotype) in *Enterococcus faecium*

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**Objectives:** As opposed to *Enterococcus faecalis*, which is intrinsically resistant to lincosamides and streptogramins A (LSA phenotype) by production of an ABC protein named Lsa(A), *Enterococcus faecium* is naturally susceptible. The LSA phenotype may be selected by lincosamides or streptogramins in *E. faecium* both in vitro and in vivo. The aim of the study was to investigate the molecular mechanism of resistance in strains of *E. faecium* with acquired LSA phenotype.

**Methods:** Six LSA-resistant mutants of *E. faecium* HM1070 selected in vitro by using lincomycin (LIN), clindamycin (CLI) and dalfopristin (DAL) were studied. Three different pairs of clinical isolates (pre- and post-exposure to quinupristin-DAL) were also included. Full genome sequence of one lincomycin-selected mutant (*E. faecium* HM1070/LSA) was determined using the 454 sequencing technology, and was compared with that of wild-type *E. faecium* HM1070. The detection of a single mutation putatively responsible for the LSA phenotype was performed by PCR for all strains. Single-nucleotide allelic replacement was carried out to confirm the role of this mutation. DNA fragments comprising resistance and susceptibility mutations were cloned into the thermosensitive vector pG1KT and introduced into *E. faecium* HM1070 and HM1070/LSA strains, respectively. MICs were determined by using the microbroth dilution method.

**Results:** By comparison of the two entire genomes, 50 point mutations were identified, including one within a 1503-bp gene coding for an ABC homologue showing 66% amino-acid identity with Lsa(A). This mutation corresponded to a transition (C1349T) leading to an amino-acid substitution (Thr450Ile) within the Walker B motif of the second nucleotide-binding domain. Strictly identical mutations were identified for in vitro and in vivo resistant strains, but none for susceptible strains. The introduction of the allele from *E. faecium* HM1070/LSA into *E. faecium* HM1070 conferred the LSA phenotype with an increase of MICs of LIN (0.25–8 mg/L), CLI (0.12–2 mg/L) and DAL (4 to >64 mg/L) whereas that of the allele from *E. faecium* HM1070 into *E. faecium* HM1070/LSA restored susceptibility to LIN (8–0.25 mg/L), CLI (2–0.06 mg/L) and DAL (>64–4 mg/L).

**Conclusion:** We identified the molecular mechanism responsible for the acquired LSA resistance in *E. faecium*. Characterization of the biochemical mechanism of resistance and the physiological role of this ABC protein will need further investigations.

**P1285** Identification of the *Enterococcus faecalis* SOS regulon

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Several antibiotics, such as fluoroquinolones and  $\beta$ -lactams, are known to induce in bacteria a survival mechanism, called the SOS response which is a complex, graded response to DNA damages or stalled replication forks that includes induction of gene products blocking cell division and promoting mutations, recombination and DNA repair. The SOS activator, RecA, after the recognition of DNA damage, cleaves the transcriptional repressor LexA that induces the expression of the genes taking part of the SOS regulon. These genes are not only involved in bacterial survival but also in formation of antibiotic resistance due to the expression of low-fidelity DNA polymerases. In *Enterococcus faecalis*, the second most frequent bacteria involved in nosocomial infections (after *Staphylococcus aureus*), the genes engaged in the SOS mechanism have not been identified to date. We present here the first study to identify the SOS regulon in *E. faecalis*.

An *E. faecalis* LexA mutant was shown to produce constitutively an induced SOS response. This mutant was constructed by a plasmid insertion in the lexA gene preventing the synthesis of the repressor. Microarray experiments were performed to compare the transcriptome

of the wild type and of the constitutive SOS-induced mutant in order to identify LexA regulated genes. The experiment was followed by in silico analysis of the presence of a LexA-box in the promoter region of the regulated genes.

A total of 41 genes (30 up- and 11 down-regulated) were identified as taking part of the SOS regulon of *E. faecalis*. Among these genes, some were known to take part of other bacteria SOS regulon but some genes appear to be unique for the SOS response in *E. faecalis* such as the upregulation of two phage integrases, the downregulation of the eight genes involved in the synthesis of aromatic amino acids and the absence of low fidelity polymerase gene regulation.

This study identified for the first time the SOS regulon of *E. faecalis* with some apparent unique features. The number of genes whose expression is modified by the cleavage of LexA is concordant with previous studies in other bacteria. This study permitted also to identify precisely the *E. faecalis* LexA-box consensus sequence and to open the door for the discovery of new antimicrobial drugs targeting this survival mechanism.

**P1286 Growth condition-dependant cell surface proteome analysis of *Enterococcus faecium***

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**Objectives:** Over the last 30 years *Enterococcus faecium* (Efm) has become the third most common nosocomial pathogen in hospitals worldwide. The aim of current study was to obtain insight in cell surface expression of Efm proteins when grown in physiological and clinically relevant conditions.

**Methods:** Cell surface protein expression of a clinical blood stream Efm isolate, E1162, grown until mid-log phase in brain heart infusion medium including 0.02% bile salts (BHI-bile), urine and biofilm was compared with BHI grown cells. After washing the cell pellet, the cell surface was "shaved" using immobilized trypsin in 50 mM sodium-bicarbonate for 45 min at 37°C. The protein fragment containing supernatant was subsequently further digested using trypsin and peptides were identified using tandem mass spectrometry. The identified peptides were matched against the EfmE1162 whole genome sequence. Finally, LocateP was used to predict the subcellular location of the identified protein. All growth conditions were performed in six biological replicates.

**Results:** For the different growth conditions a total of 15, 11, 13, 13 and 11 proteins, in at least three of the six biological replicates, were identified in BHI-bile, urine, biofilm, TSB 1% glucose, and BHI respectively. Prediction of the subcellular location of identified proteins revealed that for BHI-bile 84%, urine 67%, biofilm 76% and BHI 67% belong to either lipid anchored, n-terminally anchored, secreted, multi-transmembrane or LPxTG anchored group of proteins. In general, the expression of proteins at the cell surface was very comparable with 10 proteins, including two ABC transporters, being expressed and detected in at least three of the studied growth conditions. Differentially expressed proteins included the Penicillin binding protein 5 (PBP-5), which was not identified in urine, a hypothetical lipoprotein that was only identified in urine, and a hypothetical lipid anchored surface protein that was only identified in BHI-bile.

**Conclusion:** This study revealed that proteolytic shaving of Efm cells identified cell surface proteins expressed during growth under different conditions. Differentially expressed proteins are of special interest as they might be involved in the interaction of Efm with the human host and may represent interesting candidate targets for vaccine development. Functional characterization of these proteins will provide more insight in the adaptive mechanisms of this emerging pathogen.

**P1287 Emergence of plasmid-mediated fosfomycin resistance gene, fosB, among *Enterococcus faecium* clinical isolates**

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**Objectives:** Fosfomycin is used alone or in combination with other antimicrobials for the treatment of infections caused by multi-drug

resistant Gram-positive bacteria, and fosfomycin resistance (FR) in *E. faecium* increased from 20.4% in 2007 to 32.1% in 2009 in China. Plasmid-mediated FR determinants, fosA, fosB and fosC, have been discovered and found to be present in fosfomycin-resistant *Escherichia coli* (fosA, fosC), *Enterobacter cloacae* (fosA), *Klebsiella pneumoniae* (fosA), *Staphylococcus* spp. (fosB). In a recent study on vancomycin-resistant Enterococci (VRE), we found that fosB was present in an *E. faecium* isolate (GenBank: HQ219726). In this study, we determined the prevalence of fosA, fosB and fosC genes in *E. faecium* clinical isolates.

**Methods:** A total of 148 clinical strains of *E. faecium* were isolated from patients in 10 cities of China between 2008 and 2009. Antimicrobial susceptibility testing was performed by agar dilution method according to the Clinical and Laboratory Standards Institute (CLSI). fosA, fosB and fosC were detected by PCR amplification. Multilocus sequence typing (MLST) of *E. faecium* isolates was performed and the sequence types (STs) were analyzed through the MLST database (<http://efaecium.mlst.net/>).

**Results:** Of the 148 isolates, 12 (8.1%) were resistant to (MIC  $\geq$  256 mg/L) and 28 (19%) were intermediate (MIC 128 mg/L) to fosfomycin. fosB gene was present in 10 FR isolates with fosfomycin MICs  $\geq$  1024 mg/L, but not in other two strains with MICs of 256 mg/L or 512 mg/L. Among 10 fosB positive strains, six were VRE with vancomycin MICs  $\geq$  256 mg/L, while three of 138 fosB negative strains were VRE. The 10 fosB positive strains were analyzed by MLST and showed seven STs, including STs 18, 34, 78, 203, 389, and 559. No fosA and fosC were found in any of the isolates.

**Conclusion:** Plasmid-mediated FR gene, fosB, is emerging in *E. faecium* clinical isolates, and spreading by horizontal transfer and clonal dissemination. This is the first report of plasmid-mediated FR gene fosB in *E. faecium*.

**P1288 Contact isolation for vancomycin-resistant enterococci?**

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**Background:** Vancomycin-resistant enterococci (VRE) are primarily opportunistic pathogens with variable virulence and incalculable clinical significance. Many containment strategies lack VRE specific interventions often due to the lack of relevant and reliable epidemiological data.

The primary goal of this study was to estimate the transmissibility of in-patients with VRE bacteraemia to other hospitalized patients. The secondary goal was to document reliable epidemiological data on all VRE cases in a tertiary healthcare centre.

**Methods:** A prospective survey on in-patients colonized and/or systemically infected with VRE was conducted at a 2000-bed university hospital in Germany. All contact patients of bacteraemic VRE patients were analysed. Pulsed-field gel electrophoresis was performed to assess clonal relatedness.

**Results:** A total of 16 507 screening tests were carried out, on 9258 screened patients, of which 557 patients tested positive for VRE (6.0%). Nineteen patients suffered from VRE bacteraemia, an incidence of 0.21%. Co-morbidity was highly frequent in this cohort and exposure rates to external risk factors were high (e.g. previous hospital stay prior to admission 78.9%). The transmission rate to contact patients was low (3.6%). Contact time of negative contact patients was significantly lower than that of VRE-positive contact patients (19.3 hours vs. 72.0 hours,  $p < 0.006$ ).

**Conclusions:** Systemic VRE infection was found exclusively in multi-morbid patients, transmission occurred seldom and average contact time of positive patients was very high. Our data provide evidence that not all carriers of VRE require contact isolation, since therapeutic options are available and given that transmission is not likely. Isolation consumes massive amounts of hospital resources and inflicts psychological stress on patients.

**P1289** Prevalence of phenotypically vancomycin susceptible, but vanB-PCR positive, *Enterococcus faecium*. Do we overlook VRE vanB carrying strains in our hospitals?

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**Objectives:** Prevalence investigation of vanB positive *E. faecium* in large university hospital in the northern part of the Netherlands.

**Methods:** We screened retrospectively 108 phenotypically vancomycin susceptible *E. faecium* isolates identified by standard protocol from blood culture. In addition, we performed a prospective prevalence study, were 446 rectal swabs collected from 294 patients were cultured. Swabs were grown in BHI broth for 24 hours containing amoxicillin 16 mg/L, amphotericin B 20 mg/L, aztreonam 20 mg/L, colistin 20 mg/L. Different to usual selective detection strategies and in order to increase sensitivity for detection no vancomycin was added to the broth. BHI was subcultured on Brilliance VRE Agar (Oxoid) and read after 48 hours. Blue colonies were subcultured on blood agar and identified by MALDI-TOF MS analysis. All *E. faecium* and *E. faecalis* strains were tested for vanB and TN5382 with PCR and resistance was determined by VITEK® 2. PCR products from three selected isolated were sequenced for confirmation.

**Results:** Three out of 108 (2.7%) vancomycin susceptible clinical blood culture isolates were positive for vanB. The screening results from the prevalence screening showed that, nine patients had an *E. faecium* isolate positive for both vanB and TN5382. Hereof, three patients (1%) were identified phenotypically by Vitek as VRE with a MIC value of 8, the six other isolates (2%) were not identified as VRE by Vitek, these six strains had an MIC value of 1. Etest confirmed the low MIC values. Three sequenced vanB PCR products matched with vanB2. In total, 63 *E. faecium* and 38 *E. faecalis* strains were negative for vanB. These vanB negative strains could be distinguished on Brilliance VRE agar by limited growth.

**Conclusion:** VanB positive *E. faecium* with an MIC value of 1 are likely to be missed in routine practice. The clinical relevance of these low MIC vanB positive *E. faecium* needs further research.

**P1290** Global dissemination of vancomycin-resistant VanB *Enterococcus faecium* causing outbreaks in different countries is mainly associated with chromosomal Tn1549/5382-like platforms

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**Objectives:** Dissemination of VanB *Enterococcus faecium* (Efm) results from transfer of plasmids or, more often, large chromosomal elements. We analysed the genetic context of representative isolates recovered from different continents in order to better understand the global epidemiology of VanB-Efm.

**Methods:** We analysed 20 Efm collected in Australia, Chile, Denmark, Finland, Hungary, Italy (IT), Singapore (SI), Spain (SP), and USA (1992–2008), representative of VanB clinical outbreaks in these areas. Clonality was established by PFGE/MLST. vanB was amplified using specific primers and transposons were discriminated by comparison of DNA profiles after digestion of 5959-bp vanRSYWHBX amplicons with BspHI/DraI enzymes. Distinction between vanB1-Tn1547 and vanB2-Tn1549/Tn5382 was established by specific sequences (right junction of Tn5382; vanSB-vanYB intergenic region; pbp5-Tn5382 association). Genomic location and transfer of vanB was determined by standard procedures. Plasmid analysis included determination of size (S1-PFGE), relaxases, rep initiation proteins, and toxin-antitoxin systems (PCR, hybridization, sequencing).

**Results:** Sixteen clones (11 STs, all but one belonging to CC17) were identified. vanB1 isolates (n = 3) showed RFLP-1 associated with Tn1547 (two USA; early 1990s) and RFLP-3 linked to a new vanRSYWHBX variant (one IT; 2002). This vanB1 variant resulted from recombination between Tn1549 (vanRS) and Tn1547 (vanS), with

ends of both flanking genes (vanR, vanX) being identical to those of pMG2200::vanB2-Tn1549 from *E. faecalis* in Japan. The vanB2 (n = 17) showed RFLP-2 (n = 13) linked to Tn1549/5382 and was predominant and widespread in different continents. Variants of this platform caused by insertions of ISEnf110 or ISEnf200, and the presence of pbp5 (RFLP2\*, n = 4) were detected in Europe, America and Australia. All but the 2 RFLP-1-isolates contained rel-Tn1549. vanB was transferred in 30% of the cases. Most were located on chromosome (n = 16). The four vanB2-conjugative plasmids (50–60 kb) from SP and SI carried rep-pRUM, rep-Inc18 and/or rel-pEF1. **Conclusions:** Global dissemination of VanB enterococci is mostly due to vanB2-Tn1549/Tn5382 carried by CC17 Efm. A Tn1547-Tn1549 (vanB1) hybrid is firstly reported highlighting frequent lateral gene processes among enterococci. Differences among similar transposons indicate evolution of these elements in different locations. Predominance of vanB2 over vanB1 transposons among Efm requires further studies.

**P1291** Microbiologic and clinical characteristics of vancomycin-resistant *Enterococcus faecium* bacteraemia in Taiwan: a retrospective cohort study

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**Objective:** Bacteremia caused by vancomycin-resistant *Enterococcus faecium* (VREfm) increased rapidly in our institute recently. We aimed to evaluate if there were newly introduced VREfm clones contributing to this increase and the risk factors for 14-day mortality of VREfm bacteremia.

**Methods:** Between January 2003 and December 2010, all patients with VREfm bacteremia were enrolled to analyze the clinical features and antibiotic susceptibility, genotype of vancomycin resistance, and multilocus sequence typing (MLST) of the relevant VREfm isolates were analyzed. Primary end-point was all-cause mortality within 14 days of bacteremia.

**Results:** Vancomycin resistance among non-duplicated blood enterococcal isolates during the study period increased significantly from 3.9% to 18.9% (p < 0.0001). There were 149 episodes of VREfm bacteremia noted. One hundred and two VREfm isolates were available for further analysis. Ninety-three isolates carried the vanA gene. All isolates were susceptible to daptomycin and linezolid. Only two isolates belonging to ST18 were intermediately resistant to tigecycline. ST18 and ST414 were the emerging predominant sequence types (20.6% and 17.6%, respectively). Using of immunosuppressant agents, thrombocytopenia, high Charlson comorbidity index, and septic shock were independently associated with 14-day mortality of VREfm bacteremia. In contrast, male sex, patients received adequate treatment, and whose VREfm isolates belonged to ST414 had a significantly lower in-hospital mortality rate.

**Conclusions:** Vancomycin resistant rate increased in blood enterococcal isolates causing bacteremia. Among tested VREfm isolates, the susceptible rate to daptomycin, linezolid, and tigecycline remains high. Clonal spread of VREfm belonging to ST18 and ST414 might contribute to the increase of VRE. The sequence type of VREfm may play an important role in 14-day mortality of patients with VREfm bacteremia.

**P1292** Molecular characterisation of vancomycin-resistant *Enterococcus faecium* from a tertiary care centre in Saudi Arabia

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**Objective:** There remains a paucity of data on the molecular epidemiology of Vancomycin resistant enterococci in the Arabian Gulf Region. This study was carried out to characterize the vancomycin genotype/phenotype, carriage of putative virulence genes and genetic



relatedness of *E. faecium* isolated in a tertiary care center in Saudi Arabia.

**Methods:** From January 2009 to April 2010, *E. faecium* isolates from inpatients at the King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia were studied. Antimicrobial susceptibility testing to ampicillin, linezolid, teicoplanin, quinupristin/dalfopristin, tetracycline and ciprofloxacin was determined using Microscan Walkaway, disk diffusion and E-test. PCR assays for detection of Van genes and virulence genes for aggregation substance (Agg, Asa1), enterococcal surface proteins (esp); cytolysin (cylA, cylL, cylM), gelatinase (gelE), cell wall adhesin (EfaAfm), hyaluronidase (hyl), surface antigens (Ef) and collagen adhesin (Ace) were carried out. Genetic relatedness of isolates was determined using pulsed field gel electrophoresis (PFGE).

**Results:** The twenty-nine *E. faecium* isolates mostly stool isolates (n/N = 22/29; 75.8%) were found to show seven PFGE banding patterns (A–G). Majority of isolates (n/N = 25/29; 86%) were in three predominant pulsotypes. All isolates were positive for VanA gene and 13 exhibited VanA+/VanB+ genotype. Of these, 10 exhibited VanB phenotype and three had VanA phenotype. Eight isolates with VanA+/VanB– genotype exhibited a VanB phenotype with six of them belonging to the same PFGE pulsotype. All isolates were positive for gelE, esp and EfaAfm genes. All isolates were negative for Ace, Agg, Asa1 and CylL genes while five were positive for the CylA. The hyl and Ef genes were present in 24 and eight isolates respectively. Of the eight isolates positive for a combination of gelE, esp, EfaAfm, hyl and Ef genes, four showed VanB phenotype VanA genotype incongruence.

**Conclusions:** The findings demonstrate the first report of the emergence of VanB phenotype-vanA genotype incongruent *E. faecium* isolates in the Middle East region. Molecular typing suggests a clonal spread of these isolates. The high occurrence of virulence genes especially esp genes associated with epidemic *E. faecium* isolates is a cause for concern.

## Epidemiology of antimicrobial resistance

### P1293 In vitro activity of rifaximin against *Escherichia coli* with prevalent ESBLs and carbapenemases

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**Objectives:** Rifaximin, a semi-synthetic rifamycin antibiotic, is licensed for treatment of travellers' diarrhoea in the USA and many European countries. As it is poorly absorbed from the gut, there is a theoretical potential for its use to select rifamycin-resistant strains in the gut flora, and for these to have resistance determinants of greater public health significance. We assessed the in-vitro activity of rifaximin and comparators against 90 clinical isolates of *E. coli* that produced ESBLs and/or carbapenemases.

**Methods:** The 90 non-diarrhoeal isolates of *E. coli* produced CTX-M-15 (n = 52), CTX-M-14 (7) or CTX-M-2 (1) ESBLs, or NDM-1 (16), OXA-48 (9), KPC-2 (2), IMP-type (2) and VIM-type (1) carbapenemases. MICs were measured by CLSI agar dilution methodology. Comparator agents were rifampicin, ciprofloxacin, azithromycin, co-trimoxazole and doxycycline. All isolates non-susceptible to either rifamycin (MIC > 32 mg/L) were screened by PCR for arr genes, which encode rifamycin ADP-ribosyltransferases.

**Results:** Rifaximin was active at  $\leq 32$  mg/L (vs. mode = 16 mg/L) against 84/90 (93.3%) of the *E. coli* isolates, but MICs were >128 mg/L for 3/16 isolates with NDM-1 carbapenemase, 1/9 with OXA-48 carbapenemase, and 1/52 with CTX-M-15 ESBL. One isolate with CTX-M-15 ESBL was borderline non-susceptible (MIC 64 mg/L). Seven isolates, including the above six, were non-susceptible to rifampicin (MICs > 32 mg/L vs. mode = 16 mg/L) whilst 63.3% of the isolates were non-susceptible to ciprofloxacin (MICs > 0.5 mg/L) 44.4% to azithromycin (MICs > 16 mg/L; BSAC ECOFF for *S. Typhi*), 81.1% to doxycycline (MICs > 4 mg/L; EUCAST ECOFF for *E. coli*) and 84.4% to co-trimoxazole (MICs > 2 mg/L). Of the

seven isolates with rifamycin MICs > 32 mg/L, only two (both with NDM carbapenemase) had arr-2/-3 genes.

**Conclusion:** Rifaximin was active against most ESBL- and/or carbapenemase-producing *E. coli*. However, high-level resistance was detected in three isolates with NDM-1 metallo-beta-lactamase, one with OXA-48 carbapenemase, and one with CTX-M-15 ESBL. Rifaximin might select such strains in the gut flora of travellers to countries where these resistance mechanisms are prevalent, but there were stronger associations between ESBLs/carbapenemases and resistance to ciprofloxacin or azithromycin implying greater selectivity with these latter alternatives.

### P1294 Evolution of antimicrobial resistance of *Escherichia coli* isolates causing obstetric infections: relationship with virulence and clinical features

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**Objectives:** *Escherichia coli* is a Gram-negative bacilli that can colonize the female genital tract and become implicated in different infections in pregnant women, both from genital and urinary foci, causing septicaemia or not. The aim of this study was to determine the evolution of the antimicrobial resistance of *E. coli* isolates causing obstetric infections as well as to compare the relationship between resistance profiles, virulence factors and clinical features caused by these strains.

**Methods:** Seventy-eight *E. coli* isolates from women in the Hospital Clinic of Barcelona collected from 1987 to 2010 were included in the study. Among these strains, 24 caused genital-focus sepsis (GFS), 32 UTI-related sepsis (URS) and 22 non-bacteraemic intraamniotic infection (NII). Virulence profiles were analysed by PCR and sequencing using gene-specific primers for 17 virulence genes. Resistance profiles were determined by minimal inhibitory concentrations using the MicroScan-Negative MIC Panel Type 37. *E. coli* phylotype screening was carried out by multiplex PCR.

**Results:** The percentage of resistance in *E. coli* strains has increased for several antimicrobial agents over the years, mainly for nalidixic acid but also for ciprofloxacin, tetracycline and cotrimoxazole. There has also been a significant increase in resistant strains belonging to phylotype D ( $p = 0.04$ ). Compared to clinical features, the resistance profiles from strains causing GFS showed a greater percentage of nalidixic acid, gentamycin and kanamycin resistance compared to strains causing URS or NII. The most virulent strains were those causing septicaemia, as they possess significantly more virulence factors such as hly, cnf, papA, iha, fyu or papG3, all contained within pathogenicity islands. Regarding virulence factors related to iron recruitment, it was of note that the iut (aerobactin-siderophore receptor) was significantly more frequently found in septicaemia-causing strains ( $p < 0.0001$ ), whereas in non-bacteraemic strains iroN (catechol-siderophore receptor) was the most frequent ( $p = 0.041$ ).

**Conclusions:** The increasing percentage of resistance to quinolones in strains causing obstetric infections is a matter of concern. *E. coli* strains causing GFS are more resistant than other strains. Further studies are necessary to elucidate the role of the iron recruitment systems used by the microorganism depending on the environment it colonises.

### P1295 Clonal structure of invasive *Streptococcus pneumoniae* collected from adult patients in Spain

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**Objectives:** To analyze the clonal composition of invasive *Streptococcus pneumoniae* collected from adult invasive pneumococcal disease (IPD) in seven Spanish hospitals.

**Methods:** A prospective, active, hospital-based surveillance of all culture-confirmed IPDs in adults ( $\geq 18$  years) was performed in seven Spanish hospitals from August 2010 to June 2011. IPD was considered

isolation of *S. pneumoniae* in normally sterile fluids (blood, cerebrospinal fluid, pleural fluid...). Serotyping was performed by the Quellung reaction, dot blot assay or real-time PCR. All isolates were genotyped PFGE (SmaI). A selection of 37 isolates representative of major PFGE pattern were selected for MLST.

**Results:** A total of 191 cases were identified. The most frequent serotypes were: 3 (11.5%), 19A (9.4%), 7F (8.4%) 1 (6.3%) and 14 (5.8). A total of 72 PFGE patterns were found among 191 pneumococci analyzed, of them 19 accounted for three or more isolates and were selected for MLST. Regarding PFGE and MLST results the most frequent clones among invasive pneumococci were: Netherlands-7F-ST191 (8.4%), Netherlands-3-ST180 (7.3%), Denmark-14-ST230 (6.3%; serotypes 24F and 19A), Sweden-1-ST306 (5.2), Sweden-15A-ST63 (5.2%; serotypes 15A, 19F and 8), Spain-9V-ST156 (4.7%; serotypes 9V and 14), and ST260 (3.7%, serotype 3). Resistance to three or more antimicrobial groups, was detected in 53 isolates (27.7%) and was associated with genotypes CC230 (serotypes 24F, 19A; n = 10), CC63 (serotypes 8, 15A, 19F; n = 10), CC156 (serotypes 9V, 14; n = 5), ST386 (serotype 6C; n = 5), and ST320 (serotype 19A; n = 4).

**Conclusion:** Two antibiotic susceptible clones, Netherlands-7F-ST191 and Netherlands-3-ST180, were the most frequent cause of adult IPD in Spain. One fourth of multidrug-resistant invasive pneumococci belonged to five genotypes. Capsular switching was observed in three multidrug-resistant clones (CC230, CC63 and CC156).

#### P1296 The impact of efflux activity on drug resistance of *Burkholderia cepacia* complex isolated from non-cystic fibrosis patients

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**Objectives:** *Burkholderia multivorans* is an opportunistic pathogen with a remarkable capacity to develop resistance to an extensive range of antibiotics and disinfectants.

The aim of this study was to determine the contribution of efflux to antimicrobial resistance in a panel of highly drug resistant *B. multivorans* isolated from non-cystic fibrosis (non-CF) patients.

**Methods:** In our study we tested efflux activity of 46 strains of *B. multivorans* which were obtained from clinical specimens of patients hospitalized from 1 February to 30 September 2011 in University Hospital in Olomouc, Czech Republic. All strains were unique, obtained from different patients.

Susceptibility of isolates to tigecycline (TIG), piperacilin (PIP), ciprofloxacin (CIP) and chlorhexidine was determined according to the guidelines of the British Society of Antimicrobial Chemotherapy. Experiments were repeated in the presence of Phe-Arg beta-naphthylonide dihydrochloride (PABN) as an inhibitor of efflux. Accumulation of Hoescht 33342 was also determined fluorimetrically in the presence and absence of PABN and used as a measure of efflux activity.

**Results:** Hoescht accumulation experiments defined the optimum PABN concentration required for inhibition of efflux to be 100 mg/L. Whilst accumulation of Hoescht varied between the isolates all isolates exhibited low accumulation of the dye when compared to other proteobacteria. There were no obvious correlations between efflux activity and antibiogram of the isolates suggesting all *B. multivorans* exhibit an intrinsically high efflux activity (Table 1). The isolates demonstrated a high degree of tolerance to chlorhexidine with MIC50 and MIC90 values of 100 mg/L an MBC50 of 100 mg/L and MBC90 of more than 100 mg/L of chlorhexidine.

Table: MIC50, MIC90 and range of MICs with and without PABN of 46 *Burkholderia multivorans* strains

Drug	CIP	CIP+PABN	PIP	PIP+PABN	TIG	TIG+PABN
MIC50	0,06	0,06	1	1	1	2
MIC90	0,125	0,125	2	1	4	4
Range	0,06 - 2	0,06 - 2	1 - 4	0,5 - 1	0,5 - 8	1 - 8

Legend: CIP-ciprofloxacin, PIP-piperaciline, TIG-tigacycline, PABN-Phe-Arg beta-naphthylonide dihydrochloride

**Conclusion:** In 46 clinical isolates of *B. multivorans* there was a high degree of efflux activity in all strains, differences in resistance to TIG, PIP and CIP in these strains are not likely to be due to differential efflux activity. Clinical strains of *B. multivorans* are highly resistant to chlorhexidine, which may have a clinical impact for anti-epidemic management and allow survival and spread of these strains in the hospital environment.

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#### P1297 Faecal carriage of *Escherichia coli* O25b:h4/ST131: preliminary results of a survey in nosocomial and community settings

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**Objectives:** Intercontinental *Escherichia coli* clonal group producing different extended-spectrum beta-lactamases (ESBLs) and carbapenemases has been reported recently. This clonal group belongs to the B2 phylogenetic group, to the serotype O25b:H4 and to the multilocus ST131 and causes approximately one in every 10 extraintestinal infections at this moment in Spain, with and without multiresistant pattern. The aim of this study was to determine the prevalence of faecal carriage of this clone in hospital and community settings.

**Methods:** From April 2010 to October 2011, all clinical *E. coli* isolates recovered from hospitalized and emergency room-attended patients were screened for O25b and pabB by using PCR method. Positive isolates were studied for B2 phylogroup by multiplex PCR assay. Rectal swabs were obtained from all participants who agreed to participate. Four groups were studied: cases with infections caused by O25b/pabB/B2 positive isolates, roommates of hospitalised patients or patients who shared nursery care with the case, household members of community cases and 50 healthy volunteers. Rectal swabs were inoculated on Brilliance UTI agar and 4 mg/L cefotaxime Mac Conkey agar. All distinct morphotypes of lactose-positive colonies were selected for screening of O25b/pabB/B2.

**Results:** A total of 27 cases were included in this study: 16 inpatients and 11 patients with community-acquired infections (17 had UTI infections, six suffered bacteraemia and four soft tissue infections). Besides, 30 hospitalised roommates and 25 healthy household members were also studied. The prevalence of faecal carriage of this clone was 60% in hospitalized patients and 42% in community cases (p = 0.45). The faecal O25b *E. coli* colonization was higher in household contacts (36%) than in hospitalised contacts (13.3%) (p = 0.05) and 2% of volunteers were found to be positive. Household contacts of patients with infection who harboured this clone in the rectal flora were found to be more frequently positive for ST131, 50% vs. 23%, but not statistically significant (p = 0.23).

**Conclusions:** The prevalence of faecal carriage of ST131 clone is higher in healthy relatives of infected patients than roommates of inpatients and healthy volunteers. These preliminary results suggest transmission or common acquisition of ST131 within households.

#### P1298 The development of antimicrobial resistance patterns of commonly Gram-negative bacteria isolated from bronchial secretions in intensive care unit in anticancer hospital Thessaloniki GR

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**Objectives:** The study and evaluation of the resistance rates development of the most frequently Gram(-) bacteria isolated from bronchial secretions in intensive care unit (ICU) patients.

**Methods:** The period 2007–2010, 297 Gram(–) bacterial agents from bronchial secretions were isolated. Identification of microorganisms and susceptibility testing were performed using the WIDER semi-automatic system. Carbapenemase were detected using Hodge test with ertapenem disk and E-test (Imipenem (IMP)/IMP + EDTA). Analysis of data was performed using the WHONET software and statistical analysis with  $\chi^2$  Yates' Correction test ( $p < 0.05$  was considered as significant).

**Results:** Out of 297 Gram(–) species, the predominant pathogen was *P. aeruginosa* ( $n = 113$ ), *A. baumannii* ( $n = 69$ ) and *K. pneumoniae* ( $n = 56$ ) followed. 52/113 (46%) *P. aeruginosa* isolates presented resistance to IMP which from 50% in 2007 decreased to 38% in 2010. Metallo-beta lactamases (MBL) were detected in 33/52 (63%) strains. Statistically significant (S.S.) decrease in resistance to ciprofloxacin (CIP) observed since 2007 (45%) to 2010 (19%) ( $p = 0.003$ ). Gentamicin (GEN) showed a S.S. increase in resistance rates from 2007 (15%) to 2008 (43%) ( $p = 0.03$ ) and reduction from 2009 (19%) to 2010 (3%) ( $p = 0.04$ ). During the 4-year period all isolates were sensitive to colistin (COL). High resistance rates were observed for multidrug-resistant isolates *A. baumannii* to IMP from 2007 to 2009 (84–81%) while in 2010 turned into 30%. A S.S. reduction in Carbapenems resistance exhibited the years from 2009 to 2010 ( $p = 0.008$ ). No strain was resistant to COL. Among *K. pneumoniae* strains 28/56 (50%) were resistant to IMP. Resistance to Carbapenems increased successively from 17% in 2007 to 59% in 2010. All resistant strains had a positive Hodge test. Twelve percent (3/28) were MBL(+) while the rest possibly produce KPC Carbapenemase and requires further testing. Resistance rates to CIP increased from 17% in 2007 to 64% in 2010 ( $p = 0.04$ ). Amikacin resistance from 50% in 2008 reduced to 18% in 2010 while GEN from 19% in 2009 was reset in 2010 ( $p = 0.04$ ). All isolates were susceptible to COL.

**Conclusion:** High rates of MDR (*P. aeruginosa*, *A. baumannii*, *K. pneumoniae*) strains in ICU are threatening fact. COL remains the unique antibiotic with no resistance. Significant reduction in GEN resistance recorded in 2010. In order to reduce resurgence of multi-drug-resistant isolates, judicious selection of antibiotics is required.

#### P1299 Clinical features and outcome of infections due to panderug-resistant Gram-negative bacteria

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**Objectives:** Infections caused by panderug-resistant (PDR) gram-negative bacteria are of particular concern due to lack of therapeutic options. The purpose of this study was to describe the characteristics and outcome of gram-negative PDR bacterial infections cared for at a Greek University Hospital.

**Methods:** A series of patients with infections due to PDR gram-negative pathogens hospitalized between December 2009 and June 2011 is presented. An isolate was considered as PDR if resistant to all classes of antimicrobial agents available.

**Results:** In total 17 episodes of infection with PDR bacterial infections occurring in 17 patients have been studied. Their median age was 58 (range: 30–81). Eleven patients (65%) had been treated in the Intensive Care Unit (ICU) during the current hospitalization. Eleven patients (65%) had more than three co-morbidities. Nine patients (53%) had been treated with antimicrobials the previous 3 months including colistin in 5 (38.5%) and tigecycline in 2 (15%). The median duration of hospitalization before the isolation of the PDR microorganism were 25 days (range: 2–91). The PDR microorganisms isolated included 14 *K. pneumoniae* (74%), 3 *A. baumannii* (16%) and 2 *P. aeruginosa* (10.5%). One patient had a polymicrobial infection with three PDR bacteria (*K. pneumoniae*, *A. baumannii*, *P. aeruginosa*). The PDR bacteria were isolated from blood (five patients), pus (4), urine (3), peritoneal fluid (3) BAL (1) and cerebrospinal fluid (1). Treatment was based mainly on colistin (11 patients), tigecycline (10), carbapenem (8) and aminoglycosides (6). The median length of antimicrobial therapy was 44 days (range: 9–162) and the median

duration of hospitalization 45 (range: 2–168). The overall in-hospital mortality was 41% (seven patients), with an infection-related mortality 23.5% (4).

**Conclusions:** The study presents characteristics and outcome of infections due to PDR gram-negative bacteria associated with considerable mortality and prolonged hospitalization. Most of the patients were characterized by multiple co-morbidities, prior hospitalization and ICU admission as well as previous treatment with colistin or tigecycline. These characteristics seem to enhance the risk for developing PDR infections.

#### P1300 Invasive pneumococcal disease in HIV-infected patients in France from 2000 to 2011. Antimicrobial susceptibility and implication of serotypes for vaccination

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**Objectives:** Invasive pneumococcal diseases (IPD) remain frequent and severe in HIV-infected subjects. Changes in antimicrobial susceptibility may challenge empirical antibiotic choices. The polysaccharide 23-valent vaccine (PPV23) is recommended in HIV infected subjects, although not fully efficient. Whether PPV23 and the pneumococcal conjugate vaccine (7-valent; PCV7 or 13-valent; PCV13) cover the serotypes most frequently isolated in HIV-infected patients is unclear.

**Methods:** We retrospectively analysed all *S. pneumoniae* isolated from blood cultures, synovial fluid or CSF between 2000 and 2011 in HIV-infected patients from a single reference centre for the management of HIV infection in Paris. MIC of antibiotics were determined by E-test and serotyping was performed by the antiserum agglutination method.

**Results:** Forty HIV patients presented with IPD during the study period. Eighty percent were men, median age was 42 years (23–62), mean CD4-cell count: 251/mm<sup>3</sup>. Half were receiving antiretroviral therapy and 25% had plasma HIV-RNA <400 copies/mL. Only 5% had been vaccinated by PPV23. Resistance rates of *S. pneumoniae* to penicillin G, cefotaxime, cotrimoxazole and levofloxacin were 16.3%, 2.4%, 31.6% and 29.4%, respectively.

Serotyping was performed on 27 strains; 18 different serotypes were observed. 19A, 14, 7F and 6A serotypes were the most frequently represented (6, 3, 2 and 2 strains, respectively), which is a similar pattern to the epidemiology of IPD in the French population. The PCV7, PCV13, PPV23 vaccines and both PCV13 + PPV23 would have covered 26%, 70%, 78% and 85% of the serotypes found, respectively.

**Conclusions:** Resistance to antibiotics and in particular fluoroquinolones is of particular concern in HIV infected patients with IPD. Pneumococcal serotypes are theoretically well covered by both PPV23 and PCV13 vaccinations. Recommended pneumococcal vaccination should be more thoroughly implemented in this population.

#### P1301 Haemophilus haemolyticus as a potential reservoir for ftsI gene mutations and altered penicillin-binding protein 3 mediated resistance in Haemophilus influenzae

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**Objectives:** B-lactamase-negative ampicillin-resistant (BLNAR) strains of *H. influenzae* are being increasingly recognised. BLNAR strains are characterized by specific ftsI gene mutations and associated amino acid substitutions in the encoded penicillin binding protein 3 (PBP3). The N526K (BLNAR defining) PBP3 substitution occurs in almost all BLNAR strains and resistance is enhanced by further substitutions (BLNAR associated) at other sites. It is generally accepted that most BLNAR strains emerge from de novo ftsI mutations in previously susceptible strains although there is some evidence of inter-strain horizontal transfer of mutated ftsI genes. Significantly, there has been one report of horizontal transfer of the ftsI gene from the respiratory commensal *H. haemolyticus* to *H. influenzae*. A role was

proposed for *H. haemolyticus* as a reservoir of *ftsI* genes to mediate BLNAR type resistance in *H. influenzae* through recombination and formation of mosaic *ftsI* genes. Little is known about the frequency, nature or effect on susceptibility of *ftsI* mutations in *H. haemolyticus* and the aims of this study are to investigate this.

**Methods:** A collection of 349 strains of XV dependent *Haemophilus* spp. from a geographically restricted cohort of children with otitis media were further identified by 16sRNA PCR. Subsequently, all isolates of *H. influenzae* and *H. haemolyticus* were tested for susceptibility to ampicillin and amoxicillin-clavulanate using CLSI broth dilution, and screened for N526K associated *ftsI* mutations using single nucleotide specific PCR. The *ftsI* genes of all *H. haemolyticus* and PCR screen positive *H. influenzae* were sequenced.

**Results:** Of the 349 strains, 33 were confirmed as *H. haemolyticus*, and 248 confirmed as *H. influenzae* by 16sRNA PCR. The remaining 68 strains gave equivocal 16sRNA PCR results and were excluded from further analysis. A significantly higher proportion of *H. haemolyticus* (19/33) had the BLNAR defining N526K genotype than *H. influenzae* (80/248) and the presence of this mutation was associated with raised MICs compared to the ATCC strain. Furthermore, a range of BLNAR associated substitutions were seen in strains of *H. haemolyticus* with and without N526K, and appear to represent part of the baseline genotype.

Table 1: Deduced amino-acid substitutions in PBP3 of the 33 *H. haemolyticus* strains compared to *H. haemolyticus* ATCC and *H. influenzae* Rd control.

Strain genotype (n) <sup>1</sup>	Amino Acid at position										MIC (mg/L)	
	274	350	352	377	389	449	490	502	526	547	AMP	AMC
<i>H. influenzae</i> RD	E	D	S	M	L	I	G	A	N	V	0.5	2
<i>H. haemolyticus</i> ATCC 3390 <sup>2</sup>	D	N	G	I	L	I	G	A	N	I	0.5	0.5
<i>H. haemolyticus</i> BLNAs (n=14)	D	N	G	I	L	I	G/E	A	N	I	0.25-1	0.25-2
<i>H. haemolyticus</i> BLNAR <sup>3</sup> (n=7)					F	V		T	K	T	0.25-1	0.25-1
(n=1)							E	V	K		0.5	0.5
(n=3)	E							V	K		0.25-1	1-2
(n=2)	E		T	M				T	K		1	2
(n=2)	E	D	T	M				T	K	V	1	1-4
(n=5)									K		0.5	2

<sup>1</sup> Total number of *H. haemolyticus* strains = 33

<sup>2</sup> Boldface underlined type; known BLNAR associated substitutions in *H. influenzae*.

<sup>3</sup> Shows the deduced amino acid substitutions in PBP3 when compared to the baseline *H. haemolyticus* ATCC 3390 sequence.

**Conclusions:** There is a high prevalence of BLNAR defining and associated PBP3 substitutions in the *H. haemolyticus* strains studied here, with potential to be a reservoir for inter-species transfer of resistance to *H. influenzae*.

### P1302 Are biocides and antibiotic resistance driving the emergence of *Salmonella enterica* serotype 4,[5],12:i:- (monophasic variant of *S. Typhimurium*)?

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**Objectives:** *Salmonella enterica* serotype 4, [5],12:i:-, a monophasic variant of serotype *S. Typhimurium*, has become one of the most worldwide common serotypes related to human infections transmitted by food chain. In order to better understand if the rampant emergence of this serotype was linked to particular bacterial features we evaluate the clonal relationship and characterize the phenotype and genetic determinants associated with antibiotic (AB) and biocide (B) resistance of *S. enterica* serotype 4,[5],12:i:- from different sources.

**Methods:** Isolates from humans (n = 115), food (n = 9), environment (n = 4) and piggeries (n = 4) recovered in Portugal (2002–2010) were studied. The serotype was confirmed by PCR (fljA, fljB, hin and fljA-fljB). Susceptibility to 10 AB was tested by disk diffusion (CLSI). Clonality was studied by PFGE and MLST. Analysis of *pcdD* copper efflux, *merA* mercuric reductase, *silA* silver efflux system, AB resistance genes, plasmid and integron backbones (PCR, RFLP and/or sequencing), transferability and genomic location (I-CeuI/S1 nuclease hybridization) was performed.

**Results:** All but two isolates (99%) were resistant to AB, with 94% of multidrug-resistance (MDR, 3–8 AB). Resistance to sulfamethoxazole

(Su, 92%), tetracycline (T, 91%), streptomycin (S, 88%), ampicillin (A, 67%), chloramphenicol (C, 45%), trimethoprim (Tr, 35%), gentamicin (G, 27%), nalidixic acid (4%) or kanamycin (3%) was detected. Isolates were divided in three groups according to their features: (i) European clone (n = 48; four PFGE-types/ST34 that is a SLV of ST19)-ASSuT type (*bla*TEM, *strA*-*strB*, *sul2*, *tetB*), *pcdD*+ and *merA*+, all chromosomally located; (ii) MDR Spanish clone (n = 45; two PFGE-types; ST19 and phagetype DT104/U302) mostly ACGSSuTTr phenotype (n = 27; *bla*TEM, *cmlA1*, *aac(3)*-IV, *aadA*, *sul1*-*sul2*-*sul3*, *tetA*, *dfrA12*), with *merA*, atypical (*estX*-*psp*-*aadA2*-*cmlA1*-*aadA1*-*IS440*-*qacH*-*sul3*) and conventional (*dfrA12*-*orfX*-*aadA2*-*qacEd1*-*sul1*) class 1 integron on large non-conjugative *IncA/C* plasmids; (iii) CSSuTTr type (n = 15; ST19; *cmlA1*, *aadA*, *sul3*, *tetB*, *dfrA12*), carrying an atypical class 1 integron (*dfrA12*-*orfF*-*aadA2*-*cmlA1*-*aadA1*-*IS440*-*qacH*-*sul3*) on large, non-conjugative *IncR* plasmids.

**Conclusions:** Three MDR genotypes of globally spread clonal lineages are involved in the emergence of *S. enterica* serotype 4,[5],12:i:- in Portugal. Besides MDR, resistance to biocides used in animal production or linked to environmental pollution could account for the recent success of this serotype.

### P1303 The role of plasmids in the control of antibiotic resistance in *Escherichia coli* and *Klebsiella pneumoniae* isolated in a London hospital

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**Objectives:** The aim of this study was to investigate the infiltration of extended-spectrum beta-lactamases and their association with individual clones of Enterobacteriaceae isolated from blood cultures within a London teaching hospital.

**Methods:** One hundred Enterobacteriaceae, isolated sequentially in August–October 2009 from blood culture specimens, were obtained from St George's Hospital London. Antimicrobial susceptibility testing was determined by disc diffusion and agar dilution was used to measure the MICs of Cefotaxime, Ceftazidime, and Ceftriaxone; all tests were interpreted according to the BSAC guidelines. Multiplex PCR was used to identify CTX-M and AmpC enzymes. Pulsed-field gel electrophoresis (PFGE) with XbaI restriction was used to genotype the strains. Plasmid profiles were determined using S1 nuclease and alkaline lysis extraction and examined by PFGE. Furthermore, PCR-based replicon typing was used to identify individual plasmid types.

**Results:** Six isolates showed resistance to cefotaxime, ceftazidime and ceftriaxone, five were *Escherichia coli* and one was *Klebsiella pneumoniae*. All of them possessed the CTX-M-15 beta-lactamase gene but none of the strains harboured any of the known transferable AmpC beta-lactamases. Genotyping by PFGE revealed that two of the CTX M-15 beta-lactamase-containing isolates of *Escherichia coli* were closely related but the remaining isolates were <80% identical. In the six isolates, the plasmid profiles were not identical, ranging from 97.0 to 145.5 kb though four isolates of *Escherichia coli* did have the same plasmid of 145.5 kb.

**Conclusion:** Our data indicate the presence of plasmids carrying the CTX-M-15 beta-lactamase within this Enterobacteriaceae population and suggest that the dissemination of CTX-M-15 beta-lactamase gene is linked to both the transfer of these genes between plasmids and the transfer of those plasmids between the individual strains.

### P1304 Resistance mechanisms to amoxicillin-clavulanate in *Escherichia coli*: molecular epidemiology of inhibitor-resistant TEM and OXA-1-producing isolates

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**Objectives:** Resistance to amoxicillin-clavulanate (AMC) in *E. coli* isolates is increasing in last years in Spain. The aims of this study are to

characterize the mechanisms of resistance to amoxicillin-clavulanate (AMC) in *Escherichia coli* in Spain, and to study the molecular epidemiology of OXA-1- and inhibitor resistant TEM (IRT)-producing isolates.

**Materials and methods:** A prospective multicenter study was designed to collect AMC-resistant *E. coli* isolates from clinical samples between January 2010 and May 2010. Seven hospitals members of the Spanish network for infectious disease research (REIPI), and representing six geographical areas, participated. Each hospital collected a maximum of 44 isolates (22 community and 22 nosocomial-acquired). Susceptibility testing was carried out by the microdilution method (CLSI). Mechanisms of resistance were characterized by PCR and sequencing. Molecular epidemiology was carried out by pulsed field gel electrophoresis (PFGE) and by Multilocus Sequence Typing (MLST).

**Results:** Two hundred and fifty-eight isolates were collected. AMC resistance mechanisms were: OXA-1 (26%), plasmidic AmpC (19.4%), hyperproduction of AmpC<sub>Cc</sub> (18.2%) and IRT (17.4%). Fifty-nine isolates (22.9%) had an antibiotic resistant profile compatible with hyperproduction of penicillinase, of them 54 (21.31%) produced blaTEM-1 and five (1.5%) blaSHV-1. The IRTs identified in this study were blaTEM-40 (33.3%), blaTEM-30 (28.9%), blaTEM-33 (11.1%), blaTEM-32 (4.4%), blaTEM-34 (4.4%), blaTEM-35 (2.2%), blaTEM-54 (2.2%), blaTEM-76 (2.2%), blaTEM-79 (2.2%), and the new blaTEM-185 (8.8%). PFGE analysis of IRTs-producing isolates showed a high genetic diversity, with 43 PFGE different patterns among 45 isolates. Two well-defined clusters were detected in the 67 OXA-1-producing isolates, cluster C1 made up of 19 phylogroup A isolates, and cluster C2 made up of 19 phylogroup B2 isolates (16 produced CTX-M-15). MLST results showed 16 different STs among OXA-1-producing isolates, being the majority ST88 (37.3%) and ST131 (32.8%). C1 and C2 isolates belonged to ST88 and ST131, respectively. In IRTs isolates, 20 different STs were found, mainly ST131 (17.8%), ST23 (11.1%), ST73 (11.1%) and ST38 (6.7%).

**Conclusions:** A great diversity of mechanisms of AMC resistance in *E. coli* was detected. Clonal dissemination of OXA-1-producing isolates linked to ST88 and ST131 clones was observed. ST131 clone was detected in the 26.8% of all 112 IRTs or OXA-1-producing isolates studied.

#### **P1305** Resistance to aminoglycosides in *Corynebacterium striatum*

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**Objectives:** Coryneform bacteria are aerobic Gram-positive, non-spore forming bacilli increasingly recognized as pathogens of clinical relevance, particularly as a cause of opportunistic infections. They are mostly associated with soft-tissue infections, respiratory tract infections, bacteraemia and endocarditis. Corynebacterial infections are usually treated with glycopeptides, mainly vancomycin. Most of *Corynebacterium* are highly resistant to several antimicrobials. Corynebacteria are considered as a possible reservoir of antibiotic resistance genes, that could be transmitted to other saprophytic or pathogenic bacteria colonizing the human skin or other tissues. The objectives of this work have been: To study the susceptibility to aminoglycosides of a collection of *Corynebacterium striatum* clinical strains isolated from different origins and patients at the Hospital Marqués de Valdecilla, Santander, Spain, during the period 2006–2010. The elucidation of their resistance mechanisms.

**Methods:** Seventy-four *C. striatum* clinical isolates were included in the study. MICs were determined using the E-test system. The following antimicrobial drugs were tested: amikacin, gentamicin, kanamycin, netilmicin, streptomycin and tobramycin. Search for aminoglycoside resistance genes were performed by PCR using specific primers for aphA1, aadA1, aadB, aadD, aac(6′)-II, ant(4)-IIa, strA and strB genes. Amplification products were sequenced.

Sequences were analyzed with the BLAST tool at the website of the National Center for Biotechnology Information, USA

**Results:** Forty-five *C. striatum* strains were resistant to at least one of the tested aminoglycosides. Forty-two percent of the isolates were resistant to kanamycin, 30% to streptomycin, 18% to tobramycin, 3% to amikacin and 1% to gentamicin. Nine different resistance profiles were observed. The four most frequent profiles were: resistance to kanamycin and streptomycin (18% of the isolates), resistance to kanamycin (12%), resistance to streptomycin (12%) and resistance to kanamycin and tobramycin (8%). Thirty-three *C. striatum* carried the aphA1 gene, encoding an aminoglycoside phosphotransferase that confers resistance to kanamycin. Three strains carried the genes strA and strB, encoding resistance to streptomycin.

**Conclusion:** A high percentage of our *C. striatum* clinical isolates were resistant to aminoglycosides. Most of the resistant isolates carried the gene aphA1.

#### **P1306** Broad host-range transferable high-level gentamicin resistance in group B *Streptococcus*

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**Objectives:** A screening for Group B *Streptococcus* (GBS) highly resistant to gentamicin (HGR) collected by the National Reference Centre for Streptococci between 2007 and 2011 retrieved three unrelated clinical isolates. The aim of our study was to confirm and to characterize the genetic support of HGR of these GBS strains.

**Methods:** Gentamicin resistance was determined using disk diffusion and minimal inhibitory concentrations (MICs) were measured by E-test®. Sequencing of PCR products as well as direct chromosomal sequencing were performed to identify the aac(6′)-aph(2) gene and to determine its genetic environment. Intra- and inter-species resistance transferability was assessed by conjugation experiments.

**Results:** Three unrelated GBS isolates of capsular serotype II, III, and V were recovered. Two of these strains were responsible for invasive infection (neonatal n = 1, adult n = 1) whereas the remaining was a vaginal colonizing strain. All strains were highly resistant to gentamicin with MIC > 128 mg/L and the presence of the aac(6′)-aph(2) gene encoding the bifunctional aminoglycoside-inactivating enzyme was confirmed by PCR and sequencing. This analysis revealed that the resistance determinants were carried by a classical Tn4001 transposon in one isolate and by truncated Tn4001-like transposons in the remaining two. Resistance of the strains carrying the truncated transposons was intra- and inter-species transferable at a high (10<sup>4</sup>) and a low (10<sup>7</sup>) frequency, respectively, for one strain and intra-species transferable at a low frequency (10<sup>6</sup>) for the other one.

**Conclusion:** We described chromosomally HGR mediated by Tn4001-like transposons in three unrelated GBS isolates. Such resistance has already been reported in other *Streptococcus* species. Yet, to our knowledge, this is only the second report of HGR in GBS, stressing that it is still very rare. However, we characterize one strain displaying a broad host range of conjugal transfer, a feature which might favour the spreading of the aac(6′)-aph(2) gene to other bacteria, as already observed with other antibiotic resistance genes. Thus, continuous surveillance of gentamicin susceptibility in GBS remains necessary.

#### **P1307** Antimicrobial susceptibility and molecular characterisation of metronidazole resistance in *H. pylori* isolated from Thai patients

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**Objectives:** To investigate the prevalence of antimicrobial resistance in *H. pylori* isolated from Thai patients and to determine the mechanisms of metronidazole resistance including mutations in the rdxA gene, encoding oxygen-insensitive nitroreductase and efflux pump.

**Methods:** A total of 102 *H. pylori* isolates were obtained from patients at King Chulalongkorn Memorial Hospital and Thammasat University Hospital between August 2003 to January 2007. The MICs were determined by E-test. Mutations in *rdxA* were detected by PCR and DNA sequencing. The role of efflux pump was investigated by using efflux pump inhibitor, CCCP.

**Results:** Prevalence of antimicrobial resistance in *H. pylori* was 0.98% to amoxicillin, 20.59% to ciprofloxacin, 8.82% to clarithromycin, 0.98% to tetracycline and 19.6% to metronidazole. Amoxicillin was the most potent antibiotic against *H. pylori* (MIC<sub>90</sub> = 0.047 mg/L). Sequencing analysis of the *rdxA* gene was determined in three metronidazole-sensitive and 14 metronidazole-resistant *H. pylori*. The results showed 15 amino acid changes in RdxA in metronidazole-sensitive and 28 in metronidazole-resistant *H. pylori* isolates when compared with those of *H. pylori* 26695 from GenBank. No particular amino acid substitution was associated with metronidazole resistance. Three metronidazole-resistant *H. pylori* isolates contained nonsense mutations, leading to a stop codon at either amino acid position seven or 65. One isolate had a deletion of A522 in the *rdxA* gene, leading to frameshift mutation at amino acid position 175. The remaining 10 isolates had missense mutations at various sites (5–12 sites). The efflux pump mechanism was not detected in any isolates.

**Conclusion:** These results suggest that mutations in the *rdxA* gene are associated with metronidazole resistance. However, other mechanisms must be involved in the development of resistance.

#### **P1308** *Escherichia coli* as reservoir for macrolide resistance genes

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**Introduction:** Although macrolides is not intended in the treatment of *E. coli* infections, because of the frequent contact of this bacterium with macrolides in the digestive tract, it is susceptible to become a real reservoir for macrolide resistance genes and able to transfer them to other target bacteria. All of these genes confer full cross-resistance between erythromycin and azithromycin. We aimed to assess the resistance to macrolides in commensal isolates of *E. coli* and to characterize the mechanisms of resistance.

**Materials and methods:** A total of 70 commensal *E. coli* isolates were collected from feces of hospitalized and non hospitalized patients. Susceptibility to antibiotics was determined as recommended by the French society for microbiology. MICs of erythromycin were determined by E-test (AB-Biodisk). The macrolide resistance genes screened by PCR were *mph* (A), *erm* (A), *erm* (B), *erm* (C), *ere* (A), *ere* (B), *mef* (A), *msr* (A).

**Results:** Resistance to amoxicillin, cefotaxime and cotrimoxazole was detected for 32%, 4% and 11% of isolates respectively. MICs of erythromycin ranged from 4 to 64 mg/L. the majority of strains (47%) had high MICs ( $\geq 32$  mg/L). the *mph* (A) gene was the most common macrolide resistance gene, it was present in 11 isolates obtained essentially from patients receiving antimicrobial drugs. The *erm* (B) gene was detected in seven isolates. The six other genes were not detected.

**Conclusion:** The *mph* (A) gene giving resistance to azithromycin was the most common macrolide resistance gene detected in *E. coli*. Because *E. coli* and other species can exchange plasmids, further dissemination of resistance to macrolides in target bacteria may be predicted.

## Epidemiology of MRSA, VRE and other Gram-positives

#### **P1309** First occurrence of methicillin-resistant *Staphylococcus aureus* with a novel *mecA* homologue in a child in Switzerland

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**Objectives:** We describe a methicillin-resistant *Staphylococcus aureus* (MRSA) with a novel *mecA* homologue. The isolate attracted our interest because it exhibited phenotypic resistance to methicillin but was *mecA* negative by PCR. MRSA isolates with similar characteristics possessing the novel *mecA* homologue *mecALGA251* have been described recently in the UK, Ireland, Denmark and Germany. An association with dairy cattle has been reported.

**Methods and results:** The MRSA isolate was recovered from the blood of a 10-year-old girl who had undergone orthopaedic surgery and presented with a wound infection. An additional MRSA with the same characteristics was isolated from a wound swab. The patient had not been abroad for a year and had no contact with dairy cattle.

Routine susceptibility testing of the *S. aureus* isolate with the Vitek AST P580 card (bioMérieux) revealed resistance to penicillin, oxacillin, and ceftioxin and susceptibility to all other compounds tested. The isolate grew on a chromogenic selective agar plate (chromID MRSA, bioMérieux). Latex agglutination for PBP2a with MRSA-Screen (Denka Seiken) did not indicate PBP2a expression.

Conventional PCR was positive for *femA* and negative for *mecA*. Rapid real-time PCR with the Xpert MRSA/SA test (Cepheid) identified methicillin-susceptible *S. aureus*. PCR designed for the simultaneous detection of both the *mecA* gene and the *mecA* homologue *mecALGA251* as described by Garcia-Alvarez et al. (*Lancet Infect Dis* 2011; 11: 595–603) resulted in a 356-bp product. Sequencing of the PCR product revealed 100% homology to *mecALGA251*.

The isolate was typed by pulsed-field gel electrophoresis and compared with 621 different MRSA types consecutively isolated from humans since 1994. Analysis of the restriction patterns did not reveal any relationship with other MRSA strains except from one single isolate obtained in 2006.

**Conclusion:** To our knowledge this is the first case of MRSA with the novel *mecA* homologue *mecALGA251* in Switzerland. Molecular typing results suggest that the isolate is not associated with strains circulating in Switzerland. Clinicians and microbiologists have to be aware that MRSA isolates with the novel *mecA* homologue may go undetected by both PCR assays and agglutination tests for PBP2a.

#### **P1310** Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in Switzerland: diversity from bacteraemia represents only a small part of the diversity found in colonised/infected patients

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**Objective:** To assess the molecular epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) strains in Swiss hospitals.

**Methods:** National survey of MRSA cases identified in >500-bed Swiss hospitals from January to June 2011. Participating hospitals were asked to submit the first five successive MRSA isolates from individual patients with MRSA bacteremia and the first ten successive MRSA isolates from individual new cases (colonization or infection other than bacteremia). Molecular analysis of MRSA strains was done by Double Locus Sequence Typing (DLST), *spa*-typing, and SCCmec typing. In addition, the presence of PVL genes was also investigated. Swiss genotypes were compared to a reference database including international MRSA clones.

**Results:** Six out of eight >500-bed Swiss hospitals participated in the survey. A total of 74 MRSA isolates were sent to the reference laboratory: fourteen strains isolated from blood cultures (0–5 per hospital) and the 10 first MRSA isolates of each participating hospital. Depending on the hospitals' MRSA incidence, 9 days to 3 months were needed to obtain the first 10 isolates.

Among the 74 MRSA isolates, 40 different DLST types were observed (4–10 types per hospital). Only nine types were shared by more than one patient (2–8 patients). Seven international clones were found

among the Swiss isolates: Berlin clone (ST 45-IV-PVL neg), South German clone (ST 228-I-PVL neg), UK EMRSA-15 clone (ST 22-IV-PVL neg), New-York/Japan clone (ST 105-II-PVL neg), Lyon clone (ST 8-IV-PVL neg), Brazilian clone (ST 239-IImerc-PVL neg), European CA-MRSA clone (ST 80-IV-PVL pos). Among the 14 MRSA strains isolated from blood cultures, only nine different DLST types were found.

In the hospital with the highest MRSA incidence (18 cases/1000 admissions), 6/10 isolates were of the same type (DLST 4-4-I-neg). In contrast, in two hospitals with a lower incidence (ca one case/1000 admissions), 9/10 and 10/10 isolates were of different types, respectively. However, in the hospital with the lowest incidence (0.3 case/1000 admissions), four patients shared the same strain (DLST 2-2-II-neg).

**Conclusion:** In a country with a low to intermediate MRSA prevalence among invasive *S. aureus* (ca. 10%), strains isolated from blood cultures represent only a small part of the genetic diversity of MRSA encountered in hospitals and does not allow a representative description of the local molecular epidemiology.

**P1311 Studying recombination in the context of the population structure: the case of a recently emerged methicillin-resistant *Staphylococcus aureus* lineage**

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**Objectives:** Study the role of recombination within the context of the population structure for the recently emerged methicillin-resistant *S. aureus* (MRSA) clone Sequence Type 239 (ST239).

**Methods:** Previously, 63 isolates of the MRSA ST239 were sequenced through a second-generation sequencing platform to study the global geographic structure and intercontinental transmission of this clone. Recently, this data set was expanded with another 127 isolates, many of which were collected from hospitals in Turkey, but also from hospitals in other parts of the world. This extended data set was analyzed using Bayesian approaches and homemade perl scripts.

**Results:** We find that although recombination has introduced a significant amount of genetic variation that has affected almost 30% of the genome, it has mainly been confined to the so-called mobile genetic elements (MGE). Furthermore, the ratio of the rates at which nucleotides are substituted as a result of recombination and mutation ( $r/m$ ) is 1.2, but drops to 0.6 if the MGE are not considered, implying that recombination has been introducing slightly more than half of the variation introduced by mutation in the core genome. Additionally, we note that much of the population structure of this clone is regional and so is the pattern of recombination between the strains. Interestingly, we observe that for the core genome the older the clade, the more recombination is found. Nonetheless, we find an outsider clade that appears to be less affected by recombination, and this suggests non-constancy in the recombination rate across the clades.

**Conclusions:** Our results suggest that recombination has introduced a good deal of genetic variation, although the level of recombination has varied not only along the genome but also across the clades. In addition, the possibility of recombination between the clones is constrained by the population structure.

**P1312 High prevalence of ST228-MRSA-I blood isolates with the hVISA/VISA phenotype in the northern Italian province of Bolzano**

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**Objectives:** Methicillin resistant *Staphylococcus aureus* (MRSA) causing bloodstream infections is frequently treated with

glycopeptides; reduced vancomycin susceptibility among hVISA and VISA phenotypes is therefore disturbing. We therefore performed a microbiological and molecular survey of 81 first, non-duplicate blood MRSA isolates collected during 2002–2010 in the Province of Bolzano, Northern Italy (population size 500 000).

**Methods:** All MRSA isolates were subjected to staphylococcal cassette chromosome mec (SCCmec), accessory gene regulator (agr) and surface protein A (spa) typing together with PCR-based screening for 14 toxin genes. Representative strains were also analysed by multi-locus-sequence-typing (MLST) and pulsed-field-gel-electrophoresis (PFGE). MICs were determined by agar dilution (British Society for Antimicrobial Chemotherapy), screening for hVISA was by Macro-Etest and Etest GRD and confirmed by PAP-AUC.

**Results:** Two of the 81 MRSA isolates were VISA with vancomycin MICs of 4 mg/L and teicoplanin MICs of 4 and 8, respectively. Two MLST clonal complexes predominated: CC8 (59%) and CC5 (33%); the remainder included representatives of CC1, CC22, CC45 and CC398. All isolates were PVL negative. The major clones identified were ST8-MRSA-IVc, spa t008 (55%), ST228-MRSA-I, spa t041 (16%) and ST225-MRSA-II, spa t003 (6%); all of the ST228-MRSA-I, spa t041 isolates were hVISA or VISA. Twenty-one (26%) of the isolates were positive at the screening tests and at the PAP-AUC confirmation tests for hVISA; the difference in the frequencies of the hVISA/VISA phenotypes between the two major clones was statistically significant ( $p = 0.0001$ ). One ST8-MRSA-IVc, spa t008 isolate had ccrA2B2 together with ccrA4B4. One isolate had the genotype ST398-MRSA-V, spa t011 (CC398) associated to Livestock-Associated MRSA (LA-MRSA).

**Conclusion:** Single major MRSA clones together with sporadic isolates are present in blood isolates in a Province in Northern Italy and the high prevalence and association of a specific genotype with the hVISA/VISA phenotype is of concern clinically. A correlation of this phenotype with minor glycopeptide treatment success has been described; for this reason elaboration of the treatment outcomes for our patients is in progress to study the clinical implication of this genotype.

**P1313 Molecular typing and phenotype characterisation of methicillin-resistant *Staphylococcus aureus* Isolates from blood in Taiwan**

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**Objectives:** *Staphylococcus aureus* causes a variety of severe infections such as bacteremia and sepsis. At present, 60–80% of *S. aureus* isolates from Taiwan are methicillin resistant (MRSA). It has been shown that certain MRSA clones circulate worldwide. The goals of this study were to identify MRSA clones in Taiwan and to correlate the molecular types of isolates with their phenotypes.

**Methods:** A total of 157 MRSA isolates from bacteremic patients were collected from nine medical centers. They were typed based on polymorphisms in agr, SCCmec, MLST, spa, and dru. Phenotypes characterized included Pantone-Valentine leucocidin (pvl), inducible macrolide-lincosamide-streptogramin B resistance (MLS<sub>SB</sub>i), vancomycin (VA) and daptomycin (DAP) minimal inhibitory concentrations (MIC), and superantigenic toxin gene profiles. Difference between two consecutive samples was determined by Mann-Whitney *U*-test, and difference between two categorical variables was determined by Fisher's exact test.

**Results:** Four major MRSA clone complexes CC1, CC5, CC8, and CC59 were found, including four CC1, nine CC5, 111 CC8, and 28 CC59 isolates. These clones had the following molecular types: CC1: SCCmecIV and ST573; CC5: SCCmecII and ST5; CC8: SCCmecIII, ST239, and ST241, and CC59: SCCmecIV, SCCmecVT, ST59, and ST338. The toxin gene profiles of these clones were CC1: sec-seg-(sei)-sell-selm-(seln)-selo; CC5: sec-seg-sei-sell-selm-(seln)-selp-tst1; CC8: sea-selk-selq, and CC59: seb-selk-selq. Most isolates with SCCmecVT, ST59, spat437, and dru11 types were pvl+ (13 isolates), while multidrug resistance (<sup>3</sup> four antimicrobials) were associated with

SCCmecIII, ST239, spa t037, agrI, and dru14 (119 isolates) ( $p < 0.001$ ). One hundred and twenty four isolates with the following molecular types had higher VA MIC: SCCmecII and SCCmecIII; ST5, ST239, and ST241; spa t002, t037, and t421; dru4, dru10, dru12, dru13, and dru14 ( $p < 0.05$ ). No particular molecular types were found to be associated with MLSBi phenotype.

**Conclusion:** Four major MRSA clone complexes were found in Taiwan. Further studies are needed to delineate the evolution of MRSA isolates.

#### **P1314** Methicillin-resistant *Staphylococcus aureus* in four Balkan countries

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**Objectives:** Little is known about Methicillin-resistant *Staphylococcus aureus* (MRSA) in the Balkan countries Albania, Kosovo, Macedonia and Montenegro. The purpose of this study was to characterize hospital acquired (HA) and community onset (CO) MRSA isolated in these four countries.

**Methods:** Fifty-eight MRSA isolates were collected and tested for the presence of the mecA gene, nuc gene, spa gene and Panton Valentine leukocidin (PVL) genes. Typing of the spa region was performed on all spa PCR positive isolates and spa types were assigned using the Ridom StaphTyper. All isolates were from 2010 or 2011. Supposed CO or HA-MRSA was recorded. Multi locus sequence types (ST) and clonal complexes (CC) for each spa type were predicted when available at the Ridom SpaServer.

**Results:** Among 58 isolates we found 16 spa types and six MRSA isolates that were spa PCR negative. spa type t030 (ST239, Brazilian/Hungarian clone) was found in three countries, t041 (ST228, Southern German clone) and t969 (related to t030) in two countries, while the remaining spa types were only found in one country each. Twenty-three isolates were CO-MRSA, while 32 were HA-MRSA, and three were not defined. Seven of the spa types (t002, t005, t015, t026, t030, t041, t062 and t969) belonged to major HA-MRSA clones [1] and 70% of these were considered HA-MRSA. PVL was present in 13 isolates (22%). Five of the PVL positive isolates were CO-MRSA, seven were HA-MRSA, and one was not defined. Based on spa type, 43 of the isolates could be assigned to six CCs and three STs without CC-designation.

**Conclusion:** The finding of a high diversity in spa types and a high number of PVL positive HA-MRSA in the Balkan region was surprising. Although PVL is usually found in CO-MRSA, the same percentage of isolates in the HA-MRSA group was PVL positive (22%). Thirty-one isolates belonged to well-known pandemic HA-MRSA clones, including eight t002 isolates from Macedonia of which five were PVL positive. PVL positive HA-MRSA is not common in Europe, but in the USA the PVL positive clone USA300 has become endemic hospitals. None of the 13 PVL positive isolates belonged to the three most common PVL positive International clones USA300, ST80 (European clone) and ST30 (South West Pacific clone).

**Reference:** 1. Deurenberg RH, Vink C, Kalenic S, Friedrich AW, Bruggeman CA, et al. The molecular evolution of methicillin-resistant *Staphylococcus aureus*. Clin Microbiol Infect 2007; 13: 222–235.

#### **P1315** Characterisation of resistance, virulence, surface and immune evasion genes in Spanish epidemic methicillin-resistant *Staphylococcus aureus* strains

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**Objectives:** Methicillin-resistant *Staphylococcus aureus* (MRSA) spa-type t067 strains are currently epidemic in Spain. The aim of

this study was the characterization of resistance, virulence, surface and immune evasion genes profiles in a collection of spa-type t067 MRSA strains.

**Methods:** A total of 91 spa-type t067 MRSA strains were isolated in the Microbiology Laboratory at the Hospital CUV (Spain) in 2005, 2007 and 2008. They were subjected to analysis using spa-typing, multilocus sequence type (MLST) and ADN microarrays to characterise and assign the bacterial isolates.

**Results:** All the strains studied belong to the clonal complex CC5 and present a type II agr locus. The 62% of t067 MRSA strain in addition to carrying the genetic elements characteristic of SCCmec IV (class B mec gene complex and ccrA/B2 genes), presented additional elements such as ccrA/B4 or ccrB4 (SCCmec VI). Using MLST, the ST125 was assigned to spa-type t067; most of spa-type t067 MRSA strains are related to ST125-MRSA-IV/VI. The ST125-MRSA-IV/VI strains were mainly associated with the genotypic resistance profile mpbBM + mrsA, aadD + aphA and sat, which are responsible for erythromycin, tobramycin, and streptothricin resistance respectively, while ST125-MRSA-IV strains were characterized by the only presence of the tobramycin-resistant gene aadD. All the isolates carry the enterotoxin gene cluster egc (seu-positive variant). Correlation between the egc cluster and the sea gene (sea-N315) was also observed in the majority of the cases. This superantigen together with the sak, chp and scn genes it forms the immune evasion cluster (IEC). The IEC type F (sep, sak, chp and scn) were presented by 71% of the isolates, and IEC type B (sak, chp and scn) were detected in 4% of the isolates. The alpha-hemolysin gene and the Leukocidin/hemolysin gamma genes were negative in 15% and 13% of the respectively. The profile of surface genes was homogeneous and the variant alleles are the same ones as those of the Mu50 strain. Seven isolates presented a deletion of the bone sialoprotein-binding protein gene, five isolates were negative for the sdrD gene and another one was negative for the fibronectin-binding protein gene.

**Conclusion:** It was possible to subdivide the epidemic genotype t067 based on the acquisition or loss of resistance or virulence genetic elements. Surface and immune evasion genes profiles were homogeneous.

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#### **P1316** Clonality of methicillin-resistant *Staphylococcus aureus* and methicillin-resistant *Staphylococcus pseudintermedius* isolated from healthy and sick companion animals and humans in Portugal

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**Objectives:** To characterize the clonality, antimicrobial and biocide susceptibility patterns of methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) strains isolated from dogs, cats and humans in close contact.

**Methods:** Sixteen MRSA isolates from five dogs (one nasal colonization isolate, three pyoderma and one urinary tract infection-UTI isolates), two cats (nasal colonization) and nine humans (nasal colonization) were studied. The nine humans included seven veterinarians, one veterinary caretaker and one owner. Twenty MRSP isolates from 16 dogs (nine isolates from nasal colonization, five isolates from pyoderma, one from an otitis externa-OE and one from a surgical infection) and four cats (three isolates from an UTI and one from an OE) were analysed. Antimicrobial susceptibility testing was performed according to CLSI guidelines. MICs of several biocides and dyes were also determined. MRSA and MRSP isolates were subjected to spa typing, SCCmec typing and PFGE.

**Results:** spa typing of the 16 MRSA isolates classified them as: t032 (n = 8), t1865 (n = 3), t020 (n = 1), t910 (n = 1), t2357 (n = 1) and t002 (n = 2). All MRSA isolates except spa type t002 belonged to the MLST clonal complex (CC) 22, harboured an SCCmec IV and had indistinguishable PFGE patterns. The MRSA t002, which corresponds to MLST CC5, clustered differently from the other strains by PFGE.



Both clones, CC5 and CC22, included animal and human isolates. Fifteen MRSP isolates were spa type t02, four were t06 and one was t05. Eighteen MRSP were SCCmec III, while only two strains were SCCmec V. PFGE revealed two main MRSP clusters. All MRSA isolates were co-resistant to ciprofloxacin and three also had elevated MICs to erythromycin. MRSP strains were multidrug resistant, being resistant at least to ciprofloxacin, erythromycin, clindamycin, kanamycin and trimethoprim/sulfamethoxazole. Only the two MRSA CC5 strains had high MICs to ethidium bromide (32 mg/L), benzalkonium chloride (1 mg/L) and chlorhexidine (1 mg/L). The other MRSA and MRSP strains had no elevated MICs towards biocides.

**Conclusions:** This study shows that MRSA clones CC5 and CC22 may be disseminated in both human and companion animal populations. This underlines the importance of the animal-to-human transmission of resistant bacteria and the potential of zoonotic dissemination. MRSP strains although not frequently identified in humans are multidrug resistant but susceptible to biocides. Nevertheless, they can spread and act as reservoirs of important resistance genes.

**P1317** Nationwide survey of methicillin-resistant *Staphylococcus aureus* harbouring mecALGA251 reveals a reservoir in ruminants

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**Objectives:** In this study the epidemiological impact of isolates harbouring the recently discovered mecALGA251 in a MRSA low prevalence country was investigated.

**Methods:** Retrospective and prospective search for phenotypic positive but genotypic negative MRSA was performed. Collections screened retrospectively included all Dan-ish MRSA cases since 1988 (n = 7200) as well as all Danish bacteremia cases since 1975 (n = 38 000). Isolates previously tested resistant to methicillin, oxacillin or cefoxitin and negative for the mecA gene were further tested for the presence of the recently discovered mecA gene variant, mecALGA251, by PCR. The genetic background of all mecALGA251 positive isolates was determined by spa typing. Pro-spective screening for mecALGA251 was introduced as routine in the Danish Staphylococcus reference laboratory by August 2011. Clinical data was obtained from 22 patients by interviews and review of discharge summaries in the Zealand region. In four cases samples were taken from animals (cow, horse, dog and sheep) with suggestive contact to persons positive for mecALGA251.

**Results:** In total, 104 persons were found to be carrying or infected by mecALGA251 positive MRSA. One case dated back to 1975, one to 1992, then no isolates were found until 2003 where after the annual numbers increased to reach 28 by the 11 November 2011 constituting 2–3% of the total number of MRSA. Isolates could be grouped in two genetic lineages CC130 and CC1943.

Clinical cases from Zealand were further investigated (n = 22). Two patients had postoperative bacteremia. In the other 20 cases a wide spectrum of infections was seen, dominated by skin and soft tissue infection (n = 11), postoperative infections (n = 4) and two bacteriuria. Three individuals were symptom free and MRSA (CC130) was only detected by screening cultures.

In four cases there was a suggestive contact between patient and animals: cows, sheep, butcher (profession). In two cases with cow respectively sheep contact, similar strains by PFGE analysis were isolated from owner and animal (one of these in a patient with bacteremia).

**Conclusions:** Since 2003 the number of isolates has increased and mecALGA251 is now found in 2–3% of all new MRSA cases in Denmark. In two cases screening of livestock animals in vicinity of infected persons resulted in the first direct evidence for bo-vine and ovine reservoirs with transmission to humans.

**P1318** Analysis of the immune evasion cluster genes in clinical and commensal *Staphylococcus aureus* strains of human and animal origins

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**Objectives:** To analyze the genes that form the Immune Evasion Cluster (IEC) in a collection of clinical and commensal *S. aureus* isolates of human and animal origin. The IEC types detected were correlated with the origin and the clonal complexes (CC) of the strains.

**Methods:** One hundred and six methicillin-resistant and methicillin-susceptible *S. aureus* (MRSA and MSSA) isolates were studied: (i) 36 MRSA implicated in human bacteremia (Group 1); (ii) 15 MSSA fecal isolates of healthy humans (Group 2); (iii) 28 MSSA and MRSA of healthy human nasal samples (Group 3); (iv) 27 MRSA of human (n = 16) and animal origin (n = 11) of the clonal complexes CC398 or CC97 or CC1 (Group 4). The presence of the genes scn, sak, chp, sea and/or sep were studied by PCR and sequencing and according to the pattern of genes detected, they were classified into different IEC types.

**Results:** Sixty-four strains harbored genes of the IEC, representing 60% of all strains studied.

The following IEC types were obtained (IEC type/number of strains): Group 1, 72% with some IEC (IEC A/1; IEC B/1; IEC D/1; IEC E/1; IEC F/21; IEC G/1 and non-IEC/10); group 2, 86% with some IEC (IEC A/2; IEC B/4; IEC C/4; IEC E/1; IEC F/1; IEC G/1 and non-IEC/2); group 3, 90% with some IEC (IEC A/2; IEC B/13; IEC C/1; IEC D/1; IEC D/5; IEC F/1; IEC G/2 and non-IEC/3). The genes of IEC were detected in MSSA CC398 but not in MRSA CC398. Clinical MRSA strains from group 1 presented a high frequency of genes encoding the IEC, and were mainly ascribed to IEC type F (these strains belonged to CC5 (ST5, ST125). The IEC type B was highly detected among the strains of group 2 (50% of the positive strains), mainly related to CC15 (ST15), CC45 (ST45), CC509 (ST509), CC8 (ST1498), CC109 (ST1141), CC121 (ST121) and CC398 (ST398). The IEC type B was also found in more than 25% of positive strains of group 3 related to CC9 (ST109), CC30 (ST30) and CC59 (ST59). Strains of human origin CC97 exhibited the IEC type E. All strains of group 4 were negative for the genes of the IEC.

**Conclusions:** There is a relation between certain genetic lineages and the presence and type of IEC, highlighting the high prevalence of IEC type F among clinical isolates (MRSA) and IEC B among community isolates (MSSA). The IEC system was detected among commensal MSSA-CC398 of human origin but not among MRSA-CC398 of animal-human related origin. IEC could be a marker of interest to track *S. aureus* clones and their origin.

**P1319** Clonal transmission of MRSA between horses and staff at a veterinary teaching hospital and successful decolonisation of staff

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**Objectives:** In the context of active surveillance for MRSA among horses at a veterinary teaching hospital, performed while two horses were treated for MRSA wound infections, several horses were found to be nasal carriers. As a result, staff members at the hospital were screened to determine whether cross-transmission of MRSA was occurring.

**Methods:** Nasal swabs were obtained from staff members and community equine veterinarians and plated on CHROMagar MRSA plates. Suspected MRSA colonies were sub-cultured and definitively speciated, and antimicrobial susceptibility testing performed by VITEK2. Molecular characterization was carried out by pulsed-field gel electrophoresis (PFGE), spa typing and multi-locus sequence typing (MLST) on representative horse and human isolates. PCR for the Pantan–Valentine leukocidin (pvl) gene was performed.

**Results:** One hundred thirty-nine individuals were screened. Positive results were obtained in the following distribution: full-time equine staff, 10/20 (50%); part-time equine staff, 3/27 (11%); non-equine staff, 2/70 (3%); community equine veterinarians, 1/22 (5%). The predominant phenotype of MRSA isolates of staff and horses (89%) was non-susceptibility to erythromycin, clindamicin, gentamicin and ciprofloxacin. Horse isolates subjected to molecular testing were of spa type t535 (global frequency 0.08%), MLST type ST5 and identical pulsotype. Spa type t535 predominated among equine staff isolates as well (11 of 12 tested – 92%), and those further characterized were of identical pulsotype and MLST type to those of the horse isolates. All isolates were pvl negative.

All equine staff and other staff carriers were treated with 1 week of daily 4% chlorhexidine soap showers and thrice daily 2% intranasal mupirocin. Infection control procedures were reinforced in the equine department, and horses discharged when possible. Follow-up screening of 27 staff members after treatment yielded 0% positive results. Six-month follow-up screening of 33 staff members and 28 horses yielded 0% positive results.

**Conclusions:** Cross-transmission of a rare MRSA clone with a healthcare-associated phenotype between horses and staff occurred at a veterinary hospital caring for horses with MRSA-infected wounds. Intensity of exposure to hospitalized horses was associated with likelihood of staff carriage. An infection control intervention including decolonization of staff succeeded in eradicating carriage at 6 months post-intervention.

**PI320 High prevalence of PVL-positive *Staphylococcus aureus* skin infections among outpatients in Brussels region, Belgium**

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**Objectives:** Community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA), carrying the Panton-Valentine leukocidin (PVL), is an emerging problem worldwide. In this study, we determined the prevalence of *S. aureus* as a cause of skin and soft-tissue infections (SSTIs) among outpatients in Brussels region and evaluated risk factors for methicillin-sensitive *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) infections.

**Methods:** We enrolled patients with acute, purulent SSTIs presenting to emergency or dermatologic departments in four hospitals, during a prospective study from May to September 2011. Data for risk factor analysis (e.g. demographic data and clinical information) were collected through a case report form. *S. aureus* isolates were characterized by antimicrobial-susceptibility testing, detection of toxin and resistance genes, spa typing, and multilocus sequence typing (MLST). On MRSA isolates, we also performed typing of the staphylococcal cassette chromosome mec (SCCmec).

**Results:** *S. aureus* was isolated from 61 of 113 (54%) patients with SSTIs. The prevalence of MRSA was 6% (7/113). The clinical manifestation of MSSA and MRSA infections was similar with a predominance of skin abscess (including furuncle). The median age of patients with MSSA and MRSA was 23 and 34 years, respectively. Among the seven patients with MRSA, three patients had previously received antibiotics in the month before enrolment, and two patients had a history of hospitalization. Among patients with MSSA, 39% of patients had travelled abroad in the 3 months before enrolment. The pvl toxin gene was detected in 57% of MRSA, and 31% of MSSA. Other toxin genes were recovered rarely. The molecular profile of MSSA collected was heterogeneous whereas only three different MRSA clones were isolated (ST8, ST80, and ST45). All ST45-MRSA were isolated from patients with a history of hospitalization.

**Conclusion:** Our study underlines the high prevalence of PVL-positive *S. aureus* (35%) as a major cause of SSTIs among outpatients presenting to emergency or dermatologic departments in Brussels. Our

data also showed the importance of MRSA (6%) as a public health issue in Belgium. Surveillance should be intensified to monitor the incidence of MRSA and control its spread in the community.

**PI321 Lack of emergence of PVL-positive MRSA strains in a university hospital**

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**Objectives:** The incidence of methicillin-resistant *Staphylococcus aureus* (MRSA) strains is decreasing, while the percentage of Panton-Valentine leukocidin (PVL) positive strains is increasing. The aim of this study was to characterize the PVL-positive MRSA strains in a Swiss university hospital (USB) with a prevalence of MRSA <2%.

**Methods:** Between January 2006 and December 2010, all MRSA first isolates per patient and year isolated at the microbiology laboratory of USB were tested for PVL genes by PCR. All PVL-positive strains were further analyzed by antimicrobial susceptibility testing, molecular typing by determining the SCCmec type and sequence-based spa type, as well as by pulsed-field gel electrophoresis (PFGE). Demographic and clinical data were collected.

**Results:** During the study period, a total of 258 MRSA cases among in- and outpatients were detected. Fifty-seven (22.1%) of these were PVL-positive exhibiting no increase over time. The mean age of PVL-positive patients was  $44.9 \pm 20.6$  years compared to  $58.4 \pm 22.4$  years of PVL-negative patients. Among the PVL-positive patients, 63.2% were outpatients, 50.9% female, and 21.1% had been hospitalized during the last 12 months. Twenty-eight (49.1%) originated from Switzerland, 10 (17.6%) from the European Union (EU), and 19 (33.3%) outside from the EU. Thirty-two (56.1%) of the PVL-positive strains were isolated from infections, most common skin, soft-tissue or wound infections (90.6%). Forty-four (77.2%) of the PVL-positive strains were classified as community-associated MRSA (CA-MRSA), and 13 (22.8%) as hospital-associated MRSA. Forty-five (78.1%) isolates harboured SCCmec type IV, 8 (14.0%) SCCmec type V variant, two new variants, and one SCCmec type II. Seventeen (29.2%) strains belonged to spa-type t008 which includes USA300 (ST8), 10 (17.5%) strains were of spa-type t044, representing clone ST80, which is known to commonly circulate as CA-MRSA in Europe. Half of PVL-positive strains belong to spa type t008 or t044. Overall, 16 different spa types were detected. PVL-positive MRSA strains were non-susceptible to ciprofloxacin in 42.6%, to tobramycin in 24.1%, to clarithromycin in 22.0%, and to fusidic acid in 20.4%. All strains were susceptible to vancomycin, teicoplanin, and doxycycline. There was no evidence for an outbreak during the study period.

**Conclusion:** In contrast to most published reports, we did not observe an increase of PVL-positive MRSA strains, despite the fact that 53.1% of all strains were CA-MRSA.

**PI322 Analysis of gene distribution of *Staphylococcus aureus* strains isolated from healthy volunteers with intermittent or persistent nasal carriage and from infected patients**

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**Objectives:** The nasal carriage of *Staphylococcus aureus* is a major risk factor for further infection with this bacterium. It has been shown previously that *S. aureus* carriers can be classified into two different states, persistent and intermittent. This study was designed to describe the distribution of 185 genes in *S. aureus* strains isolated from intermittent or persistent carriers and from patients with clinical *S. aureus* infections.

**Methods:** Thirty-three *S. aureus* strains from a cohort of healthy healthcare workers were tested, including 11 nasal intermittent carriers (IC) and 22 nasal persistent carriers (PC); they were compared to *S. aureus* strains isolated at the University Hospital of Saint-Etienne between 2008 and 2010 from 32 patients with community-acquired bloodstream infection in the absence of foreign material (BSI) and 36

patients with prosthetic joint infection without bacteremia (PJI). All these strains (n = 101) were genotyped by using the StaphyType microarray (Alere, Jouy-en-Josas, France).

**Results:** Among the 185 genes analyzed, no difference was found between strains of PC and strains of clinical infection (PJI and BSI) except for the gene coding for enterotoxin H. By contrast, four genes coding for toxins (entB, entK, entQ and lukE), the ssl08 gene coding for a staphylococcal superantigen like protein and two genes coding for serin proteases (splA and splB) were more prevalent in IC strains than in strains of the three others groups. In addition, the cna gene coding for a collagen adhesin was harbored by half of the PC, BSI and PJI strains but in only one IC strain.

**Conclusion:** These results suggest that the strains of *S. aureus* isolated from infected patients are genetically closer to those of PC subjects than those of IC subjects, which is in accordance with previous works showing that PC subjects, by contrast to IC subjects, are at higher risk of *S. aureus* infection.

**P1323** Nasal carriage rate and molecular epidemiology of methicillin-resistant *Staphylococcus aureus* among medical students in a Taiwanese university

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**Background:** Methicillin-resistant *Staphylococcus aureus* (MRSA) is among the important pathogens of nosocomial infections. To investigate whether clinical exposures in the hospital affect nasal MRSA carriage among medical students, we conducted this cross-sectional study.

**Methods:** From June to September 2010, a total of 322 students of Chang Gung University, pre-clinical (n = 167) and clinical (n = 155), were recruited. Specimens from the nares of the subjects were obtained and sent for the detection of *S. aureus*. A questionnaire regarding demographics and potential risk factors for acquisition of *S. aureus* was also completed for each subject. All the MRSA isolates were further molecularly characterized.

**Results:** Overall, the carriage rate of *S. aureus* was 19.3%, with a rate of 16.8% for pre-clinical students and 21.9% for clinical students (p = 0.26); the carriage rate of MRSA was 2.2%, with a rate of 2.4% for pre-clinical students and 1.9% for clinical students (p = 0.54). There was no significant difference between the pre-clinical and clinical students in terms of nasal carriage of *S. aureus* and MRSA. All seven MRSA isolates belonged to sequence type 59, carried staphylococcal chromosome cassette type IV or VT and were categorized as community strains in Taiwan. The risk factors for acquisition of *S. aureus* included male gender, age  $\geq 23$  years, and not taking antibiotics in the past year.

**Conclusions:** A substantial proportion of medical students in northern Taiwan harbored MRSA, categorized as community strains, in their nares. The carriage of MRSA was not affected by the clinical exposure in the hospital for 1–2 years.

**P1324** Methicillin-resistant *Staphylococcus aureus* in community-, healthcare-, and hospital-associated infections: results of a 12-month observational study in a large tertiary-care Italian hospital

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**Objective:** To investigate the role of methicillin-resistant *Staphylococcus aureus* (MRSA) in community-associated (CA) and healthcare-associated MRSA (HCA-MRSA) infections in Florence University hospital, Italy, and to compare it with the role of hospital-acquired MRSA (HA-MRSA) infections in the same setting.

**Methods:** MRSA isolated from patients within 48 hours of hospitalization without risk factors for HA-MRSA were defined CA-MRSA, with risk factors were defined HCA-MRSA. HA-MRSA infections constituted the control group. A data questionnaire was used

to collect patients demographics, clinical history and risk factors. SCCmec cassette type and PVL genes presence were investigated by PCR.

**Results:** Seventy-nine patients were enrolled from April 2009 until March 2010: seven infected by CA-MRSA, 33 by HCA-MRSA and 39 by HA-MRSA. The mean ages were 68, 65 and 51 years and the average length of stay was 6, 5, 26 days, respectively. All CA-MRSA were from skin and soft tissue infections (SSTI). HCA-MRSA were from SSTI (64%), sputum or bronchoalveolar lavage (BAL) (15%), blood (21%). HA-MRSA were from SSTI (32%), sputum or BAL (50%), blood (18%). Two CA-MRSA infected patients had HIV and alcohol as risk factors. Most of HCA- (100%) and HA-MRSA (98%) infected patients had at least one risk factor. Complications (septic shock, sepsis, pneumonia) were observed in none of the patients infected by CA-MRSA but in 8/33 (24%) and in 11/40 (28%) of those infected by HCA- and HA-MRSA, respectively. Four and eight deaths (12% and 20%) were observed in the HCA- and HA-MRSA infected patients and none in those with CA-MRSA. The susceptibility rates for CA-, HCA- and HA-MRSA were, respectively: clindamycin 57%, 33%, 15%; levofloxacin 0%, 9%, 2%; gentamicin 57%, 45%, 17%; rifampicin 100%, 94%, 77%; tetracycline 71%, 97%, 92%; trimethoprim sulfamethoxazole 100%, 94%, 95%; all isolates were susceptible to vancomycin, teicoplanin, linezolid, and tigecycline. Molecular analysis showed heterogeneity of SCCmec cassettes (type I, IV in CA-MRSA, type I, II, III, IV in HCA-MRSA and type I, II, IV in HA-MRSA) and the presence of PVL genes in 3, 3 and 1 of CA-, HCA- and HA-MRSA, respectively.

**Conclusions:** HCA-MRSA infections exhibited an intermediate profile between HA- and CA-MRSA one, showing a worse prognosis than CA-MRSA infections, but a higher antibiotic susceptibility and lower length of stay respect to HA-MRSA infections. HA-, HCA- and CA-MRSA were found to share some molecular features, as recently reported.

**P1325** Nasal carriage of methicillin-resistant *Staphylococcus aureus* among hospitalised patients and healthcare workers in a Belgrade university hospital

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Methicillin resistant *Staphylococcus aureus* (MRSA) has emerged as a major hospital-acquired pathogen worldwide, but also as an important community acquired pathogen. Many MRSA infections are preceded by a period of carriage. According to our knowledge no data concerning carriage of MRSA in Serbia have been published so far. The aim of the present study was to provide the analysis of carriage of MRSA in hospitalized patients and healthcare workers (HCWs) in the largest healthcare facility in Serbia.

Nasal swab were taken from 195 patients and 105 HCWs at the Clinical Center of Serbia in Belgrade. Nasal swabs were inoculated in Mueller-Hinton broth supplemented with 2.5% NaCl at 35°C for 24 hours. Each broth sample (50 µl) was inoculated onto MRSA-ID agar (bioMérieux, France). Chromogenic media were incubated at 35°C and read after 24 and 48 hours of incubation. Identification of isolates was confirmed by PCR for nuc and mecA genes. Susceptibility to antibiotics was performed by Vitek2 System (bioMérieux, France). Molecular typing of all MRSA strains was performed using spa, SCCmec and agr typing according to previously described protocols. Detection of Pantone Valentine leukocidin (PVL) genes were performed by PCR.

Among 195 hospitalized patients and 105 HCWs, 23 (11.8%) and 8 (7.6%) respectively were colonized with MRSA. All tested MRSA strains were susceptible to fusidic acid, trimethoprim/sulfamethoxazole, vancomycin, linezolid and mupirocin, while 27 (87.1%) were resistant to gentamicin, 27 (87.1%) to tobramycin, 17 (54.8%) to erythromycin, 17 (54.8%) to clindamycin (only two strains showed inducible type of resistance), 25 (80.6%) to ciprofloxacin and 4 (12.9%) to tetracycline. There have been detected seven different spa types (t001, t041, t030, t595, t242, t005 and t044). The spa type t001 (SCCmecI, agrII, resistant to gentamicin, tobramycin, erythromycin and ciprofloxacin) was

dominant (45.2%). PVL genes were detected in 2 (6.4%) MRSA strains (t595-SCCmecV-agrI (two strains) and t044-SCCmecIV-agrIII). Out of eight MRSA strains isolated from HCWs, 4 (50%) were indistinguishable from strains isolated from patients indicating circulation of MRSA strains between patients and HCWs.

Nasal carriage of MRSA among hospitalized patients and HCWs was determined to be high. Carriage was higher in hospitalized patients than in HCWs. The spa type t001 was dominant among tested population in this study. Our study also showed exchange of MRSA between patients and HCWs.

**P1326** **Molecular epidemiology and nasal carriage of *Staphylococcus aureus* and methicillin-resistant *S. aureus* among young children attending day care centres and kindergartens in Hong Kong**

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**Objectives:** To investigate the prevalence, molecular epidemiology as well as the risk factors associated with *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA) nasal carriage in Hong Kong children.

**Methods:** Nasal and nasopharyngeal swabs were collected from children who were 2–5 years old attending 79 day care centers (DCCs) and 113 kindergartens (KGs) in all 18 school districts in Hong Kong during September 2009 to April 2010. A standardized questionnaire was also used to obtain information from parents of participating children so as to study the variables associated with MRSA carriage.

**Results:** 27.6% (610/2211, 95% confidence interval [CI] 24.8–28.5%) of the children were found to carry *S. aureus* and the overall nasal carriage rates of MRSA was 1.3% (28/2211, 95% confidence interval [CI] 0.8–1.8%). Univariate analyses of the risk factors showed that only younger age was significantly and negatively associated with *S. aureus* carriage. Molecular typing including staphylococcal cassette chromosome mec (SCCmec), sequence type (ST) and clonal cluster (CC) showed that all the 28 MRSA isolates were belonged to SCCmec IV (n = 13) or V (n = 15). In these 28 isolates, 12 isolates had community-associated-MRSA genotypes (ST59/SCCmec IV/V, ST30/SCCmec IV and ST88/SCCmec V), 10 isolates had healthcare-associated-MRSA genotypes (ST45/SCCmec IV/V, CC5/SCCmec IV and ST630/SCCmec V) and six isolates had novel genotypes (ST10/SCCmec V and CC1/SCCmec IV). Spa typing results also indicated an intra- and inter-school transmission of certain MRSA and methicillin-sensitive *S. aureus* strains.

**Conclusion:** Our findings indicate that DCCs and KGs can act as potential reservoirs for different types of antimicrobial-resistant *S. aureus*. This highlights the importance of enhancing education, infection control measures and hygiene in these crowded environments in order to reduce the transmission of these emerging bacteria in the community.

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**P1327** **The epidemiology of methicillin-resistant *Staphylococcus aureus* in elderly residents of 65 care homes in a single primary care trust of northern England**

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**Objectives:** To determine the prevalence and epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) colonising elderly residents of care homes registered in the city of Leeds, between November 2006 and February 2009.

**Methods:** Participants from 65 care homes were screened for nasal colonisation with MRSA in four surveys: November 2006–December

2006; October 2007–November 2007; May 2008–June 2008, and January–February 2009. Antibiotic susceptibility, detection of the genes encoding the Panton-Valentine leucocidin (PVL), typing of the accessory gene regulator (agr) and the staphylococcal cassette chromosome mec (SCCmec) were used to characterise isolates. Pulsed-field gel electrophoresis and spa-typing were used to genotype the isolates.

**Results:** MRSA was identified from 888 nasal swabs taken from 2492 residents. MRSA prevalence was 20%, 19%, 22% and 21% in the four sequential surveys. Isolates were commonly resistant to beta-lactam agents and two other classes of antibiotic (39%), most commonly ciprofloxacin and erythromycin (n = 243). Of the isolates examined, 34% isolates were categorised as having multiple antibiotic resistance; isolates in this category were commonly resistant to beta-lactams, ciprofloxacin, erythromycin and trimethoprim. Isolates with resistance to beta-lactam agents and no resistance to the other agents tested had a low prevalence (2.9%). No isolates carried the genes encoding PVL. The majority of isolates (81%) were related to the predominant healthcare-associated MRSA strain in the UK, EMRSA-15 (ST22-IV). The remainder belonged to five MLST clonal complexes (CC), including CC1 (n = 6) and CC5 (n = 1); lineages known to include PVL-negative strains of community-associated MRSA. During the study the prevalence of isolates belonging to the EMRSA-15 lineage decreased (87–71%) with a corresponding increase in isolates belonging to CC59 strains (10–25%). High-level mupirocin resistance (4% overall) and fusidic acid resistance (13% overall) were most commonly associated with isolates belonging to CC59 (25% overall).

**Conclusions:** These data provide a baseline for future surveillance of MRSA in the care home setting. CA-MRSA strains were uncommon; however, there was an increasing subgroup of isolates that historically have not been clearly identified as either healthcare- or community-associated MRSA strains. By tackling MRSA colonisation in both primary and secondary healthcare settings there is a greater chance of permanently reducing levels of MRSA colonisation.

**P1328** **Organisation of genetic diversity and spread of *Staphylococcus aureus* into an intensive care unit**

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**Objectives:** *Staphylococcus aureus* is an important human pathogen characterized by a high genetic diversity in healthy nasal carriers. Here, we compared the genetic diversity and population structure of *S. aureus* isolated from patients, health care workers (HCWs) and environment in an Intensive Care Unit (ICU) of Montpellier University Hospital. The objective is to genetically characterize the isolates and clarify the circulation of *S. aureus*.

**Methods:** One hundred and twenty-five *S. aureus* (110 methicillin-susceptible and 15 methicillin-resistant) were isolated between February and June 2011 from 33 patients, 26 HCWs and 36 environment samples and were divided in five groups: asymptomatic nasal carriage (NCp, n = 32), bronchial colonization (BC, n = 17) and infections (I, n = 12) in patients, nasal carriage in HCWs (NCh, n = 28), and environment (E, n = 36). The genetic diversity and population structure of *S. aureus* were investigated using MultiLocus Sequence Typing (MLST) and Double Locus Sequence Typing (DLST) with clustering of strains by the electronic Based Upon Related Sequence Type algorithm. Genotypic and nucleotide diversity ( $\pi$ ) and mean genetic diversity (H) were calculated within each group based on the 7-MLST loci concatenated sequence.

**Results:** Twenty-eight Sequence Types (STs) of which nine new STs, were found among the 125 isolates, the most prevalent being ST398 (20% of the isolates) and ST12 (11%), grouped in 12 clonal complexes. Five genotypes (ST12, ST15, ST398, ST30, ST45) were shared by patients, HCWs and environment. Nineteen DLST genotypes were detected. Variable genetic characteristics were noted among the five defined groups (Table 1). Highest and lowest genotypic diversities were observed in the groups I (0.83) and E (0.22), respectively while

**Table 1.** Sequence analysis for the seven loci of the MLST scheme in the *S. aureus* population and subpopulations (5 groups)

Group	Number of Isolates	Number of STs	Genotypic diversity	Nucleotide diversity ( $\pi$ )	Mean genetic diversity ( $\mu$ )
<b>Nasal colonization (NC):</b>					
Patients (NCp)	32	15	0.46	0.00693	0.8315
Healthy Care Workers (NCh)	28	17	0.61	0.00752	0.8428
<b>Bronchial colonization (B)</b>					
Infection (I)	12	10	0.83	0.00750	0.8247
Environment (E)	36	8	0.22	0.00707	0.7082
<b>Global population</b>	<b>125</b>	<b>28</b>	<b>0.22</b>	<b>0.00731</b>	<b>0.8564</b>

ST: sequence type

colonizing isolates displayed intermediate characteristics (Table 1). ST398 and ST12 genotypes were significantly associated with group E (Fisher Test, Odds ratio = 5.54 and 8 respectively;  $p < 0.001$ ).

**Conclusion:** High genetic diversity was observed for *S. aureus* in patients and HCWs and a gradient of genetic diversity was noted from environmental to infecting strains, suggesting that: (i) infections in critically ill patients is due to a wide variety of *S. aureus* genotypes (ii) a selection of genotypes may occur in the environment due to differential survival characteristics of the strains and/or cleaning procedures. The environmental reservoir may then favor the persistent spread of some genotypes in both patients and HCWs in the ICU.

**P1329** Incidence of community-acquired methicillin-resistant *Staphylococcus aureus* and level of non-beta-lactam resistance in outpatients with skin and soft tissue infections in Buenos Aires, Argentina

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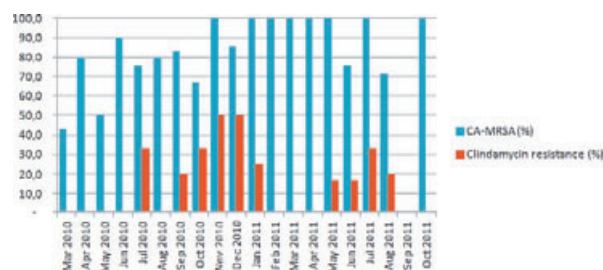
**Objectives:** Community acquired methicillin resistance *Staphylococcus aureus* (CA-MRSA) is a rapidly emerging public health problem. For empirical coverage of CA-MRSA in outpatients with skin and soft tissue infections (SSTIs) is recommended oral antibiotics including clindamycin, trimethoprim-sulfamethoxazole (TMP-SMX), a tetracycline (doxycycline or minocycline) and linezolid. However, susceptibility to these antibiotics is variable in different geographic regions, especially for clindamycin and macrolides. The aim of this study was to describe the incidence of CA-MRSA and level of non beta-lactam resistance in outpatients with SSTIs in Buenos Aires, Argentina.

**Methods:** Prospective and observational study of CA-MRSA SSTIs in outpatients attended at the Infectious Diseases Department, 'Cosme Argerich' Hospital (March 2010–October 2011). Susceptibility tests were performed by disk diffusion and Vitek 2 system (bioMérieux) according to the CLSI. For clindamycin susceptibility the D-Test method was also performed. A phenotypic rule was applied and CA-MRSA was defined as strains resistant to  $<3$  of the following non-beta-lactams antibiotics: gentamicin, erythromycin, tetracycline, TMP-SMX, rifampin and ciprofloxacin. Only one sample per patient was included.

**Results:** Of a total of 99 isolates of *S. aureus*, 79% (IC95 70–86) corresponded to CA-MRSA. Male sex: 63%. The median age was 31 years (IQR 27–53). HIV-infected patients: 8% (IC95 3–16). Clinical presentation included: cutaneous abscesses 44%, furuncles 35% and cellulitis 21%.

Resistance profile: gentamicin 8% (IC95 3–16), erythromycin 18% (IC95 11–28), clindamycin 18% (IC95 11–28) and TMP-SMX 1% (IC95 1–7). No resistance to minocycline, rifampin, vancomycin, linezolid and ciprofloxacin was observed. However it was observed 5% (IC95 2–13) of intermediate susceptibility strains to ciprofloxacin.

Strains with susceptibility to all non beta-lactam antibiotics: 73% (IC95 62–82). Resistance to clindamycin changed over time in the form of outbreaks raging from 0% to 50% in different periods of the study (Figure 1). No significant differences were seen in CA-MRSA resistance profile between HIV positive and negative patients.



**Conclusion:** A high prevalence of CA-MRSA in outpatients with SSTIs was observed. The level of resistance to clindamycin changed over time. For this reason it is crucial to surveillance the susceptibility profile of non beta lactam antibiotics in CA-MRSA infections.

**P1330** Association of MRSA colonisation and infection in adult patients hospitalised at chest intensive care units

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Study design A prospective, observational study.

**Objective:** To assess the association between the carriage of methicillin-resistant *Staphylococcus aureus* (MRSA) and the subsequent infections in patients hospitalized at chest intensive care units (ICUs) base on molecular methods

**Methods:** From November 2008 to May 2010, the patients admitted to our chest ICUs were included in this study and specimens from the nares were obtained within 3 days after admission and next week for the detection of MRSA. Genetic relatedness for all colonized and clinical isolates from each study patient with MRSA infection were analyzed and compared. Statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS software for Windows, version 16.0). Appropriate chi-square tests and Yates' correction were used for analysis. Odds ratio (OR) and 95% confidence interval (CIs) were also calculated.

**Results:** A total of 1461 patients were recruited in this study, MRSA colonization was detected in 338 patients (23%) during their chest ICU stay, and MRSA infection was noted in 432 (30%) patients during their hospitalization. Methicillin-resistant *S. aureus* colonization was significantly associated with endotracheal tube insertion, central venous catheter in site, hospitalization within 1 year, current pneumonia and previous MRSA infection, and patients with colonization had a significantly higher rate of MRSA infection, compared with those without colonization (63% vs. 20%; OR: 6.91; 95% CI: 5.25–9.09;  $P < 0.001$ ). Methicillin-resistant *S. aureus* colonization was noted for 212 (49%) of 432 patients with MRSA infections. A total of 65 clinical isolates and 43 colonized isolates from 36 patients with MRSA infection were available for genotyping analysis. Excluding four patients with multiple colonized and/or clinical isolates, colonized and clinical isolates from the same patient were indistinguishable in 24 patients (75%), highly related in six patients (18.8%), and distinct in two patients (6.2%).

**Conclusions:** Around one-fourth of the hospitalized patients were colonized with methicillin-resistant *S. aureus* during their stay in our chest ICUs; this was significantly associated with MRSA infection. Half of the patients with methicillin-resistant *S. aureus* infections had MRSA colonization and mostly an indistinguishable strain.

**P1331** Genotypic and phenotypic characterisation of methicillin-resistant *Staphylococcus aureus* strains recovered from a phase IV clinical trial for linezolid vs. vancomycin for the treatment of nosocomial pneumonia

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**Objective:** To characterize methicillin-resistant *S. aureus* (MRSA) strains responsible for nosocomial pneumonia (NP) collected during an international phase IV trial comparing linezolid (LZ) to vancomycin (VA).

**Methods:** Four hundred and thirty-five MRSA baseline isolates were collected from subjects in Latin America (LA; six countries, 45 [10.3%] strains; Table 1), Europe (EU; 10 countries, 55 [12.6%] strains), including Turkey and Russia, Asia (five countries, 67 [15.4%] strains), South Africa (three strains) and the USA (265 [60.9%] strains). Only one isolate per subject was included. PVL genes and SCCmec types were determined by PCR. All strains were subjected to PFGE and spa typing. Selected strains were evaluated by MLST. Clonal complexes (CCs) were assigned based on the spa and/or MLST results. Susceptibility (S) testing and interpretations were performed by CLSI and EUCAST methods. Inducible clindamycin (CL) resistance was assessed by D-test and isolates screened for heterogeneous resistance to VA (hVISA; Etest macromethod).

**Results:** Overall, most strains were CC5 (56.1%), which originated from the USA (CC5-MRSA-SCCmec II/IV; 70.1% [171/244]), Asia (CC5-MRSA-II; 13.9% [34/244]) and LA (CC5-MRSA-I/II; 12.3% [30/244]). The second and third most prevalent clones were CC8-MRSA-II/IV (23.4%) and CC239-MRSA-III (11.3%), respectively. Furthermore, CC5-MRSA-I/II clones predominated in Asia (50.7% within this region) and LA (66.7%), followed by CC239-MRSA-III (32.8% and 28.9%, respectively). EU strains were CC8-MRSA-IV (36.4%) or CC22-MRSA-IV (18.2%) or CC5-MRSA-IV (16.4%), while USA MRSA were CC5-MRSA-II/IV (64.5%) or CC8-MRSA-IV (28.7%). Among USA CC8-MRSA-II/IV, 73.7% (56/76) of strains (21.1% of all USA MRSA) clustered within USA300. Overall, strains were PVL-negative, except for one ST80 strain from Greece, one ST96 from Russia, one ST59 from Taiwan, one ST8 from Puerto Rico and USA300 strains from the USA. All strains were S to LZ and daptomycin, while VA and teicoplanin were active against 96.1–99.8% of strains (EUCAST). S to gatifloxacin, CL and tetracycline varied among CCs and regions. hVISA strains (14.5%) were mostly CC5-MRSA-II (63.5%; 40/63) from Asia.

Clonal complex	Number (%) of strains per region				Total
	USA	Europe	Latin America	Asia	
CC5	171 (64.5)	9 (16.4)	30 (66.7)	34 (50.7)	244 (56.1)
CC8	76 (28.7)	20 (36.4)	1 (2.2)	5 (7.5)	102 (23.4)
CC239	4 (1.5)	7 (12.7)	13 (28.9)	22 (32.8)	49 (11.3)
CC45	7 (2.6)	6 (10.9)	0 (0.0)	1 (1.5)	14 (3.2)
CC22	0 (0.0)	10 (18.2)	0 (0.0)	0 (0.0)	10 (2.3)
Total	265 (60.9)	55 (12.6)	45 (10.3)	67 (15.4)	435

**Conclusions:** Each region had two predominant clones responsible for NP. The rate of USA300 (21.1%) appears high, corroborating previous reports describing increased rates of invasive infections caused by this clone in the USA. The prevalence of hVISA was elevated in Asia and these strains appear to be associated with the CC5 lineage.

**P1332** An overview on staphylococcal linezolid resistance in Italy

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**Objective:** The most common mechanisms of linezolid resistance involve mutations in the central loop of domain V of the 23S rRNA, or

carrying of the cfr gene implicated in the methylation of A2503 in the 23S rRNA of the large ribosomal subunit, which methylation affects the binding of at least three antimicrobial classes (phenicols, lincosamides, and streptogramin A), leading to a multi-drug resistant phenotype.

The aim of our study was to investigate the rapid spread, in Italy, of staphylococci strains sharing high level resistance to linezolid, compared with the molecular mechanisms responsible of this resistance.

**Methods:** The activity of linezolid and other comparator agents was evaluated against 63 clinical *Staphylococcus* spp. isolates (53 *S. epidermidis*, five *S. simulans*, five *S. hominis* and one *S. aureus*) recovered during 2007–2011, from nine Italian hospitals, by broth dilution (MICs) and E-test methods. Mutations in the domain V of the 23S rRNA or cfr-mediated linezolid resistance were confirmed by PCR, PCR digestion with the enzyme NheI and sequencing assays. Molecular analysis was performed by PFGE, SCCmec typing (mec-complex and ccr-complex) and MLST.

**Results:** All the 63 clinical staphylococci strains, were methicillin-resistant, MDR, and showed linezolid MIC values ranging from 16 mg/L to 256 mg/L, related to G2576T mutation of 23S rRNA and presence of cfr gene. Mutational linezolid resistance was detected in 16 *S. epidermidis* strains belonging to two different PFGE types and prevalently associated to the same ST2; while, 37 *S. epidermidis* strains carried cfr gene and belonged to three different PFGE-types, widely correlated to ST 23. All *S. simulans* strains, belonging to similar PFGE subtypes (S1–S2), and all *S. hominis* strains, belonging to a unique PFGE type (H1), carried the G2576T mutation of 23S rRNA. The only strain of *S. aureus*, ST5-HAMRSA-II, carried a partial mutation of the G2576T of 23S rRNA.

**Conclusion:** This study displays a comprehensive overview of linezolid resistance among *Staphylococcus* spp. in Italy. This scenario shows the increase and rapid spread of specific clones, particularly among *S. epidermidis* strains, due to the different molecular mechanisms.

**P1333** Decreasing rates of methicillin-resistant *Staphylococcus aureus* and correlation to antimicrobial consumption pattern in a tertiary level hospital

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**Objectives:** To study prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) and effect of antimicrobial (ABC) consumption pattern in a general tertiary care hospital with high level of resistance mainly among gram-negative bacteria.

**Methods:** This was a longitudinal study of MRSA prevalence and ABC consumption rates in 15 adult hospital departments (seven internal medicine and subspecialties [MD], seven surgical and subspecialties including solid organ transplant unit [SD] and mixed ICU) from 2001 to 2010. Antibiotic consumption calculator version 3.1 (WHO) was used and annual ABC consumptions were expressed as DDDs/100 bed-days (DDDHBD). Mean annual antibiotic consumption rates were correlated (using appropriate correlation coefficient) to MRSA rates of the next year.

**Results:** MRSA rates in all departments declined from 61% to 37%,  $p < 0.001$ . Among different departments, MRSA significantly decreased in ICU (from 89% to 25%,  $p = 0.001$ ), in SD (from 67% to 41%,  $p = 0.016$ ) and after 2005 in MD (from 43% to 34%,  $p = 0.004$ ). During study period mean ABC consumption of departments increased from 112 to 136 DDDHBD ( $p = 0.004$ ). More specific, ABC consumption increased to 92% in ICU and 7% in both SD and MD. Longitudinal analysis of ABC consumption showed significant increase of penicillins (34–42 DDDHBD), carbapenems (3–10 DDDHBD), fluoroquinolones (9–16 DDDHBD) and colistin (0.2–14 DDDHBD), whereas significant decrease was found in aminoglycoside (11–9 DDDHBD) and cephalosporin (36 to 24 DDDHBD) consumption. Especially in ICU, cephalosporin consumption declined from 65 to 8 DDDHBD ( $p < 0.001$ ). No significant variations were found in consumption of glycopeptides and macrolides. Decline of MRSA rates positively correlated only to consumption of

cephalosporins ( $r = 0.76$ ,  $p = 0.017$ ) and especially of 3rd generation cephalosporins ( $r = 0.8$ ,  $p = 0.006$ ). This correlation remained significant when MRSA rates and consumption of cephalosporins (mainly 3rd generation) was studied separately in MD, SD and ICU ( $r > 0.8$ ,  $p < 0.04$ ). Negative correlations were found between MRSA rates and consumption of combination of penicillins with beta lactamase inhibitors ( $r = -0.7$ ,  $p = 0.037$ ), carbapenems ( $r = -0.8$ ,  $p = 0.007$ ), fluoroquinolones ( $r = -0.7$ ,  $p = 0.03$ ), and colistin ( $r = -0.7$ ,  $p = 0.034$ ). No correlations were found between MRSA rates and consumption of glycopeptides, macrolides or aminoglycosides.

**Conclusion:** During study period a decline of MRSA rates significantly correlated to decreasing cephalosporin consumption mainly of 3rd generation.

**P1334** Vancomycin MIC and clone type are independent predictors of persistent MRSA bacteraemia at a USA hospital

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**Objectives:** To investigate associations with persistent MRSA bacteraemias.

**Methods:** Five hundred and eighty-one patients with MRSA bacteraemia were reported at our US hospital from 1999 to 2008 and 61 isolates were selected for molecular analysis based on two factors: (i) isolates with an initial vancomycin minimum inhibitory concentration (MIC)  $\geq 2$  mg/L and/or (ii) persistent bacteraemia. A smaller cohort consisting of 59 isolates not meeting the two criteria was randomly selected from the remaining isolates. MRSA was characterised by spa type, staphylococcal cassette chromosome mec (SCCmec) allotype and MIC to vancomycin (VA), and daptomycin (DP) performed by e-test. SCCmec IV isolates were tested for carriage of the Panton-Valentine leukocidin (PVL). To explore possible associations with MICs, cut-offs of  $\geq 1.5$  and  $\geq 1$  mg/L were chosen for VA and DP respectively. Clones were defined by using Based Upon Repeat Pattern (BURP) clustering with a calculated cost between lineages of four and PVL data. Persistence was defined as bacteraemia lasting 5 days or more. Univariate analysis of contingency tables was performed using Chi-squared tests to identify variables associated with persistent bacteraemia. Multivariate binary logistic regression was used to identify independent predictors of persistent bacteraemia.

**Results:** Persistent bacteraemia occurred in 31 (25.8%) of the 120 cases (Table 1). The univariate analysis indicated that persistent bacteraemia was associated with increasing age, clone type, HIV status, and VA MIC  $\geq 1.5$  mg/L. VA MIC (adjusted odds ratio (AOR) 83.8, 95% confidence interval (CI) 9.9–710.1,  $p \leq 0.001$ ) and one clone, USA500, CC8-IV, PVL-negative (AOR 6.4, 95% CI 1.1–36.1,  $p = 0.036$ ) remained independently associated with persistent bacteraemia in the multivariate analysis. DP MIC  $\geq 1$  mg/L was not associated with persistent bacteraemia.

Table. Associations with persistent bacteraemia at a US hospital.

	No persistent bacteraemia (n=89)	Persistent bacteraemia (n=31)	Univariate P	Multivariate P	Odds ratio (95% confidence interval)
Age / years (mean +/- standard deviation)	67.3 +/- 16.7	75.5 +/- 12.1	0.02	0.445	-
Clone	n (%)	n (%)	0.01	Ref	-
CCS-II	71 (79.8)	18 (58.1)			
CC8-IV (PVL-)	9 (10.1)	10 (32.3)	-	0.036	6.4 (1.1-36.1)
Other	9 (10.1)	3 (9.7)	-	0.769	-
PVL carriage	4 (4.3)	0 (0.0)	0.07	-	-
Pigment $\geq 0.3$	49 (55.1)	17 (54.8)	0.94	-	-
Vancomycin MIC $\geq 1.5$ mg/L	30 (33.7)	30 (96.8)	<0.001	<0.001	83.8 (9.9-710.1)
Daptomycin MIC $\geq 1$ mg/L	19 (21.3)	2 (6.4)	0.06	-	-
HIV positive	16 (18.0)	0 (0.0)	0.01	0.998	-
Patient died	52 (58.4)	14 (45.2)	0.20	-	-

**Conclusion:** In our analysis, patients from a single institution with persistent MRSA bacteraemia were more likely to have a VA MIC  $\geq 1.5$  mg/L and infected with a particular clone. Other studies

have indicated that VA MIC, agr function and other factors are associated with persistent bacteraemia. Increasing age was associated with persistent bacteraemia in univariate but not multivariate analysis, which could be a function of sample size. Additional studies are required to confirm these findings and explore other possible predictors of persistent bacteraemia.

**P1335** Impact of low-level glycopeptide resistance on treatment failure in orthopaedic device-related methicillin-resistant *Staphylococcus aureus* infection

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**Objectives:** Reduced susceptibility to glycopeptides in clinical methicillin-resistant *Staphylococcus aureus* (MRSA) isolates is considered a risk factor for failure of glycopeptide therapy. We analysed the clinical impact of reduced glycopeptide susceptibility, in particular low-level teicoplanin resistance, on treatment failure of patients with orthopaedic device-related MRSA infections (ODRI).

**Methods:** Clinical and epidemiological characteristics of a retrospective cohort of patients with MRSA ODRI who were treated at the University Hospital of Geneva between 2000 and 2008 were previously reported (Ferry et al., 2010). Fifty-seven individual or multiple isolates were retrieved from 41 ODRI patients for glycopeptide susceptibility and clonality studies, including 20 patients with prosthetic joint (PJ) and 21 with osteosynthesis (OS) MRSA infections. Microbiological criteria for treatment failure were persistent ( $>5$  days) or recurrent (post-therapy) MRSA-positive infectious episodes as described (Ferry et al.). GISA isolates were detected by testing individual or consecutive, clonally-related MRSA isolates from each patient, for elevated teicoplanin or/and vancomycin MICs ( $\geq 4$  mg/L), using a previously described, modified macrodilution assay (Vaudaux et al., 2010).

**Results:** MRSA isolates showing elevated teicoplanin MICs before, during, or/and after glycopeptide therapy, were detected in 20 (49%) of the 41 MRSA-infected patients, namely 10/20 (50%) PJ-infected and 10/21 (48%) OS-infected patients. Only one isolate also showed elevated vancomycin MIC. All GISA isolates belonged to the widely prevalent MRSA clonotype ST228 found in 35/41 (85%) patients. 18/41 (43%) ODRI patients, namely nine PJ and nine OS-infected patients, experienced treatment failure. 13/20 (65%) patients with GISA-associated ODRI, namely 7/10 (70%) with PJ and 6/10 (60%) with OS, experienced treatment failure. In contrast, therapy failed in only 5/21 (24%) non-GISA-infected ODRI patients, including 2/10 (20%) with PJ and 3/11 (27%) with OS, indicating a significantly ( $p < 0.02$ ) lower failure rate in patients with non-GISA vs. GISA-associated ODRI. Emergence of low-level teicoplanin resistance could not be explained by teicoplanin administration, since 40/41 ODRI patients received vancomycin but only 5/41 teicoplanin, for therapy of the initial episode.

**Conclusion:** Low-level teicoplanin resistance in MRSA is a significant risk factor and may be a predictive marker for therapy failure, and seems to be promoted by vancomycin therapy of ODRI patients.

**P1336** A 4-year study of coagulase-negative *Staphylococcus* resistance profile in a Greek tertiary hospital

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**Objectives:** Coagulase negative staphylococci (CoNS) are recognized as one of the major causes of nosocomial infections. In this study we aimed to study the resistance profile of CoNS isolations considered as pathogen during a 4 year period.

**Methods:** From July 2007 till June 2011 we examined  $n = 977$  non duplicated CoNS isolations recovered from blood cultures of patients hospitalized in all wards of a Greek tertiary hospital. The distribution location of the collected isolates was: ICUs  $n1 = 365$  isolates, medical

ward n2 = 187 and surgical ward n3 = 233 isolates. Species identification and susceptibility testing were performed using the automated VITEK II system (Biomérieux). Additionally we used the E-test method (Biomérieux, ABI-Biodisk) in order to estimate the MIC levels of daptomycin, vancomycin, teicoplanin and linezolid when it was judged as necessary, especially to confirm some isolation resistances found by the VITEK II system. The interpretation of results was done according to the CLSI guidelines.

**Results:** Twenty three different *Staphylococcus* species were identified among the 977 CoNS isolates collected. *S. epidermidis*, *S. haemolyticus*, *S. hominis*, *S. cohnii* and *S. lugdunensis*, together, accounted for 74% of the isolates. Methicillin resistance was observed in all of the 23 species identified, with an overall prevalence of ca. 81.3%. The percentage of resistance profile of the examined CoNS isolates is shown in the following table.

All linezolid and teicoplanin resistant isolates were resistant to methicillin, too.

Period	7/2007- 6/2008 (%) (n=240 isolates)	7/2008- 6/2009 (%) (n=236 isolates)	7/2009- 6/2010 (%) (n=284 isolates)	7/2010- 6/2011 (%) (n=217 isolates)
antibiotics				
penicillin	93,2	92,8	92,1	95,4
gentamicin	41	40,2	39,1	46,8
co- trimoxazole	37	46,8	34,3	34,7
clindamycin	61,5	65	63,7	68,4
erythromycin	73,2	79,1	69,4	70,8
oxacillin	79	82,1	76,3	87,9
linezolid	0,9	1,4	3,9	12
vancomycin	-	-	-	-
teicoplanin	1,3	1,8	3,3	4,7
quinupristin/ dalfopristin	0,9	0,9	0,7	-
daptomycin	-	-	-	-

**Conclusion:** CoNS isolates present a remarkable increase of resistance to linezolid and teicoplanin in contrast to daptomycin and vancomycin which could be a very good alternative solution for treating infections caused by CoNS. These data enforced the necessity to take the appropriate measures in the hospital environment and during the clinical practice to avoid the dissemination and the amplification of these resistances.

## Genetics of antimicrobial resistance

### P1337 Role of ISKpn7 and deletions in blaKPC gene expression

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**Objectives:** The carbapenemase-encoding blaKPC gene, that is rapidly spreading worldwide, is located on a Tn3-based transposon, Tn4401. Five isoforms of Tn4401 (a, b, c, d, e), based on small deletions located immediately upstream of blaKPC gene have been described. Here, we have investigated the functional role of the different upstream located sequences on blaKPC-gene expression in respect to the major deletions observed.

**Methods:** The entire blaKPC gene with its flanking region was amplified by PCR from three clinical isolates of *K. pneumoniae* harboring Tn4401a, Tn4401b and Tn4401c, and cloned into PCR-ScriptTMCam vector resulting in pKPCprom (1, 2, 3), pKPCprom (1, 2) and pKPCprom (2) respectively. Reverse transcription and rapid amplification of cDNA ends were performed with the 5' RACE system aimed to determine the blaKPC-2 transcription initiation sites. Expression level of blaKPC gene was measured by qRT-PCR,

spectrophotometric analysis and imipenem MIC was determined by E-Test for each construct.

**Results:** We have identified three potential promoter sequences (P1, P2, and P3) upstream of blaKPC gene. Only P1 and P2 were shown to be true promoters involved in expression. P1 promoter, which is the promoter initially characterized for blaKPC gene, is absent from isoform c and d. P2 promoter was present in all isoforms, and is a composite promoter made of a -35 region located in the IR of ISKpn7, and a -10 box located in the flanking sequence. Two alternative structures surrounding blaKPC gene have been described in the literature, where both P1 and P2 promoters are absent, but where the immediately located mobile element are likely to be responsible of blaKPC gene expression. Using RT-PCR, the highest level of expression was obtained with isoform a, followed by b and c. These differences in expression led to slight differences for MIC of carbapenems. In silico analysis of DNA sequence of isoform b revealed a stem-loop structures likely responsible of strong stops, decreased expression as compared to isoform a and that could also be at the origin for the deletions observed in isoforms a and c.

**Conclusion:** Our results indicate that P1 and P2 promoters contribute to blaKPC gene expression, and that the construct with the highest expression is the isoform a, which is also the commonly encountered form in clinical isolates.

### P1338 Complete characterisation of In70-harboring plasmid pAX22 from Achromobacter xylosoxydans

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**Objectives:** VIM-type enzymes are among the most widespread metallo-B-lactamases, and their diffusion is mediated by mobile gene cassettes inserted into integrons that, in *Pseudomonas aeruginosa*, are generally chromosomally-located. Integron In70 is the most common genetic support of the VIM-1 determinant in Italy in *P. aeruginosa* and was firstly characterized in pAX22, a non-conjugative plasmid from a clinical isolate of *Achromobacter xylosoxydans*. pAX22 is the only In70-harboring plasmid known so far, and was supposed to be a donor in the acquisition and evolution of chromosomally encoded In70 by *P. aeruginosa* epidemic clones. The aim of this study was to obtain the complete nucleotide sequence of pAX22 plasmid in order to characterize the In70 genetic context.

**Methods:** The complete sequence of pAX22 was obtained by pyrosequencing using Roche 454 GS Junior, according to the manufacturer's instructions. Bioinformatic analyses were performed by Roche Newbler software; the draft sequence was finished through a bridge-PCR reaction within the terminal ends of the sequenced fragment and annotated via BASys tool.

**Results:** The complete sequence was 28 466 bp long and revealed the presence of 32 ORFs besides those composing the In70 structure; the plasmid includes genes needed for replication and maintenance functions as well as for transposition and mobilization. In70 is part of a novel transposon characterized by a Tn402-like tni module and a transposase-resolvase module upstream In70. Comparative analyses showed a high similarity with TNCP23 transposon, a mobile element that carries a different integron and is only found as a chromosomal element in *P. aeruginosa* strains; a common evolutionary origin could be hypothesized for these elements that, apart from sharing the same common ancestor, could have subsequently undergone different recombination events.

**Conclusion:** To date, pAX22 represents the unique case of a sequenced In70-harboring plasmid and one of the first characterizations of the genetic platform of VIM-1-harboring integron. The complete characterization of pAX22 structure represents an important finding and could help to get new insights on his potential role as donor source of In70-carrying elements in VIM-1 positive *P. aeruginosa*. Moreover, this study put a good base for a more comprehensive analysis of In70 genetic context in important pathogens, in order to get a better knowledge about transmission dynamics of this successful mobile genetic element.



**P1339** ICESp009, a mega-carrying conjugative genetic element in *Streptococcus pyogenes*

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**Objective:** In *Streptococcus pyogenes* efflux-mediated resistance to macrolides is mainly associated with the *mef(A)* gene that is carried by well characterized genetic elements. The *mef(E)* gene, carried by mega, has been infrequently found in *S. pyogenes*. Aim of this study was to characterize mega and its genetic context in *S. pyogenes*.

**Methods:** Fifteen *S. pyogenes* isolates showing M phenotype and carrying *mef(E)* were examined. In one isolate, MB56Spyo009, transferability of *mef(E)* was evaluated by conjugation experiments. Genomic DNA was analyzed by PFGE and hybridization assay. Nucleotide sequence of the genetic element carrying *mef(E)* was obtained by 454 technology and by direct sequencing of PCR products. Comparative analysis was performed using BLAST algorithm.

**Results:** All *S. pyogenes* isolates carried *mef(E)* in the mega element. In MB56Spyo009, macrolide resistance was transferable by conjugation both to *S. pyogenes* and to *S. pneumoniae* recipient strains. Analysis of PFGE profiles of transconjugants and hybridization assay with a *mef(E)* probe confirmed the transfer of a fragment of approximately 50 kb. Sequencing of the transferred fragment showed the presence of a 55 kb-genetic element. This element, named ICESp009, was found integrated downstream an ORF corresponding to chromosomal 50S ribosomal protein L7/L12. A free circular form was also detected. By PCR mapping, ICESp009 was also found in the transconjugants obtained and in other *S. pyogenes* isolates. In ICESp009, besides *mef(E)* and *msr(D)* encoding the two components of the macrolide efflux system, sequence analysis showed the presence of ORFs encoding a putative aminoglycoside phosphotransferase, ABC transporters, and proteins homologous to those of functional modules of ICE structures. Comparative analysis showed similarity of ICESp009 to a sequence of *Streptococcus sanguinis* and to regions of putative genetic elements of different streptococcal species, suggesting the presence of similar modules among the ICEs of streptococci.

**Conclusion:** ICESp009 is the first genetic element able to transfer *mef(E)* in *S. pyogenes* and *S. pneumoniae* by conjugation. The presence of functional modules shared among ICESp009 and genetic elements of different streptococci suggested that this element could contribute to the dissemination of antibiotic resistance among different species.

**P1340** Dynamics of digestive carriage of antibiotic-resistant integrons in new-borns during the first weeks of life

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**Objectives:** At birth, the gut of neonates is sterile but begins to be colonized on the first days of life. Resistant integrons (RI) are bacterial genetic elements able to capture and express antibiotic resistance genes. RI are largely involved in the dissemination of antibiotic resistance in Gram-negative bacteria (GNB). Three main classes of RI were described to be involved in antibiotic resistance. The aim of our study was to evaluate both by culture and by a molecular technique (cultivation-independent) the dynamics of the RI digestive carriage in term and premature new-borns.

**Methods:** One hundred and eighty-seven term and 49 premature new-borns were enrolled between October and November 2010 at the University Hospital Centre of Limoges, France. For term new-borns, meconium and the last stool before leaving the hospital (Day 2 or 3) were sampled; for premature new-borns, meconium and a stool once a week were sampled until they left the hospital. Samples were inoculated onto Drigalski agar plates; total DNA was extracted from samples using the QIAamp® DNA Stool Mini kit (Qiagen®). The three main classes of RI were detected by qPCR both from GNB and directly from samples.

**Results:** We sampled 517 specimens from term babies and 199 from premature new-borns. We isolated GNB (with a majority of *Escherichia*

*coli*) in 39% of term babies' samples and in 46% of premature new-borns' samples. We detected RI from GNB in 5.8% and 18.4% of the patients (term and premature new-borns respectively) and in 10.7% and 22.4% when we used the molecular method. We found class 1 and class 2 RI but no class 3. The proportion of RI in term new-borns was 1% from meconium samples and 9.7% from stools; in premature new-borns, the proportion was 6.1% from meconium samples and 14.8% from stools.

**Conclusion:** RI acquisition occurs rapidly during the first days of life. Few babies were colonized with RI very early, as 1–6% of meconium samples were RI-positive. Further studies are needed to investigate if the RI were acquired from the mother or from the hospital environment.

**P1341** IncFIIK plasmids conferring resistance to cephalosporins and fluoroquinolones among Enterobacteriaceae from patients hospitalised in a children's oncological clinic

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**Objective:** The aim of this study was to investigate the molecular epidemiology of extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae in a children oncological clinic in the Czech Republic.

**Methods:** From June 2009 to January 2010 a total of 51 ESBL-producing isolates were obtained from rectal swab samples of thirty patients. Further characterization with regard to ESBL enzymes, plasmid-mediated quinolone-resistance genes (PMQR), multilocus sequence types (MLST) and plasmids was performed.

**Results:** ESBL-producing strains were identified as *Klebsiella pneumoniae* (36), *Escherichia coli* (7), *K. oxytoca* (3), *Enterobacter cloacae* (3) and *Citrobacter freundii* (2). *K. pneumoniae* isolates belonged to seven MLST: 280, 321, 323, 419 and novel types 626, 627, 628. The multiresistant highly virulent epidemic *E. coli* O25b-ST131 clone was detected in one patient. The gene blaCTX-M-15 was found on large conjugative FIIK plasmids, considered as virulence plasmids specific for *Klebsiella* spp., along with blaTEM-1b, blaOXA-1, qnrB1, aac(6')-Ib-cr, strA, sul2, aac(3)-II and tetA in most isolates including the O25b-ST131 clone. Sequences of copA gene of all FIIK plasmids were identical and showed the allele type 6.

**Conclusion:** Spreading of particular clones among patients and dissemination of FIIK plasmids among bacterial species were considered to be likely involved in the ESBL bacteria infections in the clinic. This is the first study documenting these multiple antibiotic resistance elements on FIIK plasmids in different bacterial species, highlighting the evolution of IncF plasmids into new variants containing novel antibiotic resistance elements and their important role in spreading ESBL-producing bacteria among diseased children. The study was funded by Czech Science Foundation (P502/10/P083); CEITEC – “Central European Institute of Technology” (CZ.1.05/1.1.00/02.0068); Ministry of Education, Youth and Sports of the Czech Republic (MSM6215712402) and RECAMO (CZ.1.05/2.1.00/03.010).

**P1342** Comparative phylogenetics of ICEHin1056 family reveals deep evolutionary relationships of mobile genetic elements

E.R. Robinson\*, X. Didelot, D. Hood, D.W.M. Crook (Oxford, UK)

**Objectives:** Integrating and conjugating elements (ICEs) are self-transmissible mobile genetic elements. ICEs are composed of modules of conserved genes, with accessory genes at hotspots. Antibiotic resistance genes are often encoded on ICEs, leading to rapid intra and inter-specific spread of resistance. Our aim was to study ICEs with homology to ICEHin1056 in *Haemophilus influenzae* using the large number of whole genome sequences now available.

**Methods:** Members of the ICEHin1056 family were identified using tBLASTx searches on the NCBI genome database. The query sequences were concatenated core genes from ICEHin1056. Alignments were performed with the Artemis Comparison Tool

(ACT). Sequences were stored in a BIGS (Bacterial Isolate Genome Sequence) database and homologues of core genes identified. Alignments were performed in ClustalW and phylogenetic trees drawn in MEGA. Ancestral sequences were predicted using GASP (Gapped Ancestral Sequence Prediction). Predicted ancestral sequences were used as BLAST inputs to find further possible members of the ICE family and more distant relatives.

**Results:** We identified over a hundred whole or partial sequences in the ICEHin1056 family in a-, b- and g- proteobacteria. This is the largest comparative phylogenetic study of ICEs performed to date and demonstrates extensive lateral gene transfer across the whole phylum. The three core ICE modules encode: replication, type IV secretion and excision/integration. The conservation of synteny implies a powerful selective advantage of the ICE. GC content of the core modules mirrors that of the host chromosome, suggesting co-existence deep in evolutionary history.

Absence of core genes or modules represent “lifestyle” adaptations of the mobile genetic element. Absence of an integrase and presence of a replicative DNA helicase are markers of a “plasmid lifestyle”.

A variety of accessory genes are found at hotspots; they confer survival advantage in the ecological niche of the organism, which ranges from eukaryotic pathogens to extreme environments.

**Conclusion:** This large comparative phylogenetic study of ICEs allows inference about evolutionary relationships within the ICEHin1056 family. This evolutionary history is so ancient that it may link all mobile genetic elements transferred by conjugation in proteobacteria. This provides important insights into the mobile gene pool and may have implications for prediction of spread of antibiotic resistance and pathogenicity.

#### **P1343** Complete sequencing of a novel IncH plasmid carrying the blaNDM-1, blaCTX-M-15 and qnrB1 genes

L. Villa, L. Poirel, P. Nordmann, C. Carta, A. Carattoli\* (Rome, IT; Le Kremlin-Bicêtre, FR)

**Objective:** The current emergence of the blaNDM-1 gene is not related to the spread of an epidemic plasmid but rather to multiple events of acquisitions on different plasmid types. In several strains isolated from seepage water and public tap water collected from New Delhi, India, the blaNDM-1 was identified on large (>250 kb) and non-typeable plasmids (Walsh et al. 2011). The complete sequence of pNDM-MAR, a non-typeable plasmid of 250 kb identified from a *K. pneumoniae* from Morocco and carrying the genes encoding NDM-1, CTX-M-15 and qnrB1, was determined and analyzed.

**Methods:** Plasmid sequencing was performed by the 454-Genome Sequencer FLX procedure on libraries obtained on total plasmid DNA purified from an *E. coli* J53 transconjugant. Contigs with at least 15-fold coverage obtained by GS-FLX gAssembler software were assembled in continue plasmid sequences by the PCR-based gap closure method.

**Results:** Plasmid pNDM-MAR was 267 242 bp in-size and encoded 177 predicted CDS. BLASTN comparison indicated a completely novel plasmid scaffold, showing only small portions of homology with GenBank data. pNDM-MAR possessed novel replicons and transfer loci, defining a new group within the IncH plasmid family.

Plasmid pNDM-MAR carried the blaNDM-1 gene into a genetic environment that was different from those previously described for other plasmids encoding NDM-1. In addition, it encoded the ESBL CTX-M-15 and the plasmid-mediated quinolone resistance gene qnrB1. That latter was flanked by an IS26 element and the Tn3 transposase which immediately preceded the groEL-groES genes, flanking the blaNDM-1 gene. The blaCTX-M-15 gene was associated to insertion sequence ISEcp1. Plasmid pNDM-MAR carried additional resistance determinants to tellurite, mercury, quinolones, chloramphenicol, and aminoglycosides.

**Conclusion:** pNDM-MAR is the first identified plasmid carrying the blaNDM-1 gene together with an ESBL encoding gene which is the most widespread worldwide (blaCTX-M-15). It did not harbor any 16S

rRNA methylase encoding genes, whereas all the other NDM-1 encoding plasmids did. It is the largest NDM-1-encoding plasmid described so far and interestingly harbors two completely new replicons. The presence of the HIB replicon, and conservation of conjugative pilus proteins, strongly suggest that pNDM-MAR is a novel plasmid type that however derives from the IncHI plasmid family. Our study provides further insights regarding the current successful dissemination of blaNDM-1 gene.

#### **P1344** Complete sequence of an IncT-type plasmid carrying the blaOXA-181 carbapenemase gene from *Citrobacter freundii*

A. Carattoli\*, P. Nordmann, L. Villa, C. Carta, L. Poirel (Rome, IT; Le Kremlin-Bicêtre, FR)

**Objectives:** Multiple plasmids were identified in an extensively drug resistant *Citrobacter freundii* isolate in France from a patient transferred from India. That isolate co-expressed the metallo-beta-lactamases NDM-1 and VIM-1, but was additionally positive for the blaOXA-181 gene, that latter encoding a class D carbapenem-hydrolysing beta-lactamase. OXA-181 that differs from OXA-48 by four amino acid substitutions hydrolyses penicillins, carbapenems at low level, but spares broad-spectrum cephalosporins.

**Methods:** Transconjugants expressing OXA-181 were obtained at very low frequency by mating-out assays from the *C. freundii* strain to *Escherichia coli* recipient. One transconjugant contained three plasmids, two non-typeable and one assigned by PCR-based replicon typing to the IncT group. Complete sequencing of the blaOXA-181-bearing plasmid was performed using the 454-Genome Sequencer FLX procedure on libraries obtained on total plasmid DNA purified. Contigs with at least 15-fold coverage obtained by GS-FLX gAssembler software were assembled in continue plasmid sequences by the PCR-based gap closure method.

**Results:** Sequencing revealed that the blaOXA-181 gene was located onto a 83 557-bp IncT-type plasmid, named pTOXA-181. A fragment of 69 kb of pTOXA-181 derived from the IncT reference Rts1 plasmid (217 182 bp), including the replicon, the origin of transfer and partitioning proteins, but conjugation capability of pTOXA-181 was impaired by the complete loss of the transfer system, with only the TraN and TraG pilus biogenesis proteins remaining. The traG locus was actually truncated through the integration of the blaOXA-181-containing fragment, including a Tn3 transposase similar to a TnpA identified in *Acinetobacter baumannii* AbaR1 resistance island, the PinR site-specific recombinase, and the ISEcp1 insertion sequence that has mobilized the blaOXA-181 gene by a one-ended transposition process. A 34 828-bp helper IncN-like conjugative plasmid was also identified in the transconjugant, encoding a complete transfer locus. We observed that this latter plasmid had mobilized both pTOXA-181 and a small mobilizable plasmid (8969 bp) which was also identified in the transconjugant.

**Conclusion:** This study identified the genetic vehicle of the emerging blaOXA-181 carbapenemase gene. Our study shows that the plasmid at the origin of spread of the blaOXA-181 gene was totally different from that of the blaOXA-48 gene known to be vehiculated by an IncL/M plasmid.

#### **P1345** Analysis of CTX-M plasmids in *E. coli* by antisense silencing

A.R. Edwards\*, R.M. La Ragione, L. Good (London, Weybridge, UK)

**Objectives:** Extended spectrum beta-lactamases, including the CTX-M family, pose serious problems for human and animal health as they substantially reduce the number of effective therapeutic antibiotics. In enteric bacteria, ESBLs are carried on large (>60 kb) promiscuous plasmids, which are of interest because, apart from contributing resistance traits, little is known about how they benefit or burden the host bacteria. Two ESBL *E. coli* outbreak strains were isolated from a

cattle farm for study. The project objectives are: (i) Sequence and annotate the two ESBL plasmids; (ii) Establish RNA silencing of large plasmid encoded genes; (iii) determine the fitness contribution of the plasmid and certain plasmid-borne genes in relation to the host.

**Methods:** Due to difficulty in studying genes on large plasmids by traditional means, Antisense silencing is an attractive alternative for genetic and functional analyses as it can give a clearer picture of gene contribution in comparison to other techniques which may distort whole cell processes. The large ESBL plasmids were sequenced using 454 technology and this information was used to determine several targets for silencing. Peptide nucleic acid (PNA) or expressed antisense silencers or both were designed against plasmid genes of interest and their knockdown effect validated by various phenotypic assays.

**Results:** The sequence data reveals a number of genes of potential benefit to the host in addition to CTX-M. The plasmids isolated from the two strains appears to be highly homologous, but shows different lactamase activities, suggesting that genes at other loci within these large plasmids may affect the CTX-M phenotype. Work silencing the CTX-M resistance gene has shown a significant reduction in lactamase activity; however this does not initially appear to be sufficient to revert the phenotype to CTX susceptibility.

**Conclusion:** These studies show that the silencing of plasmid genes is a viable option for the study of gene function and that these ESBL plasmids do appear to contribute fitness advantages to their hosts in addition to drug resistance. Silencing of other plasmid genes is ongoing and the method validation and phenotypic effects data will be presented.

#### **P1346** CTX-M plasmid sequencing (Part 1): development of a plasmid multi locus sequence typing scheme

*M. AbuOun\*, M. Stokes, H. Preedy, L. Randall, G. Wu, R. La Ragione, N. Coldham, M. Woodward (Surrey, UK)*

**Objectives:** Enteric bacteria carrying the Extended Spectrum Beta-Lactamase (ESBL) blaCTX-M gene on large promiscuous plasmids are increasingly isolated from human and animals worldwide. These bacteria show resistance to third generation cephalosporin antibiotics and can cause both morbidity and mortality in humans. The presence of these bacteria in food producing animals is of particular public health concern as they may pose a risk of infection to humans via the food chain. Accurate identification and epidemiological tools to trace the plasmid mediated spread of blaCTX-M ESBLs within bacterial populations are essential. Current methodologies are limited to certain types of plasmids, thus a typing scheme encapsulating the range of CTX-M plasmids is required.

**Methods:** Fifteen CTX-M plasmids from various veterinary sources, CTX-M types, Inc types and of various sizes were selected for full plasmid sequencing and interrogated using BLAST analysis to identify gene/sequence candidates for the design of a plasmid multi locus sequence typing (pMLST) scheme. Six primer sets were designed to amplify and sequence 6 loci. Computer generated pMLST results from plasmids available on the NCBI database were also incorporated.

**Results:** BLAST analysis of the initial set of 15 CTX-M plasmids identified six loci, four loci allowed sequence based differentiation. The pMLST scheme presented was applied to a panel of 270 ESBL and non-ESBL plasmids. One hundred and forty-five different plasmid types were recognised by the pMLST scheme, with 42 types with more than one plasmid. There are 103 "unique" plasmid pMLST types identified. Six large pMLST types were identified containing 8–21 plasmids each. For several pMLST types, plasmids with different characteristics such as different CTX gene types, sources and incompatibility groups were present within the same pMLST type. Several pMLST types appeared to be restricted to animal source (e.g. swine).

**Conclusion:** Using newly sequenced CTX-M plasmids of animal origin, a generic plasmid typing scheme has been developed. The pMLST scheme has been used to determine the relationships between 270 plasmids from various sources and possessing a range of

characteristics. The scheme identified several groups of plasmids from related sources or CTX group. The development of this generic pMLST typing scheme will contribute much needed information for further epidemiological studies to track the dissemination of ESBL plasmids from farm to fork.

#### **P1347** CTX-M plasmid sequencing (Part 2): development of plasmid biomarkers for epidemiological studies

*M. Stokes\*, M. AbuOun, H. Preedy, L. Randall, G. Wu, I. Freire-Martin, R. La Ragione, M. Fielder, M. Woodward, N. Coldham (Surrey, Kingston, UK)*

**Objectives:** CTX-M genes are the most widely disseminated plasmid mediated Extended Spectrum beta Lactamase (ESBL), and such plasmids can be carried by different Enterobacteriaceae species. *E. coli* containing CTX-M plasmids can cause both morbidity and mortality in animals and humans, and can be difficult to treat due their resistance to 3rd and 4th generation cephalosporins. Plasmids harbouring blaCTX-M vary in size and replicon type and often have similar plasmid backbones and undergo recombination events. Such changes are typically facilitated through mobile genetic elements resulting in a mosaic and recombinant plasmids. The presence of such plasmids in bacteria from food producing animals could lead to transmission of bacteria to humans through the food chain. By developing plasmid biomarkers, it is aimed to identify the epidemiology of ESBL plasmids as well as identifying recombination events.

**Methods:** Eight plasmids ranging in size from 35 to 160 kb with various CTX-M and Inc types were isolated from *E. coli* originating from chickens, turkeys, cattle and pig which were sequenced using a Roche 454 sequencer. BLAST analysis against the NCBI database identified several plasmid specific sequences to eight of the plasmids from which the primers were designed. Primers were screened in silico using computational methods against CTX-M and non CTX-M plasmids from the NCBI database and then tested against a range of field isolates.

**Results:** The analysis of the plasmids sequences identified 39 specific sequences, which have been used to distinguish the eight sequenced plasmids. Of these 39 sequences, 14 were unique among the eight plasmid sequences. All plasmids could be distinguished using a combination of primers, including plasmids which are in the same pMLST group according to a new typing scheme (Poster by AbuOun ECCMID 2012). Plasmids from poultry and pig isolates shared a high proportion of biomarkers suggesting link between plasmids.

**Conclusion:** The sequencing of CTX-M plasmids in *E. coli* of veterinary origin has allowed identification of biomarkers which can be used to distinguish between several different plasmid types. These biomarkers could further be applied to elucidating the evolution of plasmids through recombination events as well as the epidemiology of ESBL plasmids, potentially highlighting routes of transmission and dissemination.

Accompanying abstract: CTX-M plasmid sequencing (Part 1): Development of a plasmid multi locus sequence typing scheme.

## Diagnosis and follow-up of syphilis

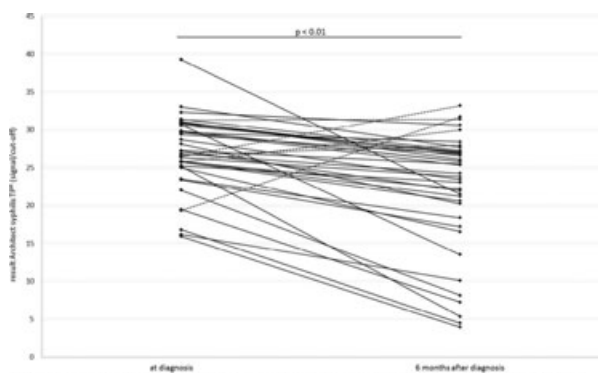
#### **P1348** Value of a treponemal test in follow-up of therapy in patients with syphilis

*S. Desmet\*, P. De Munter, K. Lagrou (Leuven, BE)*

**Objectives:** Nowadays follow-up of patients with syphilis is based on non-treponemal tests. Guidelines recommend evaluation of the non-treponemal titer at fixed time points after diagnosis. The applicability of treponemal tests in follow-up is not clear and has not been studied in detail. The aim of this study is to evaluate the value of a treponemal test in follow-up of patients with syphilis.

**Methods:** The Architect Syphilis TP<sup>®</sup> (Abbott) is a chemiluminescence immunoassay for the detection of IgG and IgM antibodies to *Treponema pallidum* (TP) in human serum or plasma. The presence or absence of anti-TP antibodies is determined by comparing the signal (S) of the reaction to a cut-off signal (CO). In this retrospective study, 54 patients with a diagnosis of syphilis and with more than one follow-up sample in a 2 years' time period were included. Diagnosis was based on clinical signs and symptoms, patient history and treponemal and non-treponemal test results. Relapse or reinfection was defined by a fourfold increase in Rapid Plasma Reagin (RPR) titer (Macro-Vue<sup>®</sup> RPR, Beckton Dickinson).

**Results:** Seven out of eight patients diagnosed with a relapse or reinfection had an increase in treponemal signal (mean increase: 6.95 S/CO) at time of relapse or reinfection compared to the signal of a sample before the new diagnosis. The increased treponemal signal corresponded to the increase of the RPR titer. All patients without relapse or reinfection during follow-up showed a minimum fourfold decrease in RPR titer within 24 months. The treponemal result remained positive in all patients in follow-up. However, patients with a follow-up sample 6 months after diagnosis and subsequent treatment, had a significant lower signal of the Architect assay compared with the signal at diagnosis (Wilcoxon;  $p < 0.01$ ) (Figure). Also 12 and 24 months after diagnosis the signal showed a significant decrease (Wilcoxon;  $p < 0.01$ , Wilcoxon;  $p < 0.01$ ) compared with the signal at diagnosis.



**Conclusions:** The findings of this study show a promising correlation between the Syphilis TP<sup>®</sup> signal and treatment response of patients with syphilis. The treponemal signal decreased significantly after successful treatment. Relapse or reinfection cases during follow-up were associated with an increase in treponemal signal in seven out of eight patients. Further studies are needed to evaluate whether treponemal tests can add value to the follow-up of syphilis.

#### P1349 Diagnostic value of the ADVIA Centaur<sup>®</sup> syphilis assay for the screening of antibodies to *Treponema pallidum*

A. Donkers\*, A. Letens van Vliet (Rotterdam, NL)

In this study, the ADVIA Centaur<sup>®</sup> Syphilis was compared to the IMMULITE<sup>®</sup> 2000 Syphilis Screen and the bioelisa SYPHILIS 3.0 assay for the detection of total antibodies specific for *Treponema pallidum*. The ADVIA Centaur<sup>®</sup> Syphilis assay uses Tp15 and Tp17 recombinant antigens, the IMMULITE<sup>®</sup> 2000 Syphilis Screen uses Tp17 rec. antigens. The bioelisa SYPHILIS 3.0 assay uses Tp15, Tp17, and Tp47 rec. antigens. All reactive samples were tested with VDRL and Euroline *Treponema* IgG and IgM Western-blot.

**Objective:** To evaluate the performance of the ADVIA<sup>®</sup> Centaur Syphilis assay.

**Methods:** A total of 1251 patient samples (of which 500 pregnancy specimens) were tested by the ADVIA Centaur<sup>®</sup> syphilis, IMMULITE<sup>®</sup> 2000 Syphilis Screen and bioelisa SYPHILIS 3.0 assay. All reactive samples were tested by Western-blot IgG and IgM, and VDRL. The ADVIA Centaur<sup>®</sup> and IMMULITE 2000<sup>®</sup> Syphilis Screen are fully automated, one-step chemiluminescent immunoassays. The bioelisa SYPHILIS 3.0 assay is a two-step enzyme immunoassay (EIA). The Euroimmun *Treponema* Western-blot is for the separate detection of IgG and IgM antibodies using the pathogen-specific *Treponema* antigens Tp15, Tp17, p22 (unspecific), Tp45, and Tp47.

**Results:** The overall agreement for the Centaur vs. IMMULITE 2000 Syphilis Screen is 100%, the agreement Centaur and IMMULITE vs. the bioelisa SYPHILIS 3.0 assay is 99.92%. The relative sensitivity, specificity, for the Centaur and IMMULITE 2000 is 100%. The Centaur and IMMULITE relative sensitivity, specificity vs. bioelisa Syphilis Screen kit is 99%, and 100% respectively. Out of a total of 100 reactive results, with all three assays, 11 samples were diagnosed as active infections based on a positive IgM blot and a VDRL  $\geq 1:8$ . One bioelisa positive, negative Centaur and IMMULITE sample was negative with the WB IgM, IgG and VDRL. The WB IgM and VDRL test results suggest the presence of reactivity to anti-TP in 0.88% (11/1251) of the tested population.

**Conclusions:** The ADVIA Centaur<sup>®</sup> Syphilis Assay has shown to be a highly specific and sensitive method for syphilis antibody screening. The follow-up of reactive syphilis results with a combination of WB IgM and VDRL is a good method to confirm the active infections and reduce the amount of FP VDRL results.

#### P1350 Evaluation of an automated chemiluminescent microparticle immunoassay for screening of antibodies against syphilis

Z.Y. Li\*, P. Liu, Z. Feng (Beijing, CN)

**Objectives:** To analyze the performance of a newly introduced chemiluminescent microparticle immunoassay (CMIA) for screening of antibodies against syphilis, CMIA was compared to the rapid plasma reagin test (RPR), ELISA for detection of antibodies against syphilis, and the *Treponema pallidum* particle agglutination assay (TP-PA) in the present study.

**Methods:** A total of 4870 samples were detected of antibodies against syphilis by CMIA, ELISA, RPR and TP-PA. When the results were inconsistent using the four methods, dot-immunoblotting test (dot-IBT) was adopted for confirming the test results. Using dot-IBT as gold standard, the sensitivity and total accordance rate of the other four methods were analyzed.

**Results:** There were 160 samples with inconsistent results. Among these, 149 samples were reactive by CMIA, 119 samples by ELISA, 116 samples by TP-PA and 58 samples by RPR. After retesting by dot-IBT, 122 samples were confirming to be reactive, with 13 equivocal results. The sensitivities of CMIA, ELISA, TP-PA and RPR compared with the results of the dot-IBT were 96.7%, 93.4%, 91%, and 46.7%, respectively. CMIA has highest sensitivity, but there were 19 false positive results. The accordance rate of CMIA with the dot-IBT was 77.5%, only slightly below the rate of ELISA and TP-PA (84.4% and 83.1%, respectively). The receiver operating characteristic (ROC) curve showed that the area under the curve of CMIA was higher than that of ELISA (0.920 and 0.901, respectively), but there was no significant difference. The diagnostic optimal cutoff determined by this analysis was  $>1.91$  S/CO for CMIA. The sensitivity and specificity obtained at this cutoff point were 90.9% and 96.0%, respectively.

**Conclusion:** The high sensitivity and specificity of CMIA, together with the fact that it was a simple, objective, and easily automated method, lead us to believe that it could be used as a screening test for syphilis.

**P1351** Comparison of two fully automated serologic tests for Lues antibodies

M. Obermeier\*, S. Müller, H. Klima, R. Bertele, S. Neifer, T. Berg (Berlin, DE; Rotkreuz, CH; Penzberg, DE)

**Background:** Screening for Lues antibodies is still performed with TPPA (Treponema pallidum particle agglutination) or TPHA (Treponema pallidum hemagglutination) in many laboratories. Especially in settings with a high throughput there is an urgent need for automatized testing, which can not be fulfilled by those assays.

**Material and methods:** We compared the Abbott ARCHITECT Syphilis TP (Micro particle enzyme immuno assay) vs. the Roche/Sekisui Cobas Mediace TPLA (Treponema pallidum latex agglutination test), which is a latex enhanced immunoturbidimetric assay. We analyzed 617 fresh serum samples sent to the Medizinisches Labor Dr. Berg for routine diagnostics in which a high prevalence of Lues antibodies was expected and 300 samples from healthy blood donors.

**Results:** In the 617 routine clinical samples 197 (31%) of the samples were tested positive and 397 (64%) were tested negative in both assays, resulting in an overall agreement between the two assays of 96%. In the 300 samples from healthy blood donors all 300 samples were detected negative with the Architect assay, while only 264 samples were detected negative by the Roche/Sekisui assay. As the Roche/Sekisui assay is based on turbidimetric measurement we analysed Serum Indices, especially the basal turbidity of the samples which is represented by the L(=lipemic)-Index. In the 36 samples which showed reactivity in the Roche/Sekisui assay, while negative in the Abbott assay, the L-indices were between 28 and 969 with a median of 347.5. Forty-five samples which were negative for both assays showed L-indices between 14 and 434 with a median of 77. This difference in L-indices between falsely reactive and correctly negative samples was statistically significant ( $p < 0.005$ ).

**Conclusions:** Both assays show excellent performance in terms of sensitivity and specificity in the routine clinical samples. In the samples from healthy blood donors a number of unspecific positive results with the Roche/Sekisui assay were observed. As the Roche/Sekisui Mediace TPLA is a turbidimetric assay we were observing some interference in samples with high turbidity (high L-index). Therefore we recommend testing of serum indices in each sample and careful evaluation of samples with high lipemic indices (L-Index  $>100$ ). We conclude that both assays are valuable tools for syphilis screening. Nevertheless, positive results should be confirmed by further serological testing.

## Diagnosis and prevention of device-associated infection

**P1352** Successful prevention of ventilator-associated pneumonia (VAP) in intensive-care setting by using VAP prevention bundle

M.A. Alhedaihy\* (Riyadh, SA)

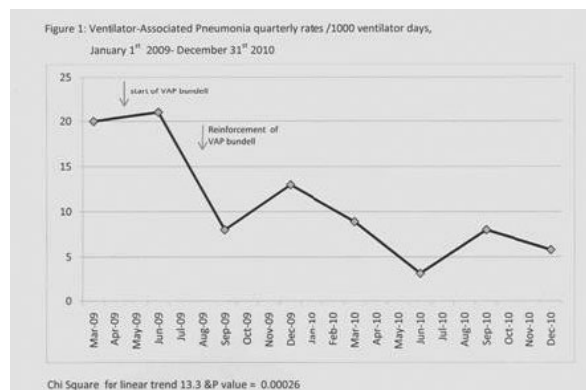
**Background:** Ventilator-associated pneumonia is one of the most common health-care associated infection (HAIs) encountered in the intensive care units with an incidence ranging from 6% to 52%. It has the highest mortality rate of any HAI. Many different interventions for preventing VAP in ICUs have been described. In this study we report the results of implementing the VAP prevention bundle suggested by the Institution of Healthcare Improvement in a private hospital, Riyadh, Saudi Arabia.

**Methods:** This study was done as part of quality improvement plan in the 14bed ICU of Dallah Hospital, a 350 bed private, general hospital in Riyadh, Saudi Arabia. VAP was defined according to CDC definition. The surveillance was carried out by trained infection control practitioners. The study period was from January 2009 until 31 December 2010. The VAP bundle consisted of Elevation of the head of the bed to  $>30^\circ$ , Daily "sedation vacations" and assessment for

readiness to extubate, Peptic ulcer prophylaxis and Deep venous thrombosis prophylaxis.

**Statistical analysis:** Data were analyzed using Epi-Info Software. 2010 was compared to 2009 regarding VAP rate and mortality rate in ventilated patients. Relative Risk (RR) and 95% Confidence Intervals (CI) were calculated. Chi square was used to assess linear trends for the 2 year period. p Value was considered significant if  $\leq 0.05$ .

**Results:** The total admission to the ICU in 2009 and 2010 were 497 and 465 with total ICU days of 3327 and 4405, respectively. The number of VAP was 18 and 10 with VAP rate of 15.3/1000 ventilator days and 4.34/1000 ventilator days, respectively ( $p = 0.0006$ ). The mortality in ventilated patients decreased significantly at 65.6/1000 ventilator days in 2009 vs. 32.6/1000 in 2010 ( $p = 0.000$ ).



**Conclusions:** VAP remains one of the leading causes of mortality and morbidity in ventilated patients. Several evidence based interventions have been shown to reduce the VAP rate and mortality in ventilated patients. The Institute for Healthcare Improvement has recommended the implementation of several of these interventions in a bundle format to decrease the VAP rate and mortality in ventilated patients. In Dallah Hospital we started a Quality Improvement Plan for several device-associated infections including VAP in 2008 using the published guidelines and tools. Our findings confirm the previously published results of the effectiveness of bundle implementation.

**P1353** Comparison of bacterial colonisation of central venous haemodialysis catheters with needle free connection device TEGO® and conventional closing cap system

S. Erb\*, P. Grosse, S. Tschudin-Sutter, U. Neff, M. Dickenmann, A.F. Widmer (Basel, CH)

**Introduction:** Catheter related blood stream infection (CRBSI) is a major problem in patients with long-term central venous catheter (CVC) for chronic hemodialysis. The closed needlefree connection device system TEGO® was developed to protect long-term CVC from tip and hub colonization and showed a 50% decrease of CRBSI in children with a hemodialysis catheter in one study (McAfee et al. 2008). The goal of our study was to determine the colonization rate of central venous catheters used for hemodialysis with the TEGO® connection device in comparison to conventional closing cap (CCC) Discofix® in the dialysis centre of the University Hospital in Basel, Switzerland – a university affiliated tertiary care center.

**Methods:** All consecutive patients receiving hemodialysis using a permanent or transient CVC with the TEGO® connection device system and 0.9% sodium chloride as catheter branch lock solution in June 2010 were included. In July 2010, the TEGO® system was replaced by conventional closing caps Discofix® using 46.7% or 30% citrate lock solution respectively. Lock solution from the arterial and the venous branch of the CVC of all patients were cultivated in aerobic blood

culture bottles (BacT/ALERT<sup>®</sup>) at a given time during the study period from 26 June 2010 until 7 June 2011 (Table 1).

**Results:** In the TEGO<sup>®</sup> group, 16 of 33 patients (48.5%) had bacterial growth of at least one microorganism from the 0.9%-sodium chloride lock solution. Bacterial colonization in the CCC-group with citrate lock solution was found in only seven of 56 tested patients (10.8%), thus significantly less frequent than in the TEGO<sup>®</sup>-group ( $p < 0.001$ ).

Coagulase-negative staphylococci were the most common pathogens detected in both groups.

Table 1. Results and baseline characteristics of the TEGO<sup>®</sup> and the CCC-Discofix<sup>®</sup> group

	TEGO <sup>®</sup>	Discofix <sup>®</sup>
Number of patients	33	56
Patients with culture positive lock solution	16 (48,5%)	7 (10,8%)*
Total isolated bacteria	21	9
CNS	16 (76,2%)	5 (55,5%)
<i>Streptococcus sanguinis</i>	1	
<i>Micrococcus sp</i>	1	
<i>Aerococcus viridans</i>	1	
<i>Bacillus non-anthraxis</i>	1	
<i>Enterococcus faecalis</i>		2
<i>Enterobacter cloacae</i>		1
<i>Pseudomonas aeruginosa</i>		1
Gram-pos cocci**	1	
Time connection device in use	15 August 2008 - 5 July 2010	6 July 2010 - on going
Culture date of lock solution	26 June - 5 July 2010	9-10 August 2010 and 6-7 June 2011
Lock solution	0,9% sodium chlorid	46,7% or 30% - citrate
Disinfection of device/tub	Ethanol 80%	Octenidindihydrochlorid (Octenisept <sup>®</sup> ) or Octenidindihydrochlorid-propranolol (Octeniderm <sup>®</sup> )
Exchange of connection device	once weekly	3 times weekly
Mean age in years (range)	67,3 (28,7-86,9)	67,5 (48,5-89,5)
Gender: female patients	23 (69,7%)	38 (67,9%)

\*OR: 6.59 (95% CI 2.08-21.58),  $p=0.00018$

CNS=coagulase-negative staphylococci (*S. epidermidis*, *hominis*, *warneri*, *capris*, *vitulans*, *simulans*). \*\* not more differentiated

**Discussion:** In contrary to other reports, we found a significant higher colonization rate of the TEGO<sup>®</sup>-sodium lock solution compared to conventional closing caps with citrate-lock solution.

The ease of use of closed needlefree connection devices without antimicrobial active lock solutions (as e.g. citrate) in hemodialysis CVC should be balanced with the infectious risk, since colonization proceeds infection.

#### P1354 Impact of multifaceted "bundle" approach in controlling catheter-related bloodstream infections outside the intensive care unit

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**Objective:** To determine the impact of a multifaceted "bundle" approach in controlling catheter-related bloodstream infections (CRBSIs) outside the Intensive Care Unit (ICU).

**Methods:** We performed a 1-day prevalence studies, in a 350-bed hospital, from 1991 to 2010, in order to know the proportion of patients with intravascular catheter (IC) and parenteral nutrition (PN), and all positive blood cultures in adults patients were prospectively followed-up. CRBSIs were defined according to CDC criteria. On November 2008 a comprehensive, multifaceted CRBSIs control program was instituted: instruction in insertion and after care of central venous catheter (CVC) and peripheral line, handwashing, appropriate use of gloves and dispensers for alcohol-based handrubs were installed in every room. For each episode of bacteremia, we collected data as: date, admission ward, germ, catheter site, PN and evolution. CRBSIs incidence per 1000 patient-days was calculated. To assess the efficacy

of our interventions, we recorded (before and after the intervention) the epidemiology and clinical features of CRBSIs in non-ICU adults patients. The X2 for trend was used to compare CRBSIs rates.

**Results:** Prevalence of IC use progressively increased until 2010. In 1991: 19.2% of the patients had peripheral IC, 1% CVC, 1.9% PN; in 2010: 67% peripheral IC, 2.1% CVC, 1.6% PN. We followed 556 CRBSIs, 491 (88.3%) of these in non-ICU patients. Out of 491 cases, 270 were confirmed by culture of the catheter tip (73.2% in CVC vs. 26.9% in peripheral IC). CVC associated bacteremia was the most frequent (298), more often related to PN (227/68) and due to coagulase negative *S.* (215/94), and less frequently by *S. aureus* (35/63) than that peripheral device associated,  $p < 0.05$ . CRBSIs attributable mortality was 4.9%, being higher during summer months (8.7%) and for infection by *S. aureus* (11.2%),  $p < 0.05$ . CRBSIs rate progressively increased until a maximum of 0.47/1000 patient-days in 2008 (51 cases). The distribution per months showed higher incidence in summer period. After the intervention, the incidence fell to 0.32/1000 patient-days in 2009 (34 cases) and in 2010 (33 cases), 32% decrease,  $p < 0.05$ . From 2008 to 2009–2010 the prevalence of IC use and the alcohol-based handrubs consumption increased, and there were no differences between other analysed variables.

**Conclusions:** The implementation of multifaceted infection control program decreased the clinical impact of CRBSIs outside the ICU.

#### P1355 Ethanol lock therapy in the prevention of catheter-related bloodstream infections after major heart surgery

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**Objective:** E-Lock has been successful in the cure and eradication of pathogens in patients with CR-BSI. Randomized trials with E-Lock as a therapeutic agent, however, are very limited and results are yet inconclusive. Furthermore, the potential value of Ethanol lock therapy (E-locks) in the prevention of catheter-related bloodstream infections (CR-BSI) has not been evaluated. Our work consist in the assessment of the value of E-Locks in the prevention of CR-BSI in the particular setting of patients with non-tunneled, short-term, Central Vascular Catheters (CVCs) in the postoperative period of patients undergoing Major Hear Surgery (MHS).

**Methods:** This is an academic, prospective, randomized, open and controlled clinical trial comparing patients with E-Lock and patients receiving conventional catheter-care (CCC). Patients with CVCs for >48 hours were invited to participate in the study and randomly allocated to one of the two arms. Patients with E-lock had CCC and all their catheters ethanol locked for a period of approximately 2 hours per day and catheter. The control group of patients received CCC according to standard recommendations. All catheters were withdrawn when clinically required and the catheter tips were systematically sent for culture. Definitions of Catheter Colonization and CR-BSI were standard (Mermel et al., CID 2009).

**Results:** The study is scheduled to finish in 31 December 2011. Up to the present time, 138 patients with 208 catheters were included. Overall 115 catheters (74 patients) received E-Locks and 93 catheters (64 patients) received CCC. Both groups were comparable in severity (Euroscore 3.78 vs. 4.31  $p = 0.21$ ). The results for the E-Lock and CCC were respectively as follows: episodes of Catheter Colonization 8.8% vs. 14.34% ( $p = 0.48$ ), colonization of catheter's hubs 4.09 vs. 4.40 ( $p = 0.88$ ), colonization of skin around catheter entrance 22.54 vs. 22.02 ( $p = 0.90$ ), CR-BSI 1 vs. 3 ( $p = 0.24$ ), median days of antibiotic use 7.76 vs. 7.79 ( $p = 0.99$ ), median length of ICU stay, 5 vs. 6 days ( $p = 0.82$ ) and median length of hospital stay, 11 vs. 14.5 days ( $p = 0.40$ ). No severe clinical side effects of E-Locks were observed. The E-Locks were much more time demanding for the nursing staff than CCC.

**Conclusion:** At this interim analysis, Ethanol Catheter Locks do not significantly prevent CR-BSIs nor improve in other surrogate markers of catheter infection. The process is well tolerated by the patients but cumbersome and time-consuming for the Health Care Workers.

**P1356 Validation of a prediction rule for drain-related meningitis complicating external cerebrospinal fluid drainage**

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**Objectives:** Drain-related meningitis (DRM) is a relatively frequent complication of external cerebrospinal fluid drainage using ventricular (EVD) or lumbar (ELD) drains. Manual surveillance of infection rates is time-consuming and prone to subjective interpretation. Therefore, an automated prediction model was previously developed to retrospectively identify patients who developed DRM (*PLoS One* 2011; 6: 8). We now present the results of model validation in an independent patient population within the same medical center.

**Methods:** Model validation was performed on patients receiving an EVD or ELD in the University Medical Center Utrecht between 1 January 2010 and 10 June 2011 (n = 137). Children, patients with multiple simultaneous drains, <1 day of follow-up or meningitis at drain placement were excluded (n = 105 in analysis). The model uses drain characteristics as well as clinical chemistry results, microbial culture results and antibiotic use from a clinical data warehouse as predictors. Model prediction was compared to routine surveillance of DRM by infection control professionals (reference standard). Missing data were imputed using multiple imputation. Recalibration of the model was performed on the derivation and validation dataset combined (time period 2004–2011), to improve prediction in future patients.

**Results:** Of the 105 included patients, 20 developed DRM as determined by the reference standard (17.4/1000 days at risk). The discriminatory power as determined by the area under the ROC curve was 0.951 (95% confidence interval: 0.914–0.988). The sensitivity and specificity of the model were 100% and 88.2% respectively, and predictive values were 58.8% positive and 100% negative. The predicted overall number of infections, as given by the summed predicted probability was 19.52 (observed = 20). Calibration of the model was adequate although the relatively large proportion of culture negative infections gave some distortions. Recalibration of the model (intercept = -0.1792, slope 1.0032) improved the group-level prediction although sensitivity at the patient-level declined slightly (99.0–97.1%)

**Conclusion:** The previously developed prediction model for drain-related meningitis performed well in an independent patient population. Recalibration further improved performance at the group level and more extensive model updating will be done to achieve optimal performance in new populations. This model will then be tested in a multi-center validation.

**P1357 Success revisited: 8-year sustainability of an intervention to prevent urinary tract infections in orthopaedic patients**

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**Background:** Overuse of urinary catheters (UC) is the main modifiable risk factor for healthcare-acquired urinary tract infections (UTI). In 2003, a multimodal intervention in the framework of a prospective controlled trial succeeded in reducing UTI (by >50%) and antibiotic use, whereas the control group sustained UTI rates (Stephan F, et al. *Clin Infect Dis* 2006; 42: 1544–1551). The objective of this study was to evaluate whether the intervention effect was sustained at 8-year follow-up.

**Methods:** Prospective incidence surveillance of all patients undergoing elective orthopedic surgery at the University of Geneva Hospitals (HUG) over 3 months starting November 2009, for UC use and UTI. We compared the results with those of the 2-year follow-up of the original study (N = 300 patients), reapplying the same definitions and methods. In addition, semi-structured staff interviews on indication, training, insertion techniques, and recall of the former intervention were performed.

**Results:** We included 336 study patients (mean age 60 years; 55% female). Among those, 10.1%, 3.6%, 3.9%, 17.6% received an UC in the current study against 15.7%, 1.0% and 3.7%, 18.7% at 2-year follow-up in the operating room, the post-anesthesia care unit (PACU), surgical wards, and overall, respectively. The UTI incidence density amounted to 2.4 per 1000 patient days in this study vs. 2.6 at 2-year follow-up (vs. 6.5 before the intervention). The 17 interviews demonstrated that the collective memory of the intervention and knowledge of guidelines was poor except in the operating room where we identified a champion opinion leader.

**Conclusions:** The intervention effect was sustained concerning the overall UTI rate and the catheterization rates (except that in PACU). Dedicated advocating by an opinion leader seems beneficial to sustainability. Qualitative inquiry was useful in confirming and explaining the quantitative results.

**P1358 Microbiology of cardiac device infections**

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**Objectives:** We aimed at studying the microbiological characteristics of implantable cardiac devices (CDs) infection observed during the 2000–2011 period at the Cardiology Unit of Cisanello Hospital in Pisa (Italy), a national reference centre for transvenous removal of the infected CD.

**Methods:** The tip or other parts of the leads were rolled onto the solid media while the material drawn from the pocket was spread directly on the culture plate. Blood culture system used was BACTEC 9240. For organism identification an automated system (API, Bio-Merieux, Marcy L'Etoile, France) was used. Antimicrobial susceptibility was tested according to the Kirby Bauer method.

**Results:** Electrodes from 1204 patients were analysed. Out of these, 854 (70.9%) tested positive. In 663 (77.6%) cases only one species was isolated, in 175 (20.5%) two species and in 14 (1.8%) more than two species. In 116 cases material from the pocket was also cultured. The result was consistent with that from the electrodes in 69 (59%) cases, including 16 cases in which culture was negative from both samples. In 359 cases a blood sample was also obtained for culture. The result was consistent with that from the electrodes in 124 (35%) cases, including 70 cases in which culture was negative from both samples. A total of 1068 strains was isolated from electrodes (Table 1). Coagulase negative staphylococci (CoNS) were 69% of isolates, *Staphylococcus epidermidis* was the most frequently isolated single agent (67% of CoNS isolates), followed by *S. capitis* (5.8%) and *S. schleiferi* (5.3%). Oxacillin-resistance was 33% among CoNS and 13% among *S. aureus*; strains were generally susceptible to glycopeptides

**Table 1. Isolates from electrodes of CDs  
Pisa, 2000-2011**

	N	%
<b>Total isolates</b>	1,068	100.0%
<b>Gram positive</b>	988	92.5%
CoNS	737	69.0%
<i>Staphylococcus aureus</i>	147	13.8%
<i>Corynebacterium spp</i>	53	5.0%
<i>Propionibacterium spp</i>	27	2.5%
<b>Gram negative</b>	65	6.1%
<i>Enterobacteriaceae</i>	32	3.0%
<i>Pseudomonas spp</i>	16	1.5%
<b>Candida spp</b>	11	1.0%
<i>Candida albicans</i>	4	0.4%
<b>Moulds</b>	4	0.4%

and linezolid. Seventeen percent of Enterobacteriaceae strains had a phenotype compatible with extended spectrum beta-lactamase (ESBL) expression.

**Conclusion:** Culture of the removed electrodes offers the possibility of an etiologic diagnosis of CD infection in the great majority of cases. Culture of material from the pocket is often consistent with that from the electrodes, while species isolated from blood cultures are often different and more likely to be the result of contamination. CD infection is more often monomicrobial, CoNS are most frequently isolated and *S. epidermidis* is largely the main single agent. The pattern of susceptibility to antimicrobials is in general that of community-acquired infections, though oxacillin resistance among CoNS and ESBL-like phenotype among Enterobacteriaceae were relevant.

#### P1359 Atrium blood culture for the diagnosis of cardiovascular implantable electronic device endocarditis

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**Objectives:** Transvenous removal of complete hardware of cardiovascular implantable electronic device (CIED) is indicated for CIED infections. In case of endocarditis, vegetations larger than 2 cm, are at risk of massive pulmonary embolism, therefore percutaneous lead extraction should be postponed or surgical removal might be preferred. The best way to see the diameter of lead or right valve vegetations is intra-cardiac echocardiography (ICE), performed intravenously via femoral vein. Once the echocardiography lead is removed, a blood sample may be taken from the atrium. Our objective was to demonstrate this new method is sensitive and specific.

**Methods:** Once ICE was performed, 20 mL of blood were taken from the atrium and an aerobic and an anaerobic culture bottle were inoculated. Blood culture system used was BACTEC 9240 (Becton-Dickinson, Milano, Italy). The incubation time was prolonged for at least 14 days.

**Results:** In the last 2 years we have performed 21 atrial blood cultures. According to our data, taking as gold standard leads cultures, atrial blood culture has a sensibility of 50% and a specificity of 42%; peripheral vein blood culture has a sensibility of 6% and a specificity of 57% (Table 1). We had to consider as false negative four patients with positive atrial blood cultures and leads cultures negative, patients treated for several months with antibiotics targeted on isolates from atrial blood.

Overall, 18 microorganisms were isolated, 14 coagulase negative staphylococci (CoNS), two *Propionibacterium* spp. and two gram negative.

Table 1. Results of atrial blood culture (ABC), CIED leads culture (LC), and peripheral vein blood culture (PVBC).

	ABC pos	ABC pos	ABC neg	ABC pos	ABC pos	ABC neg
	LC pos	LC pos	LC pos	LC neg	LC neg	LC neg
	PVBC pos	PVBC neg	PVBC neg	PVBC pos	PVBC neg	PVBC neg
Number of patients	1	6	7	3	1	3

**Conclusion:** Our cases suggest atrial blood cultures performed during the procedure for intra-cardiac echocardiography might be an useful option for better targeted antibiotic therapy in difficult to treat CIED endocarditis.

#### P1360 Sonication of explanted devices improves the pathogen detection in cardiac device infections

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**Objectives:** There is a lack of microbiological gold standard for the diagnosis of cardiac device infections (CDIs). Aim of the study was to analyze the role of sonication before culture in the diagnosis of CDIs and in asymptomatic bacterial colonization.

**Methods:** Over a 6 months period, a total of 80 explanted devices (40 generators, five grafts and 35 lead tips) collected from 20 subjects with CDI and 20 subjects without CDI was analyzed. The removed samples were inoculated in Trypticase soy broth (TSB) for 24 hours and cultured with traditional methods. In addition, they were vortexed for 30 seconds, sonicated for 5 minutes at a frequency >20 kHz and vortexed again for 30 seconds, using the BactoSonic (BANDELIN electronic GmbH and Co. KG). Statistical analysis were performed using STATA 9 software (STATA corp. LP, College Station, TX, USA).

**Results:** Overall sensitivity of sonication fluid culture was higher than standard culture and intra operative pocket swab culture (90% vs. 80% and 33.4%, respectively;  $p = 0.16$ ,  $p < 0.0001$ ). Among all the removed devices, culture after sonication yielded bacteria in 77% of the components compared with 60% of standard culture ( $p = 0.001$ ). Coagulase-negative Staphylococci (CoNS) accounted for 72% of the strains whereas *Staphylococcus aureus* and Gram-negative bacilli were found in 4% and 8% of the total, respectively. No bacterial growth occurred in 8% of CDI. Culture after sonication detected more bacteria than standard method ( $29.91 \times 10^4$  UFC/mL vs.  $21.10 \times 10^4$  UFC/mL,  $p = 0.019$ ), especially when the bacterial amount with TSB was  $<10^4$  UFC/mL ( $p = 0.0002$ ). The difference in the bacterial cell count between the two methods was particularly noticeable for the electrodes ( $19.41 \times 10^4$  UFC/mL vs.  $66.90 \times 10^3$  UFC/mL,  $p = 0.018$ ) rather than for the generators ( $47.77 \times 10^4$  UFC/mL vs.  $46.55 \times 10^4$  UFC/mL,  $p = 0.7$ ). Previous antibiotic therapy had no effect on the diagnostic value of sonication fluid culture ( $p = 0.9$ ). In the control group, standard culture yielded bacteria in 4/20 subjects (20%) compared with 8/20 (40%) of sonication fluid culture. The best cell-count value which discriminated between infected and non-infected devices in sonication fluid culture was  $10^4$  UFC/mL.

**Conclusion:** The sensitivity of culture after sonication is higher than standard culture, both in infected and non-infected cardiac devices. Sonication of explanted devices may represent a useful tool to improve microbiologic diagnosis of CDIs and to detect asymptomatic bacterial colonization.

#### P1361 Sonication cultures improved the microbiological diagnosis of orthopaedic implant infections

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Despite the progress on the management of orthopedic-implant infection, there have been difficulties for the microbiologic diagnosis of these infections as the peri-prosthetic tissue cultures can show no microorganisms growth.

**Objectives:** To compare the sensitivity for the pathogen identification obtained by conventional peri-prosthetic tissue culture samples with culture of samples obtained by sonication of explanted implants in the microbiological diagnosis of orthopedic infections.

**Methods:** In a single-center prospective cohort study from August to November 2011, 34 patients with orthopedic implants (osteosynthesis or joint prosthesis) were evaluated, five with hip prosthesis, and 29 with screw-plate and intramedullary nails undergoing partial or total removal due any cause were included. Diagnosis of implant associated infection was based upon standard criteria of infection previously published by Zimmerli et al. (NEJM 2004) and Berbari et al. (CID 1998). Minimal of three samples from the peri-implant tissue were taken and sent under sterile conditions to the laboratory for culture. The removal implants were packed into sterile containers and covered with Ringer's solution, vortexed for 30 seconds and sonicated for 5 minutes (frequency, 40 kHz; power density, 0.22 W per square centimeter). The sonicated fluid is then cultured and the microorganisms isolated were identified



according to standard method. Statistical analysis was performed using two tailed Fisher exact or Chi-square's test.

**Results:** Septic loosening was diagnosed in 16 patients (47.1%) and infection associated with orthopedic implants was diagnosed by either tissue cultures and sonicated fluid in 52.9% of patients. Sensitivity of peri-prosthetic tissue culture and sonication culture was 56.2% and 81.3% respectively ( $p = 0.001$ ). Seventy-six percent of patients with positive sonicated cultures were using antibiotics during at least 14 days. There was only one microorganism growing on tissue culture but not on sonicate culture. The organisms isolated from sets of plate-screw and hip prosthesis were: coagulase-negative *Staphylococcus* (*S. epidermidis*, *S. schleiferi*), *Staphylococcus aureus*, *Enterococcus faecium*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Enterobacter cloacae*. Of note, one *Candida albicans* infection was also diagnosed by sonication.

**Conclusion:** In our study, sonication technique improved the microbiological diagnosis of orthopedic implant infections.

**P1362 Study of sonication fluid cultures in the microbiologic diagnosis of implant-associated infections. The first Greek experience**

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**Objectives:** Sonication of removed implants using low-frequency ultrasound (35–40 kHz) has been shown to improve the microbiologic diagnosis of implant-associated infections. The purpose of this study was to compare the sonication fluid cultures with the periprosthetic tissue cultures in the microbiology laboratory of a tertiary trauma hospital.

**Methods:** During a 5-month-period, we prospectively included patients undergoing hip or knee prosthesis removal for a suspected infection. The explanted joint components were sonicated for 1 minute at frequency of 40 kHz in sterile Ringer's solution in a sterile plastic box. Aliquots of 0.1 mL sonicate were inoculated each onto aerobic and anaerobic blood agar, chocolate, SDA and McConkey agar, as well as inoculated in thioglycolate broth. The cultures were examined for 10 days and the number and identity of any distinct colony morphology was recorded. In addition, periprosthetic tissue specimens ( $\geq 5$  specimens) were collected and cultured following the usual laboratory practice. The duration of antimicrobial interruption interval before culture sampling was recorded.

**Results:** We included 32 patients undergoing hip ( $n = 28$ ) or knee ( $n = 4$ ) prosthesis removal. In 14 patients (44%), sonication fluid cultures were positive (13 hip and one knee prosthesis): nine coagulase-negative staphylococci (six methicillin-resistant), four *Escherichia coli*, three *Staphylococcus aureus* (one methicillin-resistant), one *Streptococcus mitis/oralis* and one *Pseudomonas stutzeri*; in four of 14 infected implants (29%), two pathogens were found. In periprosthetic tissue cultures, 10 of 32 samples (31%) were considered positive, all revealing same microbial species than in the respective sonication fluid cultures: only one tissue sample showed polymicrobial infection. In 16 of 32 patients (50%) in whom sonication fluid cultures were negative, the drug interruption interval before culture sampling was  $< 7$  days.

**Conclusions:** To our knowledge this is the first Greek study using sonication of removed implants for the microbiologic diagnosis of periprosthetic infections. Staphylococci (especially coagulase-negative staphylococci) were the predominant pathogen, followed by *E. coli*. The sonication fluid culture was more sensitive than the periprosthetic tissue cultures, although not reaching statistical significance (44% vs. 31%). A longer antimicrobial interruption interval ( $> 2$  weeks) before explantation of the prosthesis may further improve the culture sensitivity.

**P1363 Impact of removed prostheses sonication on prosthetic joint infections diagnosis and outcome**

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Sonication of removed implant may improve the rate of microbiologic diagnosis of prosthetic joint infections (PJI) but the value of a sonication-guided therapy is unknown.

**Objectives:** To evaluate the impact of sonication in ameliorating quality of microbiological diagnosis and consequently in improving cure rate of PJI.

**Methods:** PJI undergoing two-stage exchange consecutively observed during a 4-year period were enrolled. PJI were defined by clinical, microbiological and radiologic findings. In each case, we reported demographic, clinical and microbiological data, including the method employed to obtain microbiological cultures such as conventional culture of intraoperative specimens or culture of the fluid obtained by sonication of the removed implant. Cure was defined by disappearance of any evidence of infection during a 6-month follow-up period.

**Results:** Thirty-seven cases with PJI were observed (median age 64 [range 48–82], 16 hip implants and 21 knee implants). Microbiological investigations revealed bacterial growth in 30 (81%). *Staphylococcus aureus* was identified in 12 (40%) cases (eight methicillin resistant), Coagulase Negative staphylococci (CoNS) were identified in 10 (33%), *Pseudomonas aeruginosa* in 3 (10%), and other bacteria in 5 (17%). In 15 PJI the attempt to microbiological diagnosis was made only by conventional methods reporting a 67% success rate. In 22 cases undergoing both culture of the fluid obtained by sonication and culture of conventional intraoperative specimens, bacterial growth was obtained in 20 (90%) by sonication and 15 (68%) by conventional methods. Three cases reported discordant microbiological evidences between sonication and conventional methods and received antimicrobial therapy according to sonication. Overall, cultures were positive in 90% by sonication and 68% by conventional methods (20/22 vs. 25/37; RR 1.35, 95% CI 1.04–1.74;  $p = 0.04$ ) with an increase of CoNS isolated among those diagnosed by sonication. Failure after two-stage replacement was reported in 3 (20%) diagnosed by conventional methods and 1 (5%) diagnosed by sonication.

**Conclusion:** Sonication of the implant improved the accuracy of PJI microbiological diagnosis revealing a higher number of cases sustained by CoNS which are better revealed by sonication since are embedded within biofilm. Sonication resulted in slight better cure rate, probably because of the reduction of cases needing broad spectrum empiric therapy.

**P1364 Clinical utility of differential time to positivity and semi-quantitative culture of catheter segments for diagnosing catheter-related bloodstream infections**

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**Background:** Catheter-related bloodstream infections (CRBSIs) is one of the leading cause of infections with a significant morbidity and mortality rate. Because CRBSIs are difficult to diagnose, there have been suggested several diagnostic methods to detect CRBSIs. We evaluated the differential time to positivity (DTP) and semi-quantitative culture of catheter segments (SQCC) as a method for diagnosing CRBSI.

**Methods:** During January 2010–August 2011, 158 positive paired blood cultures which had the same organism isolated from blood cultures drawn simultaneously through the central venous catheter (CVC) and the peripheral vein were included. Positive DTP represents a DTP of at least 120 minutes earlier for the time to detection of CVC drawn than that of a peripheral vein drawn. A positive SQCC result

represents 15 or more colony-forming units from culture of catheter segments with the same microorganism isolated from peripheral blood cultures. We evaluated a clinical utility of DTP and SQCC for diagnosing CRBSIs, which were further divided into two groups: confirmed (either by DTP or SQCC) and non-confirmed CRBSIs (neither DTP nor SQCC positive).

**Results:** Sixty-five percent (103/158) of episodes were confirmed to CRBSIs. In CRBSIs, gram-positive cocci were 62.5% (65/103), non-fermenting gram-negative bacilli were 11.5% (12/103), Enterobacteriaceae were 9.7% (10/103), yeasts were 14.4% (15/103), and others were 1.0% (1/103). In non-CRBSIs, gram-positive cocci were 59.3% (32/55), non-fermenting gram-negative bacilli were 18.5% (10/55), Enterobacteriaceae were 21.8% (12/55), and yeasts were 1.9% (1/55). DTP-positive episodes were high in coagulase-negative *staphylococcus* (68%, 32/47) and yeasts (93.8%, 15/16). Among 88 cases, which were done both DTP and SQCC, 22 cases were both positive and 22 cases were both negative in DTP and SQCC, respectively, and 32 cases were positive in DTP only and 12 cases were positive in SQCC only. Among the other 70 cases, which were done DTP only, 37 cases were positive and 33 cases were negative in DTP. The sensitivities of DTP and SQCC techniques were 88.3% (91/103) and 51.5% (34/66), respectively.

**Conclusions:** The differential time to positivity was more sensitive than the semi-quantitative culture of catheter segments to diagnose CRBSIs. DTP is useful for diagnosing CRBSIs without a removal of catheter.

#### **PI365** A 1-year retrospective audit of vascular catheter tip culture – Is the laboratory workload justified?

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**Introduction:** The value of routinely culturing vascular catheter tips is debatable. According to IDSA guidelines the clinical impact of positive tip culture without accompanying bacteraemia/fungaemia is uncertain. Previous studies have reported low proportions (20–30%) of positive cultures from tips with fewer still being associated with a catheter related blood stream infection (CRBSI). The cost of culturing vascular catheter tips is substantial. A retrospective audit of vascular catheter tip culture practices in our laboratory was performed with a view to assessing the need to continue to provide a routine tip culture service.

**Methods:** A search of the laboratory database for all tips processed over a 1-year period (June 2010–July 2011) was carried out with results analysed for tip culture result, line type, referring ward and corresponding blood culture results. Peripheral line tips were excluded from further analysis.

**Results:** One thousand eight hundred and fifty-one vascular catheter tips from 1096 patients were cultured in our laboratory over 1 year. Non-tunnelled central venous line tips accounted for 73% of all tips cultured. Sixty eight percent of tips were referred for culture by a high dependency clinical unit.

Overall 463 of tip cultures were positive. Of these positive tip cultures 67% grew coagulase negative staphylococci, 8% grew gram negative bacilli, 4% grew *Staphylococcus aureus*, 4% grew *Candida* sp. and 13% were mixed cultures.

Sixty-three percent (293) of culture positive tips had no corresponding blood culture drawn in the 24 hours preceding/post line removal. Twenty-eight percent (128) had either a negative associated blood culture or a positive with a different organism to the tip culture.

**Discussion:** Only 2.3% of all vascular catheter tips sent for culture in our laboratory over the study period were associated with a proven CRBSI. Sixty-three percent of vascular tips sent for culture did not have an accompanying blood culture drawn. On the basis of this finding a comment has been introduced to the electronic ordering system indicating that blood cultures must be sent with all tips for culture. Tips not accompanied by a blood culture have a rejection comment applied and are not processed further until a blood culture is received in the laboratory.

## Molecular bacteriology: into the deep

### **PI366** Identification of carcinoma suppressive factor produced by bacteria of *Enterococcus* genus

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**Objectives:** Bacteria of *Enterococcus* genus are capable of producing several virulence factors, exerting toxic effects on host cells. The studies aimed at analysis of effects manifested by supernatants of *Enterococcus* strains supernatants on proliferation of carcinoma cells and at pinpointing the potential anti-proliferative factor.

**Materials and methods:** The studies included 23 strains, including 21 strains of *E. faecalis* and two strains of *E. durans*. All the studied strains represented clinical isolates, recovered from urine or wound. The bacteria were grown on D-Coccosel-Agar medium. Subsequently, suspensions of individual strains were prepared, manifesting density of 0.5 McF. The 0.1 of every studied suspension was inoculated to RPMI 1640 medium (Sigma), supplemented with 0.5% glucose and the cultures were incubated for 24 hour at 37°C. The cultures were filtered obtaining supernatants, which were tested against carcinoma cells (AGS, HeLa). The control involved carcinoma cells incubated without the tested supernatants. Proliferation of the cells was studied using TACS MTT Cell Proliferation Assay (Promega). The results were read as an absorbance value, A. In parallel, the supernatants of *Enterococcus* cultures were subjected to ultrafiltration under pressure of nitrogen using Amicon filtration membrane. Molecular mass of the active factor was determined using gel filtration in FPLC column. Moreover, thermal stability of the obtained fraction was tested incubating it at the temperature of 75°C for 0.5 hour.

**Results:** Mean absorbance value (A) of proliferation manifested by carcinoma cells in control experiments amounted to 1.437 ± 0.051 for AGS, for HeLa to 1.419 ± 0.046. Mean absorbance value of proliferation of carcinoma cells incubated with supernatant of *E. faecalis* or *E. durans* cultures amounted to 1.079 ± 0.094 for AGS (decrease in the percent of the proliferating cells against the control by 24.9%), 1.116 ± 0.085 for HeLa cells (decrease against the control: 21.3%). Absorbance values obtained following incubation with *Enterococcus* supernatants differed significantly from the control values (p < 0.0001). In parallel, the anti-proliferative factor produced by *Enterococcus* strains was shown to involve a peptide substance with molecular weight of 1.6 kDa, heat-stable at the temperature of 75°C for 0.5 hour.

**Conclusions:** The newly identified thermostable peptide of 1.6 kDa, produced by clinical strains of *Enterococcus* bacteria manifests anti-proliferative activity toward carcinoma cells.

### **PI367** Whole genome sequencing of *Neisseria meningitidis* serogroup C isolates reveals subtle genetic difference in the capsule locus of an outbreak strain

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**Objective:** Comparative analysis of the genomes of *N. meningitidis* serogroup C isolates in order to detect cues associated with a highly virulent phenotype.

**Methods:** Whole genome sequencing by using the standard shotgun and the 3-kb paired-end protocols provided by Roche 454 next generation platform.

**Results:** We sequenced the whole genome of two isolates of *N. meningitidis* serogroup C, which were isolated from patients with invasive disease in northern Italy during the 2007–2008 winter and belonged to the same ST-11/ET-37 clonal complex. One of the two strains was responsible of an outbreak with seven cases of meningitis, including three fatal cases, while the other strain was isolated from a sporadic case of meningitis in the same area and period. Whole-genome comparative analysis revealed local and remarkable differences between

the genomes of the two isolates and in comparison with the reference genome of strain FAM18.

Since these differences between the two *N. meningitidis* isolates could be the result of genetic variations of the same *N. meningitidis* strain within the outbreak, the most relevant changes in terms of sequence length and predicted function were selected for further investigation and validation through primer-specific cycle sequencing in the two sequenced *N. meningitidis* strains and in the other seven ST-11/ET-37 isolates collected in 2007–2008 in the same area. This analysis confirmed findings provided by next generation sequencing and bioinformatic comparison and demonstrated that the other five isolates from the same outbreak shared the same sequences of the outbreak index strain, whereas the isolate from sporadic meningitis shared sequence identity with isolates from other sporadic cases of meningitis occurring in Veneto Region both before and after the outbreak, during the same season. All genetic differences among strains occurred in the capsule locus and were compatible with horizontal acquisition of a cluster of genes involved in lipooligosaccharide biosynthesis from commensal meningococci.

**Conclusions:** The results of this study showed that an outbreak strain of *N. meningitidis* serogroup C was characterized by horizontal acquisition of a capsule biosynthesis operon that could contribute to its highly virulent phenotype.

#### **P1368** The significance of PCR in the diagnosis of post surgical meningitis

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**Background:** Post surgical meningitis is a dreaded complication of CNS surgery. As most patients after CNS surgery experience signs and symptoms of meningitis, the spinal fluid exam is pathological due surgery or recent bleeding, and many patients receive empirical antibiotic treatment the diagnosis of postsurgical meningitis is challenging. We examined whether PCR of the spinal fluid can improve the diagnosis of post surgical meningitis.

**Methods:** A random sample of patients after CNS surgery (n = 86) were enrolled. CSF samples were sent for routine examination (chemistry, gram stain and culture). In addition PCR identifying ribosomal bacterial DNA was performed on all samples. Bacterial DNA was produced and DNA samples were assessed for the presence of 16s rRNA. If positive, sequencing was done and the sequence obtained was compared to sequences available at the world gene bank with BLAST Software to identify bacteria. In addition, patients' files were reviewed and a blinded ID physician decided whether the patients had a clinical picture compatible with post surgical meningitis. PCR results were compared with culture results and the clinical diagnosis.

**Results:** Mean age was 59 years. 52% were males. Common reasons for surgical intervention were bleeding in 38% of patients and resection of a tumor in 37% of patients. 67% of patients had a clinical diagnosis of postsurgical meningitis but only 33% had a positive culture and 20% had a positive PCR result. Concordant results between bacterial growth in culture and PCR were found in only 42% of cases ( $\kappa = 0.24$ ). *Propionibacterium acnes* was the most common pathogen identified by PCR. In four cases it did not grow in culture but patients were considered to have clinical meningitis. In one additional case *P. acnes* grew in culture and was also identified by PCR but this case was not thought to be infected. The most common pathogen depicted by culture was coagulase negative Staphylococci (CONS) (n = 8). Of these, six were considered to have meningitis, one was also depicted by PCR and in two cases PCR was positive but sequencing failed.

**Conclusions:** Concordance between the clinical diagnosis, culture result and PCR remained unacceptably low. PCR did not increase the diagnostic yield except for the identification of *P. acnes*. Further improvement of the use of PCR is warranted prior to its application for routine diagnostics in post surgical meningitis.

#### **P1369** Characterisation of a molecular-based assay system (PLEX-ID) for broad range detection of bacteria, *Candida*, and antibiotic resistance from blood culture bottles

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**Background:** An assay was developed on the Ibis Biosciences PLEX-ID platform to detect diverse pathogenic bacteria and *Candida*. The assay was designed to identify 600+ species through mass spectrometric analysis of unfragmented amplicons and comparison of resulting mass signatures to known signatures in a reference database. The assay is capable of detecting the presence of genes conferring resistance to antimicrobials – methicillin (*mecA*), vancomycin (*vanA/vanB*) and carbapenems (*kpc*). We demonstrate the use of the PLEX-ID Broad Bacteria Assay as an innovative approach for both species identification and antibiotic resistance determination from positive blood culture bottle samples.

**Methods:** The PLEX-ID Broad Bacteria Assay is comprised of 18 primer pairs targeting all bacteria (16S rDNA, 23S rDNA), subsets of bacteria (*rplB*, *tufB*, *valS*, *rpoB*), antibiotic resistance markers (*mecA*, *vanA*, *vanB*, *KPC*), and *Candida* (18S rDNA, 25S rDNA, mitochondrial rDNA). The assay uses a 1.1 mL sample from positive blood culture bottles (BacT/ALERT, BD BACTEC, VersaTREK). The assay was fully characterized for sensitivity, cross reactivity, interference, breadth of coverage, and performance on relevant samples.

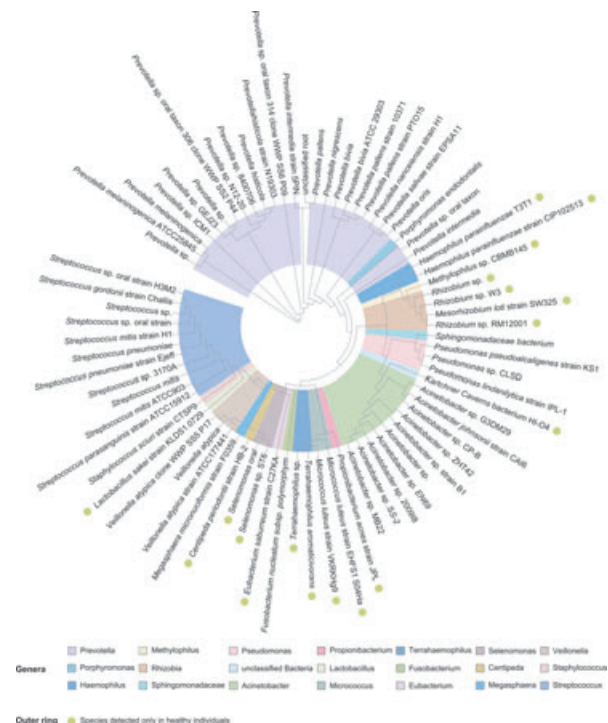
**Results:** The PLEX-ID Broad Bacteria Assay demonstrated accurate and sensitive detection of a large breadth of bacterial organisms in a wide variety of blood culture bottle media and in the presence of many different blood components and potential cross reactors. Correct identification was achieved for over 150 organisms derived from spiked blood culture bottle samples. The system was able to correctly identify antibiotic resistance markers (*mecA*, *vanA/B*, and *KPC*) for *Staphylococcus aureus*, *Enterococcus faecium/faecalis*, and *Klebsiella pneumoniae*, respectively. Over 300 positive blood culture bottles derived from clinical specimens were tested and 92.2% of the samples were correctly identified at the Genus level. 88.3% of the samples were identified at both the Genus and Species level.

**Conclusions:** Using the high throughput Plex ID Broad Bacteria Assay, we have demonstrated the ability to quickly and accurately detect and identify a wide variety of clinically relevant bacterial and *Candida* species, as well as antibiotic resistant markers, in blood culture samples using a single molecular assay.

#### **P1370** Airway microbiota in healthy state and chronic obstructive pulmonary disease

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Bacterial infection of lower respiratory tract is characteristic for chronic obstructive pulmonary disease (COPD). Routine clinical diagnostic methods include the detection of about 10 most common pathogens. New molecular-based approaches allow studying pulmonary microbiome more deeply. This paper describes the detailed structure of microbial communities found in bronchoalveolar lavage fluid of nine COPD patients compared with nine healthy individuals. The authors designed the experiments to compare two different methods to analyze species composition. They included terminal restriction fragment length polymorphism and clone library analysis. Both methods are based on the universal eubacterial 16S rRNA gene amplification. We identified 151 bacterial ribotypes. Although all probes showed high diversity, genera *Prevotella*, *Sphingomonas*, *Pseudomonas*, *Acinetobacter*, *Fusobacterium*, *Megasphaera*, *Veillonella*, *Staphylococcus*, and *Streptococcus* made up the core microbiome. Presence of *Mycoplasma* and *Pseudomonas* was characteristic for COPD state. *Pseudomonas* sp. monoinfection led to the reduction of microbial diversity. Atypical bacteria (*Mycoplasma* sp. and *Legionella* sp.) were identified by



restriction analysis, but not clone library analysis. Statistical data assessment (principal component analysis) revealed interspecies dependencies between *Pseudomonas* and *Lactobacillus* in COPD patient group. This finding can contribute to the understanding of species interaction in complex pulmonary microbial communities.

#### P1371 Duplex real-time PCR identification of *Pseudomonas aeruginosa* isolates from cystic fibrosis patients

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**Objectives:** *Pseudomonas aeruginosa* is the main infective cause of morbidity and mortality in Cystic Fibrosis patients. The CF lung environment favours conversion from motile strains of *P. aeruginosa* to atypical mucoid strains. Issues have been raised with the accuracy of standard methods for identifying atypical *P. aeruginosa* by phenotypic and biochemical testing. PCR-based assays have been shown to be a reliable alternative and are now recommended by Cystic Fibrosis Trust guidelines. This study investigates a duplex PCR assay that targets the *ecfX* and *gyrB* genes to identify *P. aeruginosa* isolates from the sputa of CF patients. The use of a simple heat-shock method of extraction was also investigated as a cost and time saving alternative to the current automated extraction method.

**Methods:** The assay was validated using 103 confirmed *P. aeruginosa* isolates from CF patients. These had been previously identified using 16S rDNA PCR. Fourteen similar organisms (such as other *Pseudomonas* spp.) were used as negative controls. A further 42 clinical isolates from CF sputa that had been previously analysed by standard laboratory methods (Vitek 2 and/or API 20NE) were collected, re-analysed using the PCR assay, and the results compared with the original laboratory findings. The heat-shock method was investigated by analysing a total of 69 known isolates.

**Results:** The assay detected all 103 confirmed positive samples. No cross-reaction was detected with the 14 similar organisms. No inhibition was detected as a result of combining the *ecfX* and *gyrB* targets into a duplex assay. The PCR assay gave results with sensitivity and specificity of 100% for *P. aeruginosa*. The PCR assay correctly identified all 42 clinical isolates as either PCR positive or negative, whilst current laboratory methods were found to have produced two

false-positive and four false-negative results upon comparison. Results from the isolates extracted by heat-shock method matched those extracted using the automated extraction method.

**Conclusion:** The assay was shown to be highly sensitive and specific for detection of *P. aeruginosa*. The heat-shock extraction method was shown to be rapid and cost-effective. The implementation of the PCR assay into the laboratory would have prevented two false-positive and four false-negative results in the 42 clinical specimens tested in this study.

#### P1372 Characterisation of drug-resistant *Mycobacterium tuberculosis* strains from Bulgaria and Georgia by bead-based multiplex ligation-dependent probe amplification

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**Objective:** In *Mycobacterium tuberculosis* (MTB) different genotypes carry characteristic genetic markers and most forms of drug resistance are encoded by distinctive single nucleotide polymorphisms (SNPs), making SNP-based identification an attractive tool for the full characterization of MTB strains. We have developed an MTB-specific Multiplex Ligation-dependent Probe Amplification (MLPA) assay. This method allows simultaneous detection of multiple dispersed drug resistance and genotype-specific markers in the MTB genome and has proved to be highly specific (Bergval et al. 2008).

**Methods:** We have transferred analysis of MLPA to a more robust and user-friendly bead-based array (Luminex MAGPIX) by modifying current MLPA-probes and protocols (manuscript in preparation). Our current MLPA assay includes 43 functional MTB-specific discriminatory markers, allowing simultaneous detection of the most important first- and second-line drug resistance mutations as well as various genotypes that are of particular epidemiological interest.

Local staff tested the performance and the practical use of the adapted MLPA on 49 multidrug-resistant (MDR) MTB strains in Bulgaria. In addition, a randomly selected panel of 100 MTB strains from Georgia was prospectively tested by MLPA in the Netherlands and France and spoligotyped in France, to predict the applicability of the assay on Georgian MTB strains. Results were compared to data previously obtained by other methods.

**Results:** MLPA results obtained in Bulgaria reveal that the majority of the selected strains carried the resistance-conferring mutations rpoB-S531L and inhA(-) C15T, rather than katG-S315T. Data on second-line drug resistance was lacking, but one XDR-TB strain was identified by MLPA. MLPA furthermore indicated that most strains (75%) belonged to principal genotypic groups 2 and 3, whereas the Georgian strains were predominantly of the Beijing genotype (33%). These results were in good agreement with MLPA and spoligotyping data obtained in France.

In the Georgian panel 12% MDR and no XDR strains were identified, as was reflected by the phenotypic data obtained in Georgia.

**Conclusion:** The overall accuracy of MLPA on the bead-based array is comparable to our original assay, with significant reduction of the turn-around-time. This system allows multi-parameter testing by MLPA and could provide a standard platform for several diagnostic and screening tests, which are traditionally performed by different, TB-specific, methods.

#### P1373 Dysbiosis of the lung bacterial community in patients with stable chronic obstructive pulmonary disease

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**Objectives:** There has been considerable controversy about the role of lower respiratory tract bacteria in the pathogenesis and exacerbation of chronic obstructive pulmonary disease (COPD). Aim of this study was

to investigate the lower respiratory tract microbioma of stable COPD patients, smokers, and healthy non-smokers subjects in order to identify microbial pathogens potentially associated with COPD.

**Methods:** Sputum was induced in six COPD patients, four smokers, and three healthy non-smoker controls. An aliquot of each specimen was subjected to microscopic examination and bacteriological culture, while another was used for deep sequencing of the 16S rRNA gene by using the 454 Life Sciences FLX system. About 10 000 good-quality reads per sample were obtained. Sequences were processed using the RDP Multiclassifier for taxonomic classification of bacteria phyla and genera.

**Results:** The composition of the microbioma in sputum samples varied widely among subjects, while it was relatively stable during time in the same subject, and included several genera from 10 bacterial phyla. *Pseudomonas*, *Streptococcus* and *Haemophilus* genera accounted for a large percentage of sequences in some patients with COPD, while *Neisseria* and *Streptococcus* genera were abundant in smokers. These genera were absent or rarely detected in healthy subjects. A "core microbioma" composed of bacteria common to all sputum samples was identified that included *Prevotella*, *Streptococcus*, *Leptotrichia*, *Veilonella*, *Fusobacterium*, *Actinomyces*, *Rothia*, *Porphyromonas*, and *Neisseria* (cutoff >1% of sequences), while some genera were specific to patient groups (e.g. *Gemella* and *Granulicatella*, detected in COPD patients and smokers). Conventional microscopy and culturing analyses did not represent the microbial diversity identified by deep sequencing.

**Conclusions:** *Pseudomonas*, *Streptococcus*, *Haemophilus*, and *Neisseria* genera were the most abundant bacteria in the deep airways microbioma of COPD patients and smokers, but not in healthy subjects, and could contribute to COPD symptoms and severe exacerbations. A "core microbioma" including aerobic and anaerobic bacteria, common to all sputum samples, was identified and considered as normal lung flora. The dysbiosis of the lung bacterial community could provide the constant inflammatory stimulus observed in stable COPD patients.

#### **P1374** Clinical significance of PCR positive, culture negative faeces for *Salmonella*, *Shigella*, *Yersinia* or *Campylobacter*

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**Objective:** Direct molecular detection (PCR) of gastrointestinal pathogens (*Salmonella*, *Shigella*, *Yersinia* and *Campylobacter* [SSYC]) from faeces samples has led to an increased sensitivity of the test and consequently a higher number of "positive" samples. The faeces PCR is used in our hospital as pre-culture screening of the faecal samples; all positive PCRs are followed by cultures on organism-specific agars. In the present study, we evaluated how often a positive PCR is confirmed by culture. In addition, since the clinical relevance of PCR positive, culture negative results is still unclear, we compared the clinical characteristics of PCR-positive patients with and without positive culture, respectively.

**Methods:** Between March 2010 and November 2011, we included all faecal samples (n = 5502) sent in for testing of SSYC by general practitioners (n = 4007), internal medicine outpatient clinics (n = 644) and internal medicine wards (n = 851). Clinical data were retrieved by detailed retrospective chart analysis in the patients seen at the internal medicine outpatient clinic or wards (n = 49).

**Results:** Using PCR, 1.8% of the samples were positive for *Salmonella*, 1.1%, for *Shigella*, 8.3%, for *Campylobacter* and 0.1% for *Yersinia*. A positive PCR was followed by a positive culture in 81% of samples tested positive for *Salmonella*, 33% for *Shigella*, 62% for *Campylobacter* and 50% for *Yersinia*. Percentage of *Salmonella* culture positivity was significantly higher in samples sent from GP (87%) than samples from the internal medicine ward (45%,  $p < 0.01$ ). There were no differences in clinical characteristics (admittance to hospital, overall antibiotic use, fever, bloody diarrhoea or CRP) between PCR-positive patients with a positive or a negative culture. A negative culture was more often seen in samples from patients who had received antibiotic

treatment before culture (29% vs. 12% without antibiotics before culture), although this was not significant ( $p = 0.14$ ).

**Conclusions:** A significant number of PCR-positive faecal samples cannot be confirmed by culture. This is only partly explained by previous antibiotic treatment. Clinical characteristics of PCR-positive patients with negative culture do not differ from patients with a positive culture. This suggests that a positive PCR is sufficient to detect clinically relevant cases of gastro-intestinal infections.

#### **P1375** Growth dynamics revealed inter- and intra-clonal fitness differences among major *Enterococcus faecalis* clonal complexes

A.P. Tedim\*, C.M. Rodríguez, F. Baquero, T.M. Coque (Madrid, ES)

**Objectives:** *Enterococcus faecalis* (Efc) has an epidemic population structure with over-representation of Clonal Complexes (CCs) CC2, CC9, CC16 and CC87, which exhibit enhanced ability to cause infection or colonize under different ecological conditions. This study evaluates growth dynamics of major Efc CCs to understand the diversification and extraordinary adaptability of this species.

**Methods:** Thirty-six Efc of CC2 (7 ST6, 2 ST2), CC9 (5 ST9, 1 ST17, 1 ST42), CC8 (4 ST8, 1 ST64), CC16 (4 ST16, 1 ST179), CC87 (2 ST28, 2 ST87), CC21 (1 ST21, 1 ST22), CC40 (2 ST40) and CC1 (2 ST1) were analysed. Isolates are CCs emblematic representatives for which comprehensive data including their mobilome content were considered. Genome size was determined by PFGE (AscI, SmaI, ICeu-I). Growth dynamics experiments were performed at 37°C and different stress conditions using Bioscreen C apparatus. Optical Density (OD) was determined every 5 minute for 24 hour, plates being agitated 10 seconds before each OD measurement. Fitness was analysed by determining Growth Rates (GR) and Generation Time (GT) using standard procedures.

**Results:** Inter and intra-clonal variations in bacterial fitness were observed (average GR =  $0.7478 \pm 0.0841$ , and average GT =  $56.48 \pm 7.12$  minute). CC8 was the less fit CC (Average GR =  $0.6745 \pm 0.1162$ ) followed by CC1 ( $0.6930 \pm 0.0185$ ), CC87 ( $0.7376 \pm 0.0261$ ), CC9 ( $0.7376 \pm 0.0666$ ), CC2 ( $0.7395 \pm 0.0499$ ), CC16 ( $0.8121 \pm 0.0320$ ), CC40 ( $0.9359 \pm 0.0120$ ), CC21 being the fittest clone ( $0.8995 \pm 0.0026$ ). Remarkable variations in the fitness of isolates within CC8, CC9, CC2 and CC16 contrast with low intra-clonal variation for CC21, CC40, CC1 and CC87. Stress conditions ( $T = 42^\circ\text{C}$ ) proved to be either an advantage or a disadvantage for some CCs, intra-clonal variation being observed. Genome size was highly variable (2–3.5 Mb) although a significant association with fitness variation was not established.

**Conclusions:** CCs associated with colonization (CC21, CC16, CC40) show a better in-vitro fitness than those linked to nosocomial infections (CC2, CC9, CC87) or some infrequent and ancient lineages (CC8, CC1). The successful expansion of CCs causing most of the clinical infections, apparently less fit than other CCs, might be due to the presence of acquired genetic elements encoding specific traits (antibiotic resistance, virulence genes) that are absent or rarely found among other CCs. Fitness variations reflects adaptation of particular lineages to different ecological conditions.

## Molecular diagnosis of bacterial infections

#### **P1376** Development of nucleic acid-based diagnostic tests for the detection of microbial pathogens using novel targets

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**Objectives:** Infectious diseases cause significant morbidity and mortality world-wide. Nucleic acid based tests provide a rapid, sensitive and specific alternative to traditional diagnostic test methods. The Molecular Diagnostics Research Group (MDRG) at NUI Galway have identified and exemplified novel molecular targets for microorganism identification. A focus of the work has been on the

development of nucleic acid based tests for microorganisms of clinical significance. While numerous assays for organisms from a range of categories have been developed, presented here are real-time PCR tests for clinically significant organisms *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Mycobacterium intercellulare*, *Bordetella pertussis* and *Staphylococcus aureus*.

**Methods:** The initial step in the process of nucleic acid test development was the identification of suitable nucleic acid targets. Following identification of a suitable target an initial sequencing program was undertaken to ensure target specificity. This involved sequencing of the target DNA from a number of geographically distinct isolates of the species of interest. It was also critical to generate sequence information for closely related species and species which are common to the target sample environment. In silico analysis of all generated sequences was performed revealing areas of sequence variability between target and non-target organisms suitable for assay design. The performance of each assay with respect to specificity and limit of detection (LOD) was evaluated on the LightCycler® 480 using Taqman probes.

**Results:** The real-time PCR assays developed for *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Mycobacterium intercellulare*, *Bordetella pertussis* and *Staphylococcus aureus* are specific for the target organism and show no cross reactivity with panels of closely related organisms tested. The limit of detection of each of the assays is between 1 and 10 cell equivalents.

**Conclusion:** The potential of a novel nucleic acid target for the development of tests for clinically significant microorganisms has been demonstrated. These real-time PCR assays represent highly specific tests with low limits of detection making them ideal for the detection of low numbers of organisms in clinical samples in a short turnaround time. The high specificity of the tests increases user confidence in determining the presence or absence of the infectious agent.

**P1377** **Detection of *Ehrlichia* and other pathogens from serial clinical specimens by PCR/electrospray ionisation mass spectrometry**

M. Eshoo\*, M. Rounds, J. Friesen, C. Crowder, H. Matthews, C. Quinn, D. Ecker, Y. Tang (Carlsbad, Nashville, US)

**Objectives:** *Ehrlichiosis* presents with non-specific, flu-like symptoms, which makes accurate diagnosis difficult. *Ehrlichia chaffeensis* and *Ehrlichia ewingii* are the two main species relating to different clinical outcomes in humans. Here we use a PCR electrospray ionization mass spectrometry (PCR/ESI-MS)-based assay designed to detect a wide range of vector-borne pathogens for the detection and identification of *Ehrlichia* species from serially collected whole blood, plasma, serum, and cerebrospinal fluid.

**Methods:** Specimens were collected from 266 unique patients from the southeastern United States with suspected tick-associated febrile illness between April 4 to September 30, 2010, many of which included of serial collections from the same patients. Nucleic acids from the initial whole blood draw were extracted for each patient using a combination of mechanical and chemical lysis and magnetic bead purification. The specimens were then tested with a multi-locus assay utilizing 10 multiplexed primers targeting different loci which detect a wide range of vector-borne pathogens, including bacteria, protozoa, and viruses. Electrospray ionization mass spectrometry (ESI-MS) of the PCR amplicons was used to determine their base compositions to identify the organisms in the specimens.

**Results:** Of the 266 patients tested, *Ehrlichia chaffeensis* was detected in 18 (6.77%) of the patients. *Ehrlichia ewingii* was detected in two patients (0.75%). *Rickettsia rickettsii* and *Plasmodium vivax* were detected in one patient each (0.38%). Fourteen patients had positive pathogen *Ehrlichia* detections in more than one serial specimen. We demonstrated pathogen detections from whole blood, serum, plasma, and cerebrospinal fluid specimens. Quantitation of pathogen genomes in the specimens revealed that the concentrations decreased but was still detectable for several days following administration of antibiotic therapy.

**Conclusion:** *Ehrlichia* was detected in whole, serum and plasma at nearly equal levels per milliliter of specimen by PCR/ESI-MS and the organisms is detectable for several days following the start of antibiotic therapy. Results of this analysis suggest that, in infected individuals, the *Ehrlichia* nucleic acids being detected may not be coming from living cells but rather free DNA in the serum/plasma.

**P1378** **Identifying pathogens in culture-negative infections: a case series exploring PCR and electrospray ionisation mass spectrometry for microbial identification**

J. Farrell, R. Sampath\*, R. Bonomo (Peoria, Carlsbad, Cleveland, US)

**Background:** The sensitivity of culture is influenced by many factors, including the site of infection, quantity of specimen, the organism, and prior or concurrent antimicrobial treatment. Timeliness of culture results is also influenced by these variables. We present seven cases suspicious for infection in which conventional culture methods failed to yield a microbiologic diagnosis. When the clinical specimens were analyzed by PCR and electrospray ionization mass spectrometry (PCR/ESI-MS), our findings were consistent with the clinical scenario. These results suggest that PCR/ESI-MS may play a unique role in culture negative infections.

**Methods:** Both conventional culture and PCR/ESI-MS were performed on the following specimens:

1 Cerebrospinal fluid (CSF) obtained by lumbar puncture from a previously healthy 26-year-old HIV-negative man with numerous brain abscesses.

2 Pleural fluid obtained by thoracentesis from a previously healthy 46-year-old woman with a right lung abscess.

3 Vitreous humor from a 53-year-old woman with cirrhosis secondary to chronic hepatitis C infection who developed hemorrhagic retinitis while hospitalized with a small bowel obstruction.

4 Wound drainage from a 38-year-old woman following fasciotomy for right lower extremity compartment syndrome.

5 Bronchioalveolar lavage fluid from a 51-year-old man with community acquired pneumonia and sepsis.

6 CSF and brain tissue from a 50-year-old man with meningoencephalitis.

7 Skin biopsy of a left thigh lesion in a 71-year-old man with acute myelogenous leukemia.

**Results:** Specimen 1: All stains and cultures were negative. PCR/ESI-MS detected *S. intermedius*.

Specimen 2: All stains and cultures were negative. PCR/ESI-MS detected *S. pneumoniae/S. mitis*.

Specimen 3: Viral cultures were negative. PCR/ESI-MS detected HSV-2.

Specimen 4: Gram stain and anaerobic cultures were negative. PCR/ESI-MS detected *Clostridium perfringens*.

Specimen 5: All stains and cultures were negative. PCR/ESI-MS detected *S. pneumoniae*.

Specimen 6: All stains and cultures were negative. PCR/ESI-MS detected *S. pneumoniae*.

Specimen 7: Fungal cultures were negative. PCR/ESI-MS detected *Fusarium solani*.

**Conclusions:** In each case, a pathogen consistent with the clinical presentation was identified. PCR/ESI-MS can assist in the rapid identification of pathogens from a variety of clinical specimens when culture results are likely to be delayed, or viable organisms are not recovered. This sensitivity can help guide antimicrobial therapy.

**P1379** **Evaluating the performance of GeneXpert in screening pregnant women for *Streptococcus agalactiae***

W. Alfouzan\*, R. Dhar (Farwania, KW)

**Objectives:** *Streptococcus agalactiae* or group B streptococcus (GBS) is a common flora of the gastrointestinal and the female genitourinary tract. Babies born to mothers colonized with the organism are at risk of

developing invasive infection. Intrapartum antibiotic prophylaxis is recommended for all pregnant women who test positive for GBS screening especially at or after 35 week of gestation and for women with intrapartum risk factors and unknown GBS colonization status. We aim to assess the diagnostic accuracy of GeneXpert GBS (Xpert® GBS, Cepheid, Sunnyvale, CA, USA) in detecting GBS as compared to culture method.

**Methods:** High vaginal swabs (HVS) collected from women attending obstetrics and gynecology out-patient clinics, in-patients using transport swab (Copan, Italy). For patients in the labour wards swabs (Venturi Transystem transport swab, Copan, Italy), which came in duplicate form were used for GeneXpert and the other for culture on 5% sheep blood agar (BA). The BA plates were incubated at 37°C for 24 hour and examined for growth of GBS which were identified by gram stained smear, catalase and grouping (Streptococcal grouping kit; Oxoid Ltd., UK).

**Results:** During the period of 3 months, a total of 485 HVS were processed in our laboratory. Of these 87 were found to be positive for GBS by culture giving the prevalence rate was 17.9% in the female genital tract. Of 84 HVS received from labour ward 23 (27%) tested positive by culture as well as GeneXpert and 56 were found to be negative by both methods. The sensitivity, specificity, positive and negative predictive values for GeneXpert were 95.8%, 98.2%, 95.8% and 98.2% respectively.

**Conclusion:** GeneXpert is considered a highly accurate point of care test to identify intrapartum GBS carriers. Not only does it helps to identify candidates who require intrapartum antibiotic prophylaxis, but also excludes the non-carriers. Its role in decreasing unnecessary antibiotic consumption in pregnant women is valuable.

**P1380 Evaluation of Xpert® GBS assay for rapid detection of group B Streptococcus in amniotic fluid among pregnant women with premature rupture of membranes after 37 weeks of gestation**

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**Objective:** Group B Streptococcus (GBS) is the leading cause of life-threatening neonatal bacterial infections and maternal chorioamnionitis. Premature rupture of membranes (PROM) is associated with an increased risk of these infections. GBS are present in the vagina of about 10–30% of pregnant women. Intrapartum antibiotic prophylaxis reduces significantly the rate of neonatal GBS colonization and the incidence of early-onset GBS disease. Recently, Xpert® GBS assay (Cepheid) was presented as an accurate and easy-of-use test to detect GBS DNA from vagina/rectal swabs specimens.

The objective of our study was to evaluate the Xpert® GBS test directly on amniotic fluid samples collected from pregnant women with term rupture of membranes before the onset of labor.

**Material and Methods:** Xpert GBS test was performed on 62 amniotic fluid samples from 62 pregnant women with PROM after 37 weeks of gestation. The results of the real-time PCR assay were compared to the results of amniotic fluid bacterial culture and GBS vaginal screening by culture at 35–37 weeks of gestation.

**Results:** The overall molecular GBS test yield was 100%. Out of the 62 amniotic fluid samples, 8 (12.7%) were found positive by Xpert® GBS. Cycle thresholds (C<sub>t</sub>) for positive samples ranged between 27.1 and 39.3. One sample had a positive Xpert GBS result (C<sub>t</sub>: 39.3) but a negative culture on blood agar plate. Another sample had a negative Xpert GBS test but a positive culture with low GBS colony density. Compared to the culture method, the Xpert GBS test for the amniotic fluids demonstrated a sensitivity and a specificity of 87.5% and 98.1% respectively and a positive and negative predictive value of 87.5% and 98.1% respectively.

Out of the eight women with a positive Xpert GBS test in amniotic fluid, five had a negative prenatal GBS culture based screening.

**Conclusion:** Xpert GBS assay is a rapid and accurate method for the detection of intrapartum GBS in amniotic fluid samples. This new tool

could enhance the exact identification of candidates for maternal intrapartum antibiotic prophylaxis. It could be helpful in the management of the delivery of pregnant women with PROM.

**P1381 Development of a high-throughput, rapid and sensitive molecular assay on the Abbott m2000 for detection of group B Streptococcus in antepartum and intrapartum women**

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**Objectives:** Group B streptococci (GBS) are responsible for most cases of neonatal sepsis. Current culture-based screening methods are time consuming and may delay the start of prophylaxis. We have developed a high-throughput, automated assay for rapid and accurate screening or detection of GBS in antepartum and intrapartum women (IMDx GBS for Abbott m2000 assay) in order to address the unmet need for the rapid screening or detection of GBS.

**Methods:** IMDx has developed NGENix™, a proprietary bioinformatics platform for designing multiplexed, real time PCR-based assays. NGENix™ identifies unique regions within sequenced genomes to iteratively design sets of primers and probes, assesses the thermodynamics of the DNA multiplex, and reviews potential cross-reactivity to other genomes. We utilized NGENix™ to design oligonucleotide solutions for a specific region of the GBS genome shown to be essential in the production of virulence factors of GBS, then assessed analytical performance on the Abbott m2000 System.

**Results:** Combined vaginal/rectal swabs or enriched culture broth samples obtained from antepartum and intrapartum women were tested with the IMDx GBS for Abbott m2000 assay to establish performance characteristics. Testing a panel of 235 samples (221 bactiswabs and 14 eSwabs), the assay resulted in 90% diagnostic sensitivity and 100% diagnostic specificity compared to standard culture methods. All eSwabs were correctly identified. The IMDx GBS for Abbott m2000 assay is highly specific for GBS, reacting with serotypes Ib, Ic, II and IV at or below the calculated LoD for the most common serotypes III and V (Table 1). No cross reactivity was observed when the assay was tested against 29 microorganisms. No interference was observed in the presence of potentially interfering substances, such as over the counter medications and prescription medications used at or around the sampling site. A total of 105 tests were performed in precision studies and demonstrated <2% CV of FCN for all variables.

**Table 1. IMDx GBS Assay Performance Characteristics**

<b>Limit of Detection</b>	722.4 CFU/swab for a serotype III isolate (95% CI: 722.27 – 722.60)
	370.12 CFU/swab for a serotype V isolate (95% CI: 370.06 – 370.16)

**Conclusions:** The IMDx GBS Assay for Abbott m2000 provides a rapid alternative to current culture methods for screening or detecting GBS in pregnant women. This assay will provide physicians an accurate and faster method to detect the presence of GBS in antepartum and intrapartum women to allow administration of preventative treatment and reduce occurrence of newborn infection.

**P1382 Development of a new diagnostic tool for the detection of Chlamydia pneumoniae and Mycoplasma pneumoniae in a duplex real-time PCR**

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**Objectives:** *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*, two atypical respiratory pathogens, are common causes of community-acquired pneumonia. Both bacteria are implicated in upper and lower respiratory tract infections among children and adults

and share clinical features, including symptoms, with many other bacterial and viral infections. A sensitive and effective identification of these agents is necessary and important in order to ensure an appropriate antibiotic therapy. Currently, culture and serological confirmation of the diagnosis of *C. pneumoniae* and *M. pneumoniae* are difficult and time-consuming. Real time PCR, sensitive, specific and rapid technology, is an effective alternative. We offer a new real-time PCR based diagnostic tool for *C. pneumoniae* and *M. pneumoniae* diagnosis.

**Methods:** Nucleic acids were extracted from nasopharyngeal specimens by using easyMag™ NucliSENS (bioMérieux). A Proteinase K (Novagen) pre-treatment was performed. Ten microlitre of purified nucleic acids were added to 15 µL of ready-to-use Chla/Myco pneumo r-gene™ amplification premix. *C. pneumoniae* and *M. pneumoniae* were distinguished in a duplex reaction. Amplification was performed on ABI7500 Fast (Applied Biosystems), Dx Real Time System (Bio-Rad), Versant kPCR System AD (Siemens) or LC480 (Roche) platforms.

**Results:** On the QCMD European Proficiency Panel 2011, 11 positive/negative samples were correctly identified with Chla/Myco pneumo r-gene™. Four positives *C. pneumoniae* and five positives *M. pneumoniae* were detected, including weak positives (0.049 IFU/100 µL for *C. pneumoniae* and 5 CCU/100 µL for *M. pneumoniae*). Analytical sensitivities study on *C. pneumoniae* and *M. pneumoniae* samples were performed in respiratory specimens. The limit of detection at 95% is 0.16 IFU/mL and 180 CCU/mL respectively for *C. pneumoniae* and *M. pneumoniae*. Specificity study performed on 80 pathogens showed no cross reaction with other respiratory bacteria or viruses. Technical studies are in progress and can also be presented.

**Conclusion:** The high quality of Chla/Myco pneumo r-gene™ kit, associated with its compatibility with the major extraction and real time PCR platforms allows immediate integration into most routine diagnostic laboratories. This tool is part of the Respiratory MWS r-gene™ brand range which represents an innovative solution to the challenges of respiratory infections.

**P1383** Evaluation of a novel high multiplexing real-time PCR array for the identification and characterisation of bacteria causative of ventilator-associated pneumonia on clinical specimens

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**Objectives:** The VAPChip is a molecular tool aiming to identify directly from clinical samples the major nosocomial pneumonia causative bacteria and beta-lactam resistance genes. We evaluated the analytical performances of the VAPChip on respiratory samples.

**Methods:** The VAPChip uses the RAP-ID technology (Real-time Array PCR for Infectious Diseases; Eppendorf Array Technologies, Belgium). It combines multiplex PCR with real-time microarray-based detection of amplification products. The VAPChip targets 13 bacterial species and 28 resistance genes or variants.

Respiratory samples (n = 45) were tested by the VAPChip and by the following reference methods: quantitative culture with identification by Vitek2 or biochemical tests and antimicrobial resistance profiles by Vitek2 or by disk diffusion. The presence of resistance genes was detected by PCR. Discrepancies were resolved by Maldi-tof and by CT103 array (Check-Points, the Netherlands).

**Results:** The comparison of the VAPChip vs. reference methods showed a sensitivity of 97.4% and 100% and a specificity of 71.4% and 94.1% for identification and detection of resistance mechanisms respectively. The culture of 30 out of the 45 samples yielded growth of 38 significant isolates targeted by the VAPChip. Species recovered by culture were distributed as follow: *E. coli* (n = 3), *K. pneumoniae* (n = 5), *E. aerogenes* (n = 1), *E. cloacae* (n = 1), *S. marcescens* (n = 1), *H. influenzae* (n = 7), *P. aeruginosa* (n = 8), *S. maltophilia* (n = 2), *A. baumannii* (n = 1), *S. aureus* (n = 6), *S. pneumoniae*

(n = 3). The VAPChip missed 1 *S. aureus* but detected two *H. influenzae* and two *K. pneumoniae* not recovered by culture. In addition, two *C. koseri* were misidentified as *K. oxytoca* by the VAPChip. Resistance genes detected by reference methods were: mecA (n = 1), blaTEM non-ESBL (n = 3), blaSHV ESBL (n = 1), blaSHV non-ESBL (n = 1), blaCTX (n = 1) and oxacillinase-23 (n = 1). All these mechanisms were detected by the VAPChip. The VAPChip detected one blaSHV- and one blaTEM-non ESBL genes that were not detected by the reference methods. One methicillin-susceptible *S. aureus* isolate was misidentified as methicillin-resistant by the VAPChip.

**Conclusion:** The VAPChip is a rapid diagnostic tool able to identify resistant bacteria directly on clinical samples. Some discrepancies could be explained by high sensitivity of VAPChip. This hypothesis should be confirmed by further analysis. In addition, clinical trials are needed to evaluate the clinical impact and cost-effectiveness of the new promising tool.

**P1384** "Direct PCR" on sputa is faster and as sensitive as PCR with extracted nucleic acids

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**Objectives:** To assess the performance of reagents that enable direct PCR without traditional extraction of nucleic acids.

**Methods:** The direct PCR reagents were developed and donated by Shimadzu as a prototype for a qualitative end-point PCR assay. We used 50 stored sputa, frozen at -80°C, previously shown by PCR to contain Influenza A (34 samples) or B (16 samples). The samples were thawed and a 5 µL aliquot of raw material was used for direct PCR; the raw material was heated to 90°C for 1 minute with 5 µL of direct PCR reagent before the mastermix was added. Further 200 µL aliquots of the same raw sputa were re-extracted into 60 µL eluates by two commercial methods, an EasyMag (Biomérieux) and an RNA viral mini kit (Qiagen). The extracted RNA and the raw samples were subjected to an influenza PCR with the same primers and detection system, a bead based array on a Luminex instrument, but different mastermixes. The Qiagen one step RT-PCR reagent was used with RNA extracted with the EasyMag and the RNA viral mini kit but, as stipulated by the manufacturer, the Invitrogen SuperScript III Platinum one-step End Point PCR reagent was used for the direct PCR. The work was performed in one day by two people.

**Results:** Influenza was detected in 44 (88%) samples by direct PCR, 43 (86%) samples extracted with the Qiagen kit and 38 (76%) samples extracted with the EasyMag instrument.

A further 50 positive and 29 negative samples were tested in a similar exercise using half the volumes, 2.5 µL of raw sample, with a real time PCR protocol. The direct PCR failed to detect any cases. When the direct method was applied to the same samples with gel based detection, it detected influenza in 47 of the 50 positive samples but none of the 29 negatives.

**Conclusion:** Direct PCR was easy and fast. It delivered a similar performance to PCR with nucleic acids extracted with commercial systems that used much larger volumes: these results demonstrate the inefficiency of methods for extracting RNA and point to sample preparation as an area of potential for further refinement. The direct PCR reagents did not work with our real time assay, reminding us that reagents cannot be assumed to work across all platforms. Direct PCR opens up an avenue for significantly reducing the total time to a result by about an hour. Automation of direct PCR methods would allow real competition with existing extraction platforms.

**P1385** Molecular identification of pathogens in pleural infection

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**Objectives:** The detection of bacteria in pleural fluid culture, using routine microbiological techniques, typically occurs in only 40% of



cases. Nucleic acid amplification testing (NAAT) is emerging as a new tool for diagnosing infection. This study aimed to improve the poor diagnostic yields in this disease, using these molecular techniques.

**Methods:** Three hundred and seventy-seven pleural fluid samples from the Oxford Pleural Infection collection underwent standard microbiological culture. DNA from these samples was also extracted (FastPrep, MP Biomedical) and subjected to two molecular diagnostic strategies: A 625 bp fragment of the ribosomal 16S RNA gene (present in all bacteria) was amplified, using primers targeting flanking conserved regions, and then capillary sequenced (Seq); alongside a nested multiplex PCR (mPCR), initially targeting a 1070 bp fragment of the ribosomal 16S RNA gene, and then amplifying genus-specific sequences of this gene in several multiplex reactions. The mPCR panel comprised of bacterial genera previously identified in pleural infection. Negative samples were depleted of human DNA by human-specific digestion and subtraction, followed by repeat molecular testing.

**Results:** Standard culture detected a pathogen in 41.9% (Fig. 1) of pleural fluid samples. When combined with the molecular testing, this positive rate increased to 89.4%. When samples were positive by all three tests, concordance was 84.4%, compared to 90.0% for mPCR and Culture positive, and 98.9% for mPCR and Seq positive. Superiority of a test was defined as detecting the taxonomic classification to the highest precision, and/or the ability to detect multiple pathogens. Overall, Seq was the most superior test (36.9% of concordant results), followed by mPCR and Seq together (15.6%). Culture was only superior in 9.1% of cases, whereas no overall superiority was determined for 16.8% of concordant results.

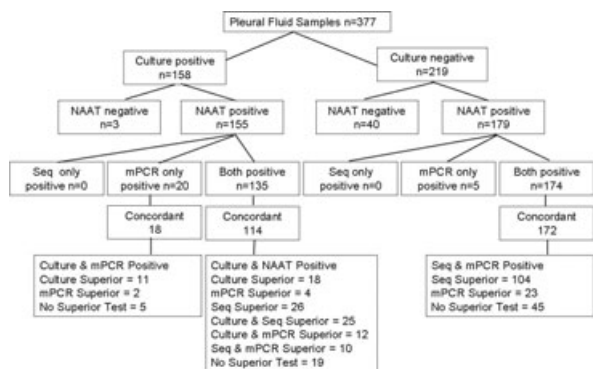


Figure 1. Diagnosis of Pleural Infection. Pleural fluid samples were subjected to standard culture methods to determine infection. Samples were then tested by two nucleic acid amplification techniques (NAAT), Seq and mPCR. When samples were positive by more than one method, only concordant samples were analysed for superiority of testing. Superiority of a test was defined as detecting the taxonomic classification to the highest precision, and/or the ability to detect multiple pathogens.

**Conclusions:** To our knowledge, this is the highest diagnostic yield of any study of this type conducted to date. In our study, NAAT was substantially superior to standard diagnostic methods. Sensitivity and specificity values for combined NAAT are 98.1% (confidence interval 94.6–99.4%) and 18.3% (confidence interval 13.7–23.9%) respectively, using culture as a “gold standard”. However, this poor result was due to NAAT identifying 47.5% more positive samples compared to culture, which leads us to question whether it should still be considered the “Gold Standard” for diagnostic techniques.

#### P1386 Spectrum of pathogens identified by molecular methods in patients with acute bacterial meningitis

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**Objectives:** Acute bacterial meningitis is a progressive illness with high morbidity and mortality. Fast diagnosis and early treatment are therefore crucial. The aim of this study was to characterise a spectrum of pathogens in Czech patients with acute bacterial meningitis.

**Methods:** Two hundred and sixty-eight samples of cerebrospinal fluid or brain abscess collected from adult patients with acute meningitis from 19 April 2004 to 14 November 2011 were included into this study. DNA was extracted using enzymatic pretreatment and QIAamp DNA Blood Mini Kit (Qiagen) and analysed by 16S rRNA broad-range PCR and direct sequencing. The results were available within 24 hour in a routine clinical setting.

**Results:** Bacterial DNA was detected in 104 (38.8%) cases, remaining 164 materials were negative. Out of positive samples, 80 (76.9%) belonged to aerobic or facultative anaerobic pathogens, 7 (6.7%) to anaerobic pathogens and causative agent was not specified due to poor PCR signal in 17 (16.3%) cases. *Streptococcus* spp., *Staphylococcus* spp., and *Neisseria meningitidis* were responsible for the vast majority of cases (67.3%). *Haemophilus influenzae* and *Listeria monocytogenes* were identified sporadically. Out of anaerobic bacteria, *Fusobacterium* spp. was detected in four and *Peptostreptococcus* spp. in three samples.

**Conclusion:** Broad-range 16S rRNA PCR followed by direct sequencing was shown to be a rapid and useful tool in detection of a wide range of acute bacterial meningitis causative agents. In addition, pathogens were detected even in cases of previous antibiotic application or presence of atypical or fastidious pathogens. Results should be interpreted carefully with respect to clinical and other laboratory signs of infection and possible contamination, mainly in cases of poor quality PCR signal and/or coagulase-negative staphylococci.

#### P1387 The nasopharyngeal microbiota in infants with acute otitis media

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**Objective:** Interspecies interactions of the nasopharyngeal microbiota are likely involved in the pathogenesis of acute otitis media (AOM). Capturing the breadth of microbial interactions requires a detailed description of the microbiota during health and AOM.

**Methods:** The nasopharyngeal microbiota of 163 infants with (n = 153) or without (n = 10) AOM was characterized using nasopharyngeal swabs (NPS) and multiplexed pyrosequencing of 16S rRNA. NPS were collected during four winter seasons between 2004 and 2010 for infants with AOM and during 2010 for controls.

**Results:** Fifty-eight bacterial families were identified of which Moraxellaceae, Streptococcaceae and Pasteurellaceae were the most frequent. Commensal families were less prevalent in infants with AOM compared to controls. In infants with AOM, prior exposure to antimicrobials and administration of the heptavalent conjugated pneumococcal polysaccharide vaccine (PCV7) were also associated with reduced prevalence of distinct commensal families (Streptococcaceae and Corynebacteriaceae). In addition, antimicrobial exposure increased the prevalence of Enterobacteriaceae and the abundance of Pasteurellaceae. Other factors, such as age, sex, day care and a history of recurrent AOM did not influence the microbiota.

**Conclusion:** The infant’s nasopharyngeal microbiota undergoes significant changes during AOM, and after exposure to antimicrobials and PCV7, which is mainly due to reduced prevalence of commensal bacterial families.

#### P1388 Detection and typing of *Streptococcus pneumoniae* by RT-PCR in clinical samples from children with acute otitis media

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**Objectives:** Isolation and typing of *Streptococcus pneumoniae* is routinely based on the classical microbiological methods, such as culturing and serological reactions, that require viable bacteria. The wide use of antibiotics and delay in microbiological analysis may preclude detection of *S. pneumoniae* in clinical samples justifying utilization of molecular methods for this purpose.

**Methods:** Nasopharyngeal swabs, tympanocentesis material or ear discharge in case of tympanum perforation were taken from children (age <5 years) with acute otitis media (AOM) by an eSwab collection and preservation kit (Copan). After swab inoculation for culture, DNA was extracted from the liquid preservation medium and used for RT-PCR with primers targeting specific *S. pneumoniae* *lytA* alleles. In the *lytA*-positive samples, separate RT-PCRs were done with primers for selected serotypes 19F, 6A/B, 23F and 14 that are the most prevalent serotypes in Russia. Serotyping of isolated *S. pneumoniae* was performed by Neufeld reaction.

**Results:** In total, we examined 67 samples from 31 patients with AOM. In 30 samples (45%) growth of relevant bacteria was found: *S. pneumoniae* – 8 (27%), *S. aureus*, *H. influenzae*, *M. catarrhalis* – 7 each (23% each), *S. pyogenes* – 1. In 15 samples, no growth was observed, in the remainder 22 samples physiological flora was present. Pneumococcal *lytA* RT-PCR was positive in 42 out of 67 samples (63%) including all eight samples where *S. pneumoniae* was isolated by culture. In 34 *S. pneumoniae* culture-negative/*lytA*-positive samples, no growth of relevant flora was observed. Serotype-specific RT-PCR with the selected serotype primers was positive in 26 samples (63%): 19F (n = 11, 26%), 23F (n = 5, 12%), 6A/B and 14 (n = 4, 10% each), 9V (n = 2, 5%). In each sample, only one serotype-specific RT-PCR was positive. RT-PCR results were in accordance to Neufeld serotyping in isolated *S. pneumoniae* strains (19F, n = 2; 9V, n = 2; 6A, n = 1; 23F, n = 1). Two isolates were typed only by Neufeld reaction (serotypes 19A and 38), specific RT-PCR for these serotypes was not used.

**Conclusion:** Our study demonstrates that PCR-based detection and typing of *S. pneumoniae* may be directly applied to clinical samples. Higher yield of RT-PCR-positive samples in comparison to culture is unlikely to reflect the presence of etiologically irrelevant bacterial debris because the *lytA*-positive samples were negative for other pathogens and RT-PCR gave similar to serological methods serotype distribution pattern.

**P1389** **Detection of *C. burnetii* DNA from patients with acute Q fever using a fluorescent nucleic acid dye (SYTO9) in a real-time PCR assay**

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**Objectives:** This study aimed to assess whether a real-time polymerase chain reaction (PCR), using a novel fluorescent nucleic acid dye (SYTO9) and primers targeting the *com 1* gene, was able to reliably detect *C. burnetii* from acute phase sera in patients from an area with a high incidence of Q fever.

**Methods:** Serial 10-fold dilutions of *C. burnetii* DNA at known concentrations, extracted from the avirulent Nine Mile phase 2 (clone 4) strain, were tested by real-time PCR to obtain a standard curve and to ascertain the reportable linear range. The analytical sensitivity (limit of detection) was determined by Probit analysis for 60 samples with low copy numbers. Primer specificity was determined by testing DNA extracts from a selection of non-Coxiella bacteria. The assay was then tested on stored acute phase sera from patients with acute Q fever (confirmed by serological criteria) as well as clinical samples without serological evidence of Q fever to determine sensitivity and specificity. Performance of the assay was further compared with a reference real-time PCR method on a selection of blinded samples.

**Results:** The assay was found to have a reportable linear range of between  $10^1$  and  $10^5$  copies/ $\mu$ L. The lower limit of detection for 95% of samples was 4 copies/ $\mu$ L. No positive results were found when tested against non-Coxiella bacterial extracts. There was 88% agreement between the assay and a routine reference PCR when tested on 84 samples = 0.74 [0.59–0.88];  $p < 0.0001$ . In clinical specimens (37 with confirmed acute Q fever and 24 with no Q fever), the assay had a sensitivity of only 40.5% (24.8–57.9%), but a specificity of 100% (85.8–100%). However, when a subset of Phase 2 IgM non-reactive samples were analysed the sensitivity increased to 66.7% (34.9–90.1%).

**Conclusions:** The SYTO9-based real time PCR assay was able to detect *C. burnetii* from acute phase human sera. While the assay was very specific, the sensitivity was modest but improved when tested on Phase 2 IgM non-reactive samples. The assay was less sensitive than a reference PCR using oligonucleotide probes and two primer targets. Incorporation of PCR into routine testing algorithms for acute Q fever, especially if the Phase 2 IgM is non-reactive, would improve the early detection of *C. burnetii*. Although this SYTO9-based assay has some advantages in relative low cost and improved specificity by melt-curve analysis, its lesser sensitivity may limit its utility

**P1390** **Identity and quantity of micro-organisms in necrotising fasciitis determined by culture based and molecular methods**

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**Objectives:** Necrotising fasciitis (NF), commonly known as flesh eating disease is a fast progressing, potentially lethal infection of the subcutaneous tissue/fascia. Treatment includes high doses of intravenous antibiotics and aggressive surgical debridement. Accurate identification of the microorganisms may add to the knowledge of NF pathogenesis and influence the administration of antibiotics, and thereby potentially improve the outcome for the patients. Here we investigate the applicability of different molecular methods compared to standard culture-based methods.

**Methods:** Twenty one samples were obtained during debridement of NF patients (n = 8). Samples were investigated by standard bacteriological examination (culture and microscopy) at Copenhagen University Hospital, Denmark, and a range of molecular methods. The best DNA extraction protocol was chosen based on tests of five protocols performed on five NF samples. Molecular methods included full-length 16S rRNA gene analysis, real-time PCR, the Ibis T5000 Biosensor System, and 454 pyrosequencing of partial 16S rRNA genes.

**Results:** No growth of bacteria was observed in nine samples (43%); of these microscopy revealed Gram-positive cocci in chains in two samples. Molecular methods were positive for all samples. Culture identified *Streptococcus* sp. in most cases (48%). By molecular methods the incidence was increased (90%) and identification to species level was possible, with *S. pyogenes* being dominant (85% of samples with *Streptococcus*). Real-time PCR showed dominance of *Streptococcus* sp. in the samples, however some additional bacteria were found. The remaining samples were found to contain yeast by culture and a mixture of *Mycoplasma* sp., *Fusobacterium necrophorum* and *Candida albicans* by molecular methods. Overall polymicrobial findings were more common by molecular- than culture-based methods.

**Conclusion:** Correspondence between findings by culture-based and molecular methods indicates that the latter may be an appropriate method. The advantages of using molecular methods are: (i) identification of the pathogen(s) even when antibiotics have been administered and (ii) less time-consumption than conventional culture. The challenge is the interpretation of the significance of the findings by molecular methods. The use of the molecular methods will potentially enable a more rapid adjustment from empiric antibiotic treatment to defined treatment directed at identified microorganisms.

**P1391** **Flow cytometry assay of adenylate cyclase toxin of *Bordetella pertussis* CyaA preparations on phagocytosis**

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**Introduction:** Using flow cytometry, the phagocytic ingestion of *E. coli* by human peripheral blood cells from healthy donors, J774.2 mouse macrophages and U937 human monoblastic cells was determined after exposure to different concentrations of CyaA .

**Material and Methods:** This technique was developed for the evaluation of phagocytosis activity in human peripheral blood (neutrophils and monocytes) and other cells. Ingestion activity was measured as the mean fluorescence intensity (MFI) produced after 20 minutes of incubation with FITC-conjugated *E. coli*. All tubes were read by flow cytometry at a wavelength of 488 nm after quenching and DNA staining.

**Results:** Exposure of granulocytes or J774.2 cells to different concentration of CyaA and CyaA\* (0.05, 0.1, 0.2 mg/mL final concentrations) resulted in significantly lower mean neutrophil ingestion in the presence of CyaA. The results also showed that phagocytosis was significantly impaired by increases in CyaA concentrations for granulocytes and J774.2 cells compared to cells treated with PBS. With 0.2 mg protein/mL of CyaA there was almost complete (92%) inhibition of phagocytosis by J774.2 cells and 63% inhibition of phagocytosis by human granulocyte cells.

**Conclusions:** The flow cytometry histograms that represents the amount of ingested *E. coli*, also indicated the dose-dependent inhibitory effect of CyaA on J774.2 and granulocytes.

Overall the results of this assay would imply that inhibition of phagocytosis by CyaA was due mainly to the AC enzymic activity of the protein.

**P1392** *Bordetella* PCR methods in Europe. A EUVAC.NET/ ECDC supported study

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**Objectives:** The study was performed in order to evaluate the differences and the quality of PCR methods for diagnosis of *Bordetella pertussis* infections around Europe. The study was initiated by the EUVAC.NET organization on an ECDC grant.

**Methods:** Twenty four laboratories from 19 European countries participated in the study. Eight coded samples were sent to the laboratories for analysis by their respective in-house PCR methods in use for laboratory diagnosis of *B. pertussis* infections. Five samples contained *B. pertussis* DNA in varying concentrations, one sample contained *B. parapertussis* DNA, one contained *B. holmesii* DNA and the last sample was a negative control.

**Results:** The PCR methods in use around Europe were very different. None of the laboratories were able to identify all eight samples in detail and only one laboratory was able to correctly identify the *B. holmesii* DNA. Ten (42%) laboratories tested only for the presence of *B. pertussis* DNA and were thus not able to diagnose infections by *B. parapertussis* or by other bordetellae; three of these laboratories identified the *B. parapertussis* DNA as a *B. pertussis* sample. Fourteen (58%) of the laboratories identified the *B. holmesii* DNA as *B. pertussis*. As expected, real-time PCR was generally capable of detecting lower concentrations of DNA than block-based PCR. However, in two instances real-time PCR identified the negative control as a weak positive. All laboratories correctly identified the high concentrations of *B. pertussis* DNA.

**Conclusions:** When laboratory-surveillance data around Europe are to be compared there is a need for standardized methods. This study underlines the need for standardization of PCR methods for diagnosis of *B. pertussis* as large differences were observed in the ability to identify DNA from different species of *Bordetella*. Infection by *B. parapertussis* is not uncommon, and the laboratories need to be able to discriminate between this and *B. pertussis*. Infection by *B. holmesii* is less common, but it is however important that laboratories are aware of the possible false-positive *B. pertussis* results caused by *B. holmesii* DNA.

**P1393** Phage mini-antibodies and their use for detection of microbial cells by using electro-acoustic sensor

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Nowadays, cell detection in molecular biology is performed with the help of genetic engineering technologies for cloning recognizing

fragments (hypervariable immunoglobulin domains). These technologies are cheaper and can be competitive in selectivity with hybridoma technologies. One such method is antibody phage-display.

**Objectives:** Obtaining the phage-displayed mini-antibodies to *Azospirillum brasilense* Sp245 as example, and their use for detection of microbial cells by using electro-acoustic sensor.

**Methods:** All experiments were conducted by specially manufactured sensor based on a resonator with a transverse electric field in the frequency range 6–7 MHz.

**Results:** The first preparation of phage mini-antibodies to *Azospirillum brasilense* Sp245 surface antigens was made by using a combinatorial phage library of sheep antibodies. The prepared phage mini-antibodies were used for cell detection by dot assay, electro-acoustic analysis of cell suspensions, and transmission electron microscopy. It has been found that the frequency dependencies of the real and imaginary parts of the electrical impedance of the resonator loaded by the cell suspension *A. brasilense* Sp245 with the phage mini-antibodies, significantly differs from ones of the resonator with the control cell suspension without phage mini-antibodies. The electro-acoustic results were in good agreement with the electron microscopic data. We have shown that the concentration limit of a possible determination of the microbial cells in their interaction with the phage mini-antibodies is equal  $10^3$  cells/mL. It has been also found that the detection of cell *A. brasilense* Sp245 using the phage mini-antibodies is possible even in the presence of other cultures.

**Conclusion:** These results demonstrate the promise of analysis of microbial suspensions using a lateral electric field excited piezoelectric resonator and show the possibility of developing a biological sensor for the quantitative detection of microbial cells. This is the first reported possibility of employing phage mini-antibodies in bacterial detection aided by electro-acoustic analysis of cell suspensions.

**P1394** Bacterial and fungal infections of the cystic fibrosis lung: what can we learn from combining culture, RFLP and pyrosequencing approaches?

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**Objectives:** Given the polymicrobial nature of pulmonary infections in patients with cystic fibrosis (CF), it is essential to enhance our knowledge on the composition of the microbial community to improve CF management. Our aim was to identify bacterial and fungal communities present in CF sputa by combining culture-based and independent approaches, including a new pyrosequencing approach.

**Methods:** Results from conventional microbiological culture for fungi and bacteria in four sputum samples from adult CF patients (followed at Lille Hospital, France) were compared with those from molecular methods.

Terminal Restriction Fragment Length Polymorphism (T-RFLP) analysis was used to examine the dominant bacterial community members by resolving ribosomal RNA (rRNA) gene fragments. Fungal populations were characterized by performing an ITS-RFLP method followed by cloning and sequencing of gene products. A pyrosequencing method targeting the 16S rRNA gene and ITS2 locus was also applied. Results of deep-sequencing, RFLP and conventional methods were compared.

**Results:** Using RFLP methods, we identified 6.5 bacterial species (range 2–15), and 2 (range 1–4) micromycetes. This was confirmed by cloning. A comparable mean number of microbial species per sputum was obtained using the pyrosequencing method. In addition, a detailed phylogenetic analysis showed high molecular diversity at the sub-species level for the main fungal and bacterial taxa identified in the present study.

Culture-based findings were compared to the culture-independent data and species richness as well as evenness of fungi and bacteria is discussed in relation to clinical data on the CF patients followed.

**Conclusion:** The present pyrosequencing approach evaluates more extensively the diversity of fungi and bacteria compare to culture-based

methods. Further work is required to explore the clinical pertinence of pyrosequencing for informing therapy.

Grants: This work was supported in part by PFIZER and by APHP.

**P1395 Variants in the colony-stimulating-factor 1 region and risk of childhood meningococcal meningitis: a Danish genome-wide association study**

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**Objectives:** To identify host genetic susceptibility variants in cases of childhood meningococcal disease and controls and to validate potential single-nucleotide polymorphism (SNP) variations in a separate case control analysis.

**Methods:** Through the registries at the Danish National Neisseria and *Streptococcus* Reference Center, Statens Serum Institut, we identified children below age of 5 years who had invasive meningococcal disease in the period 1982–2006. For each case we selected a control matched on age and place of birth.

We identified neonatal dried bloodspots on potential cases and controls in the Danish Neonatal Screening Biobank and extracted DNA. We used the Illumina Infinium HD HumanOmni1-Quad chip array for the initial genome-wide association study (GWAS). We required call rates >95%, a minor allelic frequency >0.1, and a significance level for Hardy Weinberg >0.001 (Fisher's exact test). Following the GWAS we selected SNPs based on either biological function or strength of the association. We validated selected SNPs using the KASPar technique.

**Results:** We identified 841 children who presented with meningococcal meningitis before age 5. For the initial GWAS we obtained DNA from 376 of these children and 440 controls without any episodes of meningococcal disease. For validation we additionally obtained DNA from 274 children who presented with meningococcal bacteraemia and 274 controls.

In the GWAS analysis two SNPs encoding colony stimulating factor 1 (CSF-1) were found among the 500 SNPs with strongest association to disease status. For rs333949 the minor allele frequency among cases was 0.18 and among controls it was 0.25 corresponding to an odds ratio (OR) for meningococcal disease of 0.68 ( $p = 0.003$ ). For rs333951 the minor allele frequency among cases was 0.22 and among controls it was 0.30 corresponding to an OR for meningococcal disease of 0.68 ( $p = 0.002$ ).

In the validation study the minor allele frequency for rs333949 was 0.19 among 270 cases and 0.27 among 268 controls, the corresponding OR was 0.61 (95% confidence interval [CI], 0.45–0.81). For rs333951 the minor allele frequency was 0.23 among 263 cases and among 268 controls it was 0.33, OR 0.60 (95% CI, 0.45–0.79).

**Conclusion:** In this study including more than 600 Danish children who had an episode of meningococcal meningitis or meningococcal bacteraemia before age 5 we found that variation in the CSF-1 region may be associated with host susceptibility to meningococcal disease.

**P1396 MALDI-TOF mass spectrometry based methods for the characterisation of enterococci**

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**Objective:** Vancomycin-resistant enterococci have a leading position among the pathogens causing nosocomial infections. Their rapid identification to species as well as differentiation in accordance with their drug sensitivity is very important to choose the correct treatment strategy. In our work we applied a mass spectrometry based approach for identification and differentiation of enterococci.

**Methods:** In total 162 enterococci isolates were collected. Susceptibility testing to glycopeptides (vancomycin, and teicoplanin) was done for each strain. Identification to species level was performed by MALDI Biotyper technique (Bruker Daltonics, Germany). The

correctness of identification was confirmed by 16S rRNA gene sequencing and PCR using species-specific primers of the *ddl* gene encoding Ddl-ligase enzymes. Also the presence of genes conferring the resistance to glycopeptides (*vanA*, *vanB*, *vanC-1*, *vanC-2/3*, *vanD*, *vanE*, *vanG*, *vanL*, *vanM*) was estimated by PCR. Cluster analysis of mass spectra collected from enterococci with different resistant profiles was carried out using software package MALDI Biotyper 3.0.

**Results:** The 95 *E. faecalis*, 56 *E. faecium*, 1 *E. raffinosus*, 6 *E. gallinarum*, 2 *E. avium*, 1 *E. casseliflavus*, 1 *E. hirae* were correctly identified at the species level. All *E. faecalis* appeared to be susceptible to vancomycin and teicoplanin and did not carry any of the drug resistance markers. Among *E. faecium* there were 21 drug susceptible isolates without any of the *van* genes present. All 35 drug resistant isolates carried *vanA* ( $n = 20$ ), *vanB* ( $n = 10$ ), *vanC-1* ( $n = 1$ ) or both *vanA* and *vanB* ( $n = 4$ ) genes.

Preliminarily a small group from *E. faecium* was selected for thoughtful cluster analysis of mass spectra. There were four isolates of a sensitive profile, and eight resistant isolates – four of a *VanA* genotype, and four of a *VanB* genotype. Composition analysis as well as dendrograms undoubtedly divided collected mass spectra into two groups in accordance with phenotype characterization without any differences between isolates carrying *vanA* or *vanB* genes.

**Conclusion:** Novel reproducible and efficient approach based on the MALDI ToF MS is reliable of species identification and characterization of enterococci. The differentiation of vancomycin-resistant from susceptible *E. faecium* has to be confirmed with further isolates from different regions.

**P1397 Evaluation of the QuickFISH BC test, a rapid method to distinguish *Staphylococcus aureus* from coagulase-negative staphylococci in positive blood cultures**

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**Objective:** the reduction of turn around time (TAT) for blood cultures is a major goal in clinical microbiology. Recently, a ‘‘peptide nucleic acid fluorescence in situ hybridisation test’’ (PNA-FISH, AdvanDX) has become available in clinical practice, allowing the identification of some microorganisms directly from positive blood culture bottles in 90 minutes. A modification of this technique (Staphylococcus QuickFISH BC, AdvanDX) is currently under evaluation: it allows the distinction between *Staphylococcus aureus* (SA) and coagulase negative staphylococci (CNS) with a 20 minutes procedure. Aim of this study was to evaluate the performance of this test on different sets of blood cultures positive for Gram-positive cocci in clusters.

**Methods:** the blood cultures were analyzed using the Bactec system (Becton Dickinson, USA). The samples showing Gram positive cocci in clusters were examined using the Staphylococcus QuickFISH BC, performed according to the manufacturer procedure, and the coagulase tube test. Quick-FISH BC slides were read independently by different test operators and blinded to the final results with traditional techniques. The identification of the microorganisms grown from cultures were performed using Vitek2 (BioMerieux, France).

**Results:** Fifty-nine blood cultures of 44 different patients were analyzed. Sixty staphylococcal isolates were identified (one bottle yielded two different strains). In particular 23 *S. epidermidis*, 21 *S. aureus*, 7 *S. hominis*, 4 *S. capitis*, 3 *S. haemolyticus*, 1 *S. warneri* were identified by Vitek2; one coagulase negative strain was not identified at species level. The direct coagulase tube test was negative, after incubation at 36°C, both after 4 hour and after 24 hour for all the CNS. Among the 21 *S. aureus*, 18 were positive after 4 hour incubation, two were negative after 4 hour but positive after 24 hour, one strain was still negative after 24 hour. There was no discrepancies between the QuickFISH test results and the phenotypic identification.

**Conclusion:** This study demonstrates the excellent agreement between QuickFISH test and standard laboratory techniques in identifying staphylococcal strains from positive blood cultures. Although the direct coagulase tube test is cheaper, QuickFISH BC appears quick, reliable

and easy to perform. For its reduced time of analysis, this test can be performed also in the late afternoon, when direct coagulase tube test is useless. This is very important for patient with *Staphylococcus aureus* bacteremias.

## Molecular epidemiology of bacterial infections

### P1398 Characterisation and typing of *Staphylococcus aureus* isolates from deep neck space infections in India

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**Aims and objectives:** To screen for methicillin resistance (MRSA) and to determine the antimicrobial susceptibility pattern of all the isolates of *Staphylococcus aureus* from deep neck space infections (DNSIs). To characterize all the *Staphylococcus aureus* isolates by phage typing; and the MRSA isolates by biotyping and typing by antibiogram, in addition.

**Materials and methods:** A total of 56 cases of DNSIs from a 1600-bedded tertiary care teaching hospital of New Delhi were recruited. Aspirated pus from the patients were cultured and the *Staphylococcus aureus* isolates were identified by conventional techniques. The antimicrobial susceptibility against a wide range of antimicrobial agents was performed by the disc diffusion method employing the modified Stokes' technique. The MICs of vancomycin, teicoplanin, amikacin, ciprofloxacin, mupirocin, fusidic acid were determined by the E test. Screening for methicillin resistance was performed both by cefoxitin disc method as well as oxacillin agar screening method. All the isolates were subjected to bacteriophage typing by the 23 conventional (basic) set of phages (Blair et al). All the MRSA isolates were further subjected to biotyping (Coia et al.) and a novel typing method using the antibiogram.

**Observations:** *Staphylococcus aureus* was isolated in 46.4% of cases (n = 56). Of the 26 isolates of *Staphylococcus aureus* obtained, 3(11.5%) were identified as MRSA. Penicillin resistance was found to be 100% among *S. aureus* isolates. Cotrimoxazole resistance was seen in 100% of MRSA and 69.6% of methicillin Sensitive *S. aureus* (MSSA) isolates, while ciprofloxacin resistance in 66.6% and 56.8% respectively. However, all the *S. aureus* isolates were uniformly sensitive to vancomycin and teicoplanin at MIC90 value of 2 µg/mL for both. An overall phage typeability of 53.9% was observed for the isolates with maximum in the mixed group of phages (39.1%). Biotyping of MRSA isolates showed that all the three MRSA isolates belonged to biotype A. Based on the antibiogram pattern, the three different isolates of MRSA showed three distinct resistant phenotypes.

**Conclusions:** All the MRSA isolates were found to be non multi resistant oxacillin resistant *Staphylococcus aureus* (NORSA).

### P1399 Correlation between molecular types and minimal inhibitory concentration to vancomycin among methicillin-resistant *Staphylococcus aureus* isolates from sterile sites: TIST study, 2006–2010

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**Objectives:** This goal of this study was to illustrate the molecular and phenotypic characters of methicillin-resistant *Staphylococcus aureus* (MRSA) strains from sterile sites and delineate the correlation between them.

**Materials and methods:** MRSA isolates from sterile sites of patients with invasive MRSA infections were collected from 22 teaching hospitals in Taiwan during a 5-year study period (Tigecycline In-vitro Surveillance in Taiwan – TIST, 2006–2010). MRSA were confirmed with resistance to cefoxitin disc and presence of *mecA* gene. All isolates were analyzed with molecular typing methods including staphylococcal chromosome cassette *mecA* (SCCmec), gene encoding accessory gene regulator (*agr*), and direct repeat unit (*dru*) and phenotyping method

with minimal inhibitory concentration (MIC) to vancomycin by agar dilution.

**Results:** Totally 688 isolates labeled as MRSA were collected from sterile sites of patients with invasive infections. Among these, 670 MRSA isolates (mostly from blood, 93.7%) were confirmed with cefoxitin resistance and *mecA* existence. The median and mean of vancomycin MIC of these isolates were 1 and 1.31 mg/L, respectively (range: 1–3 mg/L). The highest mean MIC occurred in 2007 (1.57 mg/L) and gradually decreased to the lowest value (1.10 mg/L) in 2010 significantly ( $p < 0.001$ ). SCCmecIII (256, 38.2%), IV (144, 21.5%), II (131, 20%) and VT (77, 11.5%); *agr* group I (495, 73.9%) and II (139, 20.7%); and *dru*14 (216, 32.2%), 9 (140, 20.9%), 4 (116, 17.3%), and 11 (77, 11.5%) accounted the majority of MRSA isolates. Strains with SCCmec II and III were found to have higher MICs than those with SCCmec IV, V, and VT. The decrease in mean MIC to vancomycin in the 5-year study period was associated with the increase in strains with SCCmecIV, V, and VT and the subsequent decrease in strains with SCCmecII and III ( $p < 0.001$ ).

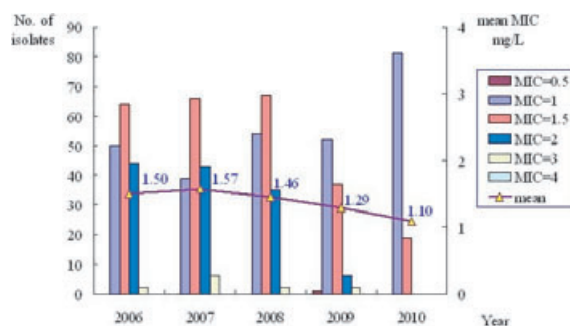


Figure 1. Distribution of minimal inhibitory concentration (MIC) of 670 MRSA isolates from sterile sites and the trend of mean MIC from 2006 to 2010

**Conclusion:** No vancomycin creep was observed in the 5-year period. The trend with decreased MIC to vancomycin was associated with the changing molecular epidemiology of MRSA isolates, which reflected the increasing numbers and proportion of molecularly community-associated MRSA (CA-MRSA) strains in clinical settings.

### P1400 Molecular characterisation of Panton-Valentine leukocidin-positive *Staphylococcus aureus* in southwestern England

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**Objectives:** In the last decade there has been an increase in PVL toxin-producing (PVL+) methicillin sensitive *S. aureus* (SA) community-acquired infections in one city in South West England. The objective of this study was to assess whether this was linked to higher nasal carriage rates compared to other cities and to determine whether molecular typing could identify clonal relationships among PVL+ nasal and clinical strains circulating in this region.

**Methods:** In 2009–2010, swabs were sent to a representative sample of adults in three cities of SW England. Self-taken nasal swabs were returned and cultured for SA. These strains, plus SA isolated from community-acquired infections in each location, were screened for *pvl* and *mecA* by PCR. All PVL+ SAs, MRSAs and 181 randomly selected MSSAs were genotyped by *spa* typing. The multilocus sequence type clonal complex (CC) was then inferred from *spa* types where possible.

**Results:** SA was recovered from 553 of 1952 nasal swabs (28.3%). Six isolates (1.0%) were PVL+ with no statistically significant association with geographic locality (Chi square test  $p > 0.05$ ). In contrast, 28 of 578 (4.8%) clinical isolates were PVL+; 4.3% in city A, 0.6% in city B and 7.8% in city C ( $p < 0.05$ ). Only two PVL+ MRSA were detected;

one (a nasal isolate from city C) corresponded to the CA-MRSA USA 300 clone, the other (a clinical isolate from city B) corresponded to the emerging multi-resistant CA-MRSA Bengal Bay clone. PVL+ isolates comprised 18 spa types and nine CCs. All PVL+ nasal isolates had different spa types and CCs. Spa types t008 (CC8) and t355 (CC152) were common in both nasal and clinical PVL+ isolates. In contrast, spa type t417 (CC22) was the most common among clinical PVL+ isolates (10/28, 35.7%), but was not found among any nasal or PVL negative isolates. Other CC22 spa types were common among both clinical and nasal isolates.

**Conclusions:** Community-acquired infection with PVL+ SA is uncommon in SW England but rates vary geographically. However carriage rates in the nares does not reflect this variation, indicating that community-based nasal screening poorly predicts pvl rates among isolates causing infection. This is consistent with the failure to identify the spa type (t417) accounting for over a third of PVL+ SA clinical isolates among nasal isolates. This clone was not geographically restricted, and the typing data did not suggest any explanation for the high incidence of PVL associated infection in city C.

**P1401** Community-acquired methicillin-resistant *Staphylococcus aureus* encoding the Pantone Valentine leukocidin genes are rarely detected in Istanbul and belong to ST22 and USA300 clones

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**Introduction:** Community-acquired methicillin resistant *Staphylococcus aureus* (CA-MRSA) containing the genes encoding Pantone Valentine leukocidin (PVL) have spread all over the world with various incidences and in different genetic backgrounds. For instance, the ST8 USA300 clone is more frequently detected in North and South America and Spain, whereas the ST80 clone is the major European clone, but is also frequently detected in Algeria; the PVL+ ST30 clone being more frequently detected in Oceania and Asia. Multiple other PVL+ CA-MRSA clones have been reported worldwide but few information are known about the epidemiology of PVL+ MRSA in Istanbul, Turkey.

**Methods:** From 2007 to 2011 we have collected all the MRSA isolates at the Istanbul Medical Faculty hospital in Istanbul. The resistance to methicillin was detected with cefoxitin disc, MRSA-agar and MRSA detection kit. The presence of the PVL genes was detected by PCR. The strains were also characterized using DNA microarrays obtained from Clonediag® and covering 334 target sequences corresponding to 185 distinct genes and their allelic variants. Sequence types were assigned by the microarray results analysis.

**Results:** On a total 88 MRSA, PVL positive MRSA were detected in four isolates only (4.5%), with the following characteristics:

**1** Two PVL positive MRSA isolates were assigned to the ST22 clone. One isolate was resistant to penicillin, gentamycin, tobramycin, kanamycin, erythromycin and the another one to penicillin, tobramycin, kanamycin, erythromycin, levofloxacin. Both of them carried the Staphylococcal Cassette Chromosome (SCC) mec type IV gene and were ermC-positive.

**2** Two PVL positive MRSA isolates were assigned to the ST8-USA300 clone. One of them carried the SCCmec type IV and arginine catabolic mobile element (ACME) genes and was resistant to penicillin, kanamycin, erythromycin. The other one carried only SCCmec type IV gene and was resistant to penicillin, kanamycin, erythromycin, levofloxacin.

These four PVL positive MRSA strains were isolated from outpatients with skin-soft tissue infections and considered to belong to community-acquired infections. The PVL positive ST80 European clone was not detected in Istanbul, whereas this clone is frequently detected in Greece that has common boarder with Turkey).

**Conclusion:** This first report described the characteristics of PVL positive CA-MRSA clones detected in Istanbul, Turkey: their rate of detection was low and they belonged to the ST22 and USA300 clones.

**P1402** Prevalence and significance of in vivo *mecA* gene deletions from the chromosome of *Staphylococcus aureus*

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Infection due to Methicillin Resistant *Staphylococcus aureus* (MRSA) causes a range of health-care associated infections (HCAIs). MRSA infection is associated with high morbidity and mortality, prolonged hospitalisation and increased health-care costs. As an aid to infection control, rapid screening for MRSA by molecular techniques has been introduced into many NHS hospitals. There are, however, problems with the specificity and sensitivity of the molecular techniques currently available.

Staphylococcal resistance for broad spectrum beta-lactams including methicillin, is encoded by the *mecA* gene. In *Staphylococcus aureus*, this is carried within a mobile genetic element termed SCCmec, which is inserted into the *S. aureus* genome at the *orfX* junction. As a result of selective pressures the *mecA* gene can be deleted from the SCCmec resulting in Methicillin Sensitive *Staphylococcus aureus* (MSSA). At present many molecular methods for the detection of MRSA use primers that specifically target the SCCmec. Therefore, methods detecting SCCmec only could lead to a false MRSA positive interpretation.

**Objectives:** The aim of this research is to enumerate the prevalence and identify the significance of *SCCmecA* gene deletions in the patient populations screened by the Microbiology Department, Countess of Chester NHS Foundation trust.

**Method:** The study population included 503 *S. aureus* isolates from the hospital and community and 50 clinical samples from the Countess of Chester Hospital.

Nucleic acid sequenced based amplification (NASBA) was performed on all the 503 Clinical isolates and the 50 clinical samples using NuclisensEasyQ (bioMerieux®). The Nuclisens EasyQ MRSA detection kit has primers for both the *mecA* gene and the SCCmec cassette *orfX* junction.

**Results:** 11.3% (57/503) of the clinical *S. aureus* isolates tested contained *mecA* gene deleted *S. aureus*.

Of the 50 clinical samples tested, MRSA was not detected, however 4% (2/50) of the clinical samples tested contained *mecA* gene deleted *S. aureus* which would have resulted in a false positive result by other methods.

**Conclusion:** The percentage of *mecA* gene deleted *S. aureus* present in the sample population would cause a significant false positive rate, therefore the need for a method which contains primers for the SCCmec cassette *orfX* junction and the *mecA* gene is essential.

**P1403** *Streptococcus agalactiae* serotype Ia ST23/ST24 are dominant among invasive infections in Portugal

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**Objectives:** We have characterized 225 group B streptococci (GBS) isolates recovered from invasive infections in non-pregnant adults in Portugal. Our aim was to document the prevalence of serotypes, antimicrobial resistance patterns and genetic lineages in this population as compared to the previously characterized neonatal population.

**Methods:** The GBS isolates were serotyped and assigned to clones according to their pulsed-field gel electrophoresis (PFGE) profiles and MLST-based sequence types. Susceptibility to penicillin, chloramphenicol, tetracycline, levofloxacin, erythromycin and clindamycin was tested by disk diffusion according to the CLSI guidelines. Macrolide-resistant phenotypes were determined by PCR by detection of the *erm* and *mef* genes. The presence of the surface protein genes *bca*, *alp2*, *alp3*, *alp4*, *eps* and *rib* genes was also tested by PCR.

**Results:** Our analysis revealed a GBS population showing a more diverse clonal composition when compared to that of neonates, consistent with the broader spectrum of disease presentation in these patients. Invasive disease in this population increased with age and was more frequent among men.

Whereas serotype V is dominant among invasive isolates recovered from non-pregnant adults elsewhere, serotype Ia was the most frequent in Portugal. Serotype Ia was represented mainly by a single PFGE cluster defined by ST23 and surface protein gene *eps*, and ST24 and *bca*, as previously seen in neonatal invasive infections in Portugal. This indicates that the same genetic lineages can be responsible for invasive disease in all age groups. Serotype V isolates were distributed into two genetic lineages, one defined by ST1, *alp3* and macrolide resistance, and another presenting with ST2, *eps* and fully susceptible to all antimicrobials tested. The *erm(TR)* gene was more frequent among erythromycin resistant isolates and the *tet(M)* gene was nearly ubiquitous.

**Conclusions:** The dominance of serotype Ia highlights the importance of this serotype in GBS pathogenesis as a leading cause of invasive infections in adults, regardless of age, contrasts to that found elsewhere but was already noted among neonatal infections in the Iberian Peninsula. Furthermore, the high prevalence of ST24, as opposed to rare descriptions elsewhere, suggests that this lineage has enhanced invasiveness and is probably expanding as a regionally successful clone that may disseminate more globally.

**P1404** First NDM-1-producing *Acinetobacter baumannii* in Belgium

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**Objectives:** To characterize the genetic environment of a NDM-1-producing *Acinetobacter baumannii* (Ab) isolated from a patient who was repatriated to Belgium from Algeria in 2011.

**Methods:** Antimicrobial susceptibility testing was performed by microdilution method according to CLSI guidelines. Resistance genes were characterized by PCR, DNA microarray (Check-points CT102) and sequencing. The genetic background of blaNDM-1 was analysed by PCR mapping. Transferability of beta-lactam resistance was tested by broth mating-out assays and transformation of plasmids. Typing of the Ab strain was performed by multilocus sequence typing (MLST).

**Results:** A multidrug and carbapenem-resistant Ab isolate 11314 was recovered from a rectal swab specimen of a 20-year old patient in vegetative state transferred from Algeria to Belgium after severe traffic injury. The isolate was highly resistant to all beta-lactams including meropenem and imipenem (MIC  $\geq$  32 mg/L), aminoglycosides (gentamicin, tobramycin and amikacin), and quinolone (ciprofloxacin) but it remained susceptible to colistin (MIC = 0.5 mg/L), and tigecycline (MIC < 0.25 mg/L). PCR/sequencing and Check-points micro-array revealed the presence of blaNDM-1 only. A single non-typeable plasmid of 174 kb was detected but transfer experiments failed to demonstrate the presence of any carbapenem-resistant trait, suggesting that the blaNDM-1 was located on the chromosome. PCR-mapping revealed that blaNDM-1 was located between two direct repeat of ISAbA125 element in a transposon similar to the one reported in Ab 161/07 isolated in Germany (Pfeifer et al. JAC 2011; Accession Nr HQ857107). By MLST, Ab 11314 presented an allele profile 1-3-3-2-2-7-3 corresponding to sequence type (ST) 92 (Bartual et al., JCM 2005), a ST already described in many different regions of the world (mostly in association with OXA-23-producing Ab strains). Sequencing analysis of the intrinsic blaOXA-51-like and blaADC genes showed that this strain harboured the blaOXA-64 and the blaADC-26, neither of which were preceded by ISAbA1 nor ISAbA125.

**Conclusions:** Here, we report for the first time an *A. baumannii*-producing NDM-1 isolated in Belgium belonging to ST92. The blaNDM-1 gene was located in a composite transposon structure composed of two copies of the insertion sequence ISAbA125, very similar to the structure described for another *A. baumannii* strain (Ab 161/07) from Germany.

**P1405** Identification of IS1006 interrupting an ISAbA3 upstream of blaOXA-58 in *Acinetobacter baumannii* from a cancer patient in Egypt

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**Objectives:** *A. baumannii* is an important opportunistic infection that is commonly found in hospitals, particularly intensive care units. Class D carbapenemase blaOXA-58 is increasingly identified in *A. baumannii* as plasmid-mediated and is believed to confer carbapenem resistance when associated with insertion sequences (IS) upstream. We hereby report a carbapenem-sensitive *A. baumannii* strain isolated from a paediatric cancer patient in Egypt, harbouring blaOXA-58 with IS1006 interrupting an ISAbA3 upstream.

**Materials and methods:** The isolate was obtained from a catheter tip culture of a paediatric cancer patient in Cairo, identified by 16s-23s rRNA restriction analysis as well as by amplification and sequencing of the blaOXA-51-like gene. The presence of Class D Carbapenemases was performed by Multiplex PCR, and the upstream region of blaOXA-58 was amplified and sequenced accordingly. Minimum inhibitory concentrations (MICs) were determined according to BSAC guidelines

**Results:** The isolate possessed the chromosomal blaOXA-64 gene, now commonly found in the Middle East. In addition, it was also positive for blaOXA-58. Sequencing directly upstream of blaOXA-58 revealed the presence of ISAbA3, which would normally provide a suitable promoter and be associated with carbapenem resistance. However, the MICs of Imipenem and Meropenem were 4 and 1 mg/L, respectively. Sequencing also revealed that the ISAbA3 was interrupted by the incorporation of another insertion sequence, IS1006, 176 bp upstream of the start codon of blaOXA-58, which is interfering with the promoter of ISAbA3.

**Conclusion:** ISAbA3 has been previously reported upstream of blaOXA-58 conferring carbapenem resistance in *A. baumannii*. Our results suggest that the insertion of IS1006 in the ISAbA3 serves to switch off the resistance promoter, hereby rendering the isolate susceptible to carbapenems.

**P1406** Characterisation of invasive non-type B *Haemophilus influenzae* disease in children in England and Wales, 2003–2010

A.A. Vickers\*, D. Litt, S. Ladhani, M. Slack (London, UK)

**Objectives:** Characterisation of all non-serotype b invasive *Haemophilus influenzae* (Hi) isolates from children 1 month to 10 year of age in England and Wales (2003–2010).

**Methods:** The UK Health Protection Agency (HPA) routinely collects all invasive Hi isolates from hospitals in England and Wales as part of prospective, enhanced national surveillance of Hi disease. All non-type b encapsulated (serotypes a [Hia], c [Hic], d [Hid], e [Hie] and f [Hif]) and non-capsulated (NTHi) isolates of Hi from children aged 1 month–10 year received by the HPA between January 2003 and December 2010 were characterised by multilocus sequence typing (MLST), antibiotic susceptibility testing (BSAC agar dilution) and biotyping. 390 Hi isolates were examined (316 NTHi [81%], 58 Hif [15%], 13 Hie [3%], 2 Hic [ $<$ 1%] and 1 Hia [ $<$ 1%]).

**Results:** A small but steady increase in incidence of invasive non-b Hi disease was observed during the study period. MLST showed that capsulated isolates were highly clonal, clustering around a dominant ST within each serotype (Hie around ST18, ST124 Hif). Conversely, NTHi isolates exhibited a high degree of diversity, with 57 STs among 316 isolates. 59 novel STs were identified. No ST was found in isolates of different serotype. 93% (69/74) of capsulated strains were biotype I. By contrast, NTHi were diverse in biotype (42% biotype II, 22% biotype III, 16% biotype I, 16% biotype V and  $<$ 5% biotypes IV, VI, VII and VIII). 38% of strains were resistant to trimethoprim, 11% to ampicillin,

5% to amoxicillin-clavulanate, 2% to ceftriaxone and <1% to chloramphenicol, erythromycin, levofloxacin, rifampicin and tetracycline. 98% (42/43) of ampicillin-resistant strains were beta-lactamase positive. 21% (4/9) strains with intermediate susceptibility to ampicillin (MIC = 1 mg/L) carried known beta-lactamase-negative ampicillin-resistant (BLNAR) amino acid substitutions in *ftsI* (encoding PBP3). All BLNARs identified were NTHi.

**Conclusions:** The incidence of invasive non-type b Hi disease in children aged 1 month–10 year increased over the study period. Capsulated strains exhibited less genotypic and phenotypic diversity than NTHi strains. Rates of resistance to ampicillin, chloramphenicol and tetracycline were consistent with those observed in a study of invasive Hi disease in England and Wales between 1985 and 2004; trimethoprim resistance rates were substantially higher (38% c.f. 11%) than reported previously.

This study was funded by a grant from GlaxoSmithKline.

#### **P1407** Clonal structure of *Vibrio cholerae* El Tor strains isolated at the cholera outbreak in Yuzhno-Sakhalinsk, 1999

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Epidemiological complications of cholera in Siberia and the Far East during 7-th pandemic were associated with introducing infection from other part of the world. Here we consider a largest outbreak of cholera in this region at last decade and a half. For retrospective outbreak analysis we used classical microbiological and epidemiological methods and modern molecular genetics techniques. The main aim of study was to identify of genetics features and clonal structure of outbreak *V. cholerae* strains. A total of 21 *V. cholerae* eltor strains isolated during cholera outbreak in Yuzhno-Sakhalinsk in 1999 were studied. Six of them were isolated from patients and six – from vibriocarriers. Demographic, social, epidemiological and clinical data were collected for each patient. Nine analyzed strains were isolated from environments at the same period. Standard microbiological procedures were used for strains identification. Complex genetics analysis was carried out by PCR-based biovar-specific and pathogenicity-associated genes detection (*ctxB*, *rstR*, *rstC*, *TLC*, *ctxAB*-promoter domain), *ctxB* and *ctxAB*-promoter sequencing analysis, MLST-typing (nine “housekeeping” genes – *dnaE*, *lap*, *recA*, *pgm*, *gyrB*, *cat*, *gmd*, *chi*, *rstA*) and PFGE-typing (with *CciNI* restriction enzyme).

During August–September, 1999, in Y.-Sakhalinsk 11 patients and 11 vibriocarriers were detected. Fourteen *V. cholerae* strains isolated from environment samples. Pending the epidemiological investigation probable ways and factors of infection's spread were revealed. All tested in present study strains (*n* = 21) belonged to *V. cholerae* serogroup O1 biovar eltor by its phenotypic features. Genetics structure of *ctxB* corresponded to classical biovar (*ctxB1* variant). All strains had identical biovar-specific and pathogenicity-associated genes profiles (*ctxB1* + *rstREI* + *rstRCl* + *rstC* + *TLC*- *tr4*) and sequence type and belonged to one genotype. Analysis of PFGE-patterns revealed seven genotypes (two cluster formed and five unique). We observed significant association between PFGE genotypes and individual foci within outbreak. Obtained DNA sequences have been deposited in GenBank under accession numbers HM366179, HM590455-56 (*ctxB*) and JN571738, JN579649-54 (*dnaE*).

PFGE-profiles polymorphism shows that clonal structure of *V. cholerae* eltor strains is heterogeneous. It may be associated with multiple importations of pathogen and forming unlinked foci of infection.

#### **P1408** Characterisation of invasive *Listeria monocytogenes* isolates in Poland, 1996–2010

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**Objectives:** *Listeria monocytogenes* is the causative agent of invasive listeriosis that affects mainly newborns/neonates, pregnant women, the

elderly and immunocompromised patients. The aim of the study was to obtain the antimicrobial susceptibility data and serological profile of invasive isolates causing infections in Poland.

**Methods:** The study was performed on 196 invasive *Listeria monocytogenes* isolates (one isolate per patient) from 68 medical centres located in different parts of Poland, collected in the National Reference Centre for Bacterial Meningitis (NRCBM) between 1996 and 2010. All clinical isolates were identified according to standard procedures. PCR reactions were run to confirm species identification, serotype determination, and affiliation to epidemic clones (ECI, ECII or ECIII) according to protocol described by Chen et al. (2007) and Borucki et al. (2003). MICs were determined for 10 antimicrobials (ampicillin, penicillin, gentamicin, meropenem, co-trimoxazole, erythromycin, tetracycline, levofloxacin, vancomycin and rifampin) by the broth microdilution method and interpreted according to the EUCAST, BSAC and SFM guidelines.

**Results:** All but one isolates were grouped into three serotypes: 4b (59.2%), 1/2a (32.0%) and 1/2b (8.2%). The EC markers were found in 106 (54.0%) isolates tested. The most common represented was ECII (36.0%), followed by ECI (16.5%) and ECIII (1.5%). All the isolates were susceptible to ampicillin, penicillin, gentamicin, meropenem, erythromycin, levofloxacin, vancomycin and rifampin. Only one isolate (0.5%) showed resistance to tetracycline (MIC = 64 mg/L) and according to new co-trimoxazole breakpoints proposed by the EUCAST, 15 isolates (7.7%) with MIC values of 0.06 mg/L were resistant to this antibiotic.

**Conclusions:** Most of the invasive *Listeria monocytogenes* isolates in Poland belonged to the epidemic clones responsible for food-borne outbreaks worldwide. Almost 60% of isolates represented the serotype 4 b. Proposed by the EUCAST new breakpoints for *L. monocytogenes* reinforce the need for microbiological surveillance to determine adequate treatment of invasive listeriosis.

#### **P1409** Characterisation of six clinical isolates representing a novel *Actinomyces funkei*-variant

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**Objectives:** The genus *Actinomyces* comprises 37 described species but clinical significance of some recently described species still has to be documented. We isolated Gram-positive, non-sporeforming branched rods from six clinical specimens. On the basis of partial 16S rRNA gene sequence analysis, the strains were presumptively identified as *Actinomyces funkei*. However, further analysis of the entire 16S rRNA gene revealed significant differences between all six investigated strains and the *A. funkei* type strain indicating that the strains represent either a variant of *A. funkei* or a novel *Actinomyces* species.

**Methods:** The strains were isolated from hospitalized patients from Basel, Switzerland, between 2007 to 2010. They were characterized phenotypically and with routine biochemical methods. Partial and complete 16S rRNA gene sequences of the six isolates were determined and compared with each other and to the sequences deposited in MicroSeq and GenBank databases.

**Results:** Three strains were isolated from abscesses (gluteal, liver and vulva), two from deep wound swabs (epididymis and navel), and for one strain no clinical data were available. All isolates were facultatively anaerobic and catalase-negative. The identification by means of API Coryne kit gave the identification result *Arcanobacterium haemolyticum* for two strains and no identification for the remaining four strains. Species identification on the basis of partial 16S rRNA gene sequence comparison allocated the strains to *A. funkei*. However, sequencing of the whole 16S rRNA gene (HQ906497) showed 12 mutations/gaps compared to the *A. funkei* type strain AJ404889 which is consistent with 99.2% identity. Full 16S rRNA gene sequences from our six isolates were 100% identical to each other. Three strains have been deposited at CCUG Culture Collection in Göteborg, Sweden.



**Conclusion:** All strains were isolated from deep materials and involved in suppurative processes including abscess formation. The isolates could not be identified with routine biochemical methods. The entire 16S rRNA sequence differences between our investigated strains and the type strain of *A. funkei* indicate a variant of *A. funkei* which may represent a novel *Actinomyces* species. This has to be clarified with further taxonomic tools such as DNA-DNA hybridization analysis.

**P1410 Evaluation of a monitoring system for nosocomial pathogens in a burn centre by three molecular typing methods**

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**Introduction:** Burn wound patients are continuously at increased risk of developing infection by endogenous and exogenous pathogens. Early recognition of colonization and concomitantly typing of the colonizing strain may provide early infection prevention.

**Objective:** To determine a suitable monitoring program for infection prevention purposes of *Staphylococcus aureus* (SA) and *Pseudomonas aeruginosa* (PA) strains in a burn centre using molecular typing methods.

**Methods:** During a six-months period all SA and PA strains from 136 patients admitted at the Burn Centre, were isolated from the nose, throat, and perineum on admission and from wounds and infection sites on admission and thereafter twice a week. All strains were typed using High Throughput MLST (HiMLST) using NGS, AFLP and Raman spectrometry (RS) (SA only). Clustering of patients was defined as a group of at least two patients carrying an identical SA or PA strain.

**Results:** Using HiMLST, 12 clusters of patients carrying SA with unique sequence types (ST) could be defined. These ST-clusters comprising 2–8 patients can be subdivided into one to five AFLP types per cluster. This results in an average of 2.6 AFLP types per ST-cluster. If AFLP is used to cluster patients, these clusters can be subdivided into one to three ST per cluster. The number of ST per AFLP cluster is, however, lower than the opposite: 1.5. This implies a significantly higher discriminatory power of AFLP in contrast to HiMLST ( $p = 0.0185$ ). Comparable results were found using RS instead of AFLP although its discriminatory power is even higher.

Only three ST-clusters of patients with PA were found in which only one comprised two AFLP types. Conversely, both AFLP-clusters contained only one ST.

**Conclusions:** A high diversity of SA infections with a notable number of possible transmissions based on both HiMLST and, to lower extend, on AFLP and RS respectively, was found. Since AFLP and RS clusters can contain more than one ST, use of them in a monitoring system can result in a lower positive-predicted value to trace an outbreak. Moreover, AFLP data are prone to day-to-day variation and RS require strict standardized culture methods. HiMLST data however, can be stored easily without loss of quality and can be compared to custom-made and international databases.

In our view, the combination of HiMLST for continuously monitoring transmission and AFLP for occasional outbreak management is a feasible and reliable system for infection prevention.

**P1411 The ex-unibus plurum evolutionary concept applied to understand the dynamic of epidemics**

M.-C. Turrientes\*, F. Baquero, A. Ripoll, M.-R. Baquero, R. Cantón, J.-C. Galán (Madrid, ES)

**Objective:** We investigate the adaptive changes in metabolic functions that occur when a homogeneous population (clonal expansion in epidemics) begins to diversify.

**Methods:** A clone from an *Escherichia coli* clinical strain (homogeneous population) was submitted to serial passages during 200 days in unstructured environment. Every day, the culture was diluted 1:100 in fresh medium giving rise to evolved population during

1500 generations. After 1000 generations six evolved clones were chosen and biochemical profiles analyzed. Genomic DNA from these evolved clones was extracted, XbaI-digested and DNA fragment separated in CHEF-DRIII system. In addition, genomic RNA was obtained in order to determine global gene expression profiles in the evolved clones respect to ancestral clone using the AffymetrixGenechip technology. We analyzed the gene expression in only two metabolic pathways (tricarboxylic acid cycle, TCA, and glycolysis/gluconeogenesis) and repair pathways involved in the generation of adaptive mutations.

**Results:** PFGE and biochemical profiles of all the isolates were exactly the same suggesting that the evolved clones were identical to ancestral clone and the original population remained homogeneous. However, the global gene expression profiles yielded unexpected results. The differences in the number of genes with altered expression respect to ancestral clone ranged between 8.9% and 36.6%, suggesting different evolutionary trajectories in the adaptive process. The gene expression in TCA showed two different patterns suggesting two different subpopulations. Four clones showing the same TCA-pattern could be discriminate in two glycolysis/gluconeogenesis-patterns and each of these patterns could be differentiate in two patterns when the profile of repair pathways were compared. Therefore transcriptomic analysis of six clones with identical PFGE and biochemical profile allowed us to identify, at least, five different subpopulations derived of single ancestral clone coexisting in the same environment.

**Conclusion:** During the clonal expansion process of a winner clone in an epidemic, the initial homogeneous population explores all adaptive possibilities, increasing its survival probabilities and decreasing the possibility to be eradicated. When the population is homogeneous at the beginning of epidemics (ex-pluribus unum), the winner clone must be easier eliminated than when the same clone is diversified in multiples subpopulations (ex-unibus plurum).

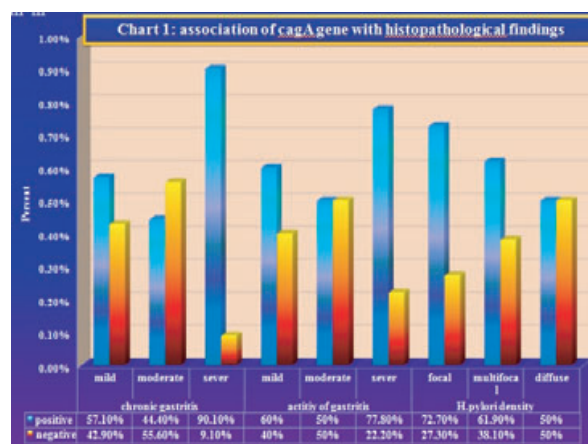
**P1412 Relation of clinical and histological finding with prevalence of *cagA* gene of *H. pylori* in Iranian population**

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**Background/Objective:** the aim of this study was to determine the relation among the cytotoxin associated gene (*cagA*) status of *Helicobacter pylori* isolates, the associated clinical diseases, and histopathological features of gastric disease in Iranian populations.

**Methods:** DNA was extracted from paraffin embedded gastric biopsies obtained from dyspeptic patients, and the *cagA* status determined by the PCR. The prevalence of *cagA* gene in three clinical groups, gastritis, gastric ulcer, and gastric malignancies was compared. The histological features in sections from antral and corpus biopsies were graded according to Sydney classification system criteria. The grades were compared with *cagA* gene status, and with clinical outcomes.

**Results:** Isolates from 86 patients were included. Forty-six (63.9%) were *cagA* positive. The prevalence of *cagA*+ strains in peptic ulcer



patients (43.5%) was rather greater than in those with gastritis (30%). Chronic inflammation, activity of gastritis, *H. pylori* density were all significantly more severe in the cagA+ than in the cagA- group (chart1).

**Conclusions:** *Helicobacter pylori* strains showing cagA positivity genotype are associated with infiltration of inflammatory cells in to gastric mucosa and accumulation of bacteria in infected area. but this virulence factor do not appear to determine the overall pattern. The pattern is closely linked to clinical disease. Therefore, the nature of the disease is determined by host, environmental factors, and bacterial factors determine the risk of developing such disease.

## Good to know: drug potency, purity, prescribing, errors and toxicity

### PI413 More potency assay results for EX-USA generic piperacillin/tazobactam lots and initial meropenem generic lots marketed in the USA

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**Objective:** To further assess piperacillin/tazobactam (P/T) generic lots in EX-USA nations and to initiate screening of meropenem (MER) generic lots recently (2011) marketed in the USA. P/T potency results expand prior experience reported in 2008 and 2009, performed by a precise, incremental MIC assay as published by Jones et al. (2008).

**Methods:** An additional 15 P/T generic lots (eight manufacturers; marketed in India, Chile, United Kingdom, and Sweden) were analyzed as part of an ongoing worldwide (EX-USA) screen that now includes results from 61 generic product lots (through 12/2010). Each lot was directly compared to a reference branded (Zosyn; Pfizer) lot or RBL using a previously described and validated assay method. MER lots (Hospira and Sandoz) from the USA were also tested and compared to a Merrem reference branded lot (TM0052; expirations of lots ranged from 06/2012–02/2013).

**Results:** The results (in 2010) of 15 P/T generic lots supplement reports of 46 other lots tested from EX-USA nations published in 2008 and 2009. Vials ranged from 2.25 to 4.5 g each and all were tested within labeled expiration dates. Orchid (three lots), Aurobindo (one lot), Libra (one lot), Wockhardt (two lots), Hospira (two lots), Sandoz (two lots), Fresenius-Kabi (two lots) and Stragen (two lots) generic products were assayed. Variations compared to P/T RBL were -23% to +3% (average, -10%; prior 46 lot experience was -16%). USA MER generic lots (Hospira [four lots], Sandoz [two lots]) exhibited potencies equal to Merrem RBL, without any significant variation, see Table.

Table Meropenem assay (MIC) results using branded (Merrem®) and five generic lots marketed in the USA.\*

Meropenem sample (exp. date)	Replicate MIC (µg/ml) for			
	<i>P. aeruginosa</i> ATCC 27853	<i>P. aeruginosa</i> 4-48716A	<i>E. cloacae</i> 106-49157A	<i>S. aureus</i> 104-37827A
Merrem TM0052 (06/2012)	0.313	0.156	0.045/0.038	0.045
Hospira 609A029 (06/2012)	0.313	0.156	0.045	0.045
Hospira 609B013 (12/2012)	0.313	0.156	0.038	0.045
Hospira 609B020 (12/2012)	0.313	0.156	0.038	0.045
Sandoz CA0710027A (04/2012)	0.313	0.156	0.038	0.045
Sandoz BN1589 (02/2013)	0.313	0.156	0.038	0.045
Variation (%)	(0.0)	(0.0)	(0.0)	(0.0)

a. Triplicate testing of four selected assay strains (3 wildtype [WT] by methods described earlier [Jones et al. 2008, Moet et al., 2010 and Sutter et al., 2011]. Vial strength was 0.5 or 1.0 gram for each product.

**Conclusions:** P/T generic lots marketed outside of the USA continue to demonstrate sub-optimal activity averaging 10–16% less activity when compared to the RBLs. Some lots, however, show comparable or acceptable activity. MER lots, FDA-approved for use in USA, exhibited equal activity compared to Merrem via this validated in-vitro assay

method. Hospital formulary practices should consider these documented differences between lots as well as between generic and branded products when making therapeutic choices.

### PI414 Particulate matter in combined intravenous antibiotics

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**Introduction:** The manufacturing processes to combine antibiotics are typically more complicated than those of a single one. Variation in particulate contamination was reported earlier in multi-source IV antibiotics.

**Objectives:** To study the powder characterization and particulate matter in combined IV antibiotics, collected from the Southeast Asia region including products of ampicillin/sulbactam (A/S), amoxicillin/clavulanate (A/C), cefoperazone/sulbactam (C/S), and piperacillin/tazobactam (P/T).

**Method:** This was a double blinding study. Powder characterization was investigated using scanning electron microscope (SEM). Two laboratory methods were applied to investigate the contamination of particulate matter after reconstitution/storage, that is, microscopic (SEM) and electrical sensing zone methods. The samples of A/S, A/C and C/S were freshly reconstituted with particle-free water before testing. Only P/Z samples were dissolved in acidic particle free water with spike zinc concentration and stored for 24 hour at room temperature before analysis, based on the previous US pharmacopoeial recommendation.

**Results:** There were 16 commercially available products, collected during 2010–2011, that is, A/S (n = 3), A/C (n = 6), C/S (n = 3) and P/Z (n = 3). Most of combined antibiotics were manufactured by dry-fill sterile blend, except for one of the P/Z products that was made by lyophilization in primary vials. Difference in particle burden was found among samples, esp. for A/S, A/C and P/Z. The microscopic method revealed heavy load of the subvisible particles with sizes smaller than the routine quality control limit, that is, <10 µm for some samples of A/C and P/Z. The finding supported the necessary application of buffer and chelating agent in P/Z formulation. However, the sizes and shapes of the particles found in A/C samples revealed that they were unlikely to be the incomplete dissolving drugs. Most of the samples were complied with pharmacopoeial standard, that is, the numbers of particles with sizes ≥25 and ≥10 µm were not more than 600 and 6000 particles, respectively.

**Conclusion:** The commercially available products of combined antibiotics A/C and P/Z from Southeast Asia region were different in particulate matter, esp. with sizes <10 µm. The serious load of these small particles could possibly lead to inferior clinical safety/efficacy when used in critically ill patients with microvascular compromise.

### PI415 Error analysis in prescribing and administration of intravenous anti-infective therapy with standard infusion pump technology

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**Objectives:** The study was conducted to gain a better understanding of the nature of errors that happen during the prescribing and administration of continuous/intermittent intravenous (IV) anti-infective therapy with standard infusion pump technology. The aim was to characterize the different types of errors and find out the level of adherence to the hospital IV anti-infectives prescribing and administration protocols, The Society of Australian Hospital Pharmacists of Australia (the SHPA) Australian Injectable Drugs Handbook as well as international prescribing guidelines. Further we assessed preventability with smart pump technology.

**Methods:** Within a 3-months-period data was collected at the University Hospital of Frankston, Australia. Three data sources were reviewed: the doctor's handwritten prescription, the IV medication

additive label on the infusion bag and the displayed rate and volume on the infusion pump. Errors in terms of deviations (i.e. non-adherence) to protocols/guidelines and administration as well as formal prescribing errors (e.g. incomplete order/missing instructions) were defined. Each observed IV medication could contain one or more errors; each error was documented and evaluated independently. Data collection and evaluation were conducted by two different persons to ensure objective decisions. Preventability using the smart pump technology was determined based on a retrospective evaluation.

**Results:** IV medication errors occurred frequently and were widespread: almost half (42 of 100) anti-infectives prescribed with standard infusion pump technology were associated with errors. Errors happened with nearly all anti-infectives used. Non-adherence to protocols in prescribing or administration was identified as the most common source for errors (n = 35) followed by administration errors (n = 12). Thirty-two out of 47 (68%) non-adherence to protocols or administration errors were considered to be preventable if smart pump technology had been implemented. Details are outlined in Table 1.

Anti-infective Agents	No. Infusions	No. Infusions with Error(s)	Error Details			
			No. Non-Adherence to Protocol	No. Administration Error(s)	No. Prescribing Error(s)	No. Preventable with Smart Pump Technology
Aciclovir	7	2		2		
Amikacin	5	2	2		1	3
Benzylpenicillin	1					
Cefepime	2					
Ceftriaxone	4	1	1			1
Cefuroxime	7	4	3	1		2
Ceftazidime	2	2	4			2
Fluconazole	4	2	2			2
Flucanazole	4	2		2		
Miconazole	1					
Miconazole	5	2	1	1		1
Penicillin	2	1	1			1
Piperacillin-Tazobactam	1					
Ticarcillin-Clavulanic acid	13	10	9	2		9
Vancomycin	42	14	12	4	2	11
Total No.	100	42	35	12	3	32

TABLE 1. Anti-infective Agents Administered with Standard Infusion Pump Technology and Associated Errors

NOTE: \* More than one error type possible

**Conclusion:** Errors happened frequently with the use of standard infusion devices. Overall, non-adherence to the protocols was shown to be a major issue with rate deviations occurring most commonly. Administration was identified as the stage in the medication-use process that is most susceptible to errors. The most severe errors were those likely to be reduced by the implementation of smart pump technology.

#### P1416 Fluoroquinolones and QTc prolongation in the hospitalised population: first results from a case-series ongoing study

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**Objectives:** Although the effect of fluoroquinolones in QTc has long been described in either animal models, healthy volunteers or case reports, there are only sparse data regarding the clinical significance of such an effect in the hospitalized population. This prospective clinical study was designed in order to compare this effect in a hospitalized population, to identify the risk factors that potentially influence it and to clarify its clinical significance.

**Methods:** One hundred and seven patients receiving moxifloxacin, levofloxacin and ciprofloxacin were included in the study. QTc interval was recorded upon admission and at 72 hour of fluoroquinolones' initiation. The difference between the two prices was calculated. Patients' clinical characteristics, baseline renal and liver function, fluoroquinolone dosage and site of infection were registered. Renal function was reevaluated at 72 hour. Any cardiac events during hospitalization were recorded. Data analysis was performed with SPSS 17.

**Results:** Median age was 69 years. There were 56 men (52.3%). Nineteen Patients suffered from chronic kidney disease (17.8%). Twenty three patients (21.5%) already received medications known to interfere with QTc. Fifty-two patients were treated with levofloxacin, 35 with ciprofloxacin and 15 patients with moxifloxacin. QTc difference at 0 and 72-hour was estimated at 15.5 ms, which is statistically significant ( $p < 0.001$ ). QTc difference at 0 and 72-hour was estimated at 12.61 ms for the levofloxacin group, 16.93 for moxifloxacin and 19.34 for ciprofloxacin. Despite a tendency towards a longer prolongation for ciprofloxacin, no statistical significance was reached ( $p = 0.08$ ). Interestingly, patients with chronic kidney disease showed a shorter QTc prolongation (10.93 ms) than those with normal renal function (18.56 ms). Only two patients experienced supraventricular arrhythmia. No other serious adverse event was recorded.

**Conclusion:** Our data show that QTc was significantly prolonged in all patients under fluoroquinolones. However, only a trend toward a longer prolongation for ciprofloxacin was implied. Gender, age and preexisting cardiac disease did not influence this effect. In patients with normal renal function this phenomenon was more pronounced. QTc prolongation was not translated in adverse cardiac events, rendering fluoroquinolones a safe option for these predisposed, at-risk patients. As these are the first results from an ongoing study, caution is warranted before safer data can be extracted.

#### P1417 Effect of a suprathreshold dose of ceftazidime avibactam on the QTc interval in a thorough QT study

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**Objectives:** Avibactam is a non-beta-lactam beta-lactamase inhibitor that has been shown to restore the in vitro activity of ceftazidime against extended-spectrum beta-lactamase-producing pathogens. A key aim of this study was to assess the potential effects of a suprathreshold dose of ceftazidime avibactam (CAZ-AVI) on cardiac repolarisation in a thorough QT (TQT) study.

**Methods:** This was a double-blind, randomised, placebo-controlled, 4-way crossover study in healthy males (n = 51; age 18–45 year; BMI 19.4–30.0 kg/m<sup>2</sup>). Each subject received, in randomised order: CAZ-AVI 3000 mg/2000 mg infused over 30 minute (following a 30-minute placebo [saline] infusion for blinding); ceftaroline fosamil avibactam 1500 mg/2000 mg infused over 60 minute (split into two infusion bags administered sequentially for 30 minute each); placebo (administered as for ceftaroline fosamil avibactam); and a single open-label oral dose of moxifloxacin 400 mg as a positive control for assay sensitivity. All treatments were separated by ≥3 days washout. Electrocardiogram (ECG) readings and safety were assessed. This abstract focuses on the CAZ-AVI ECG data. The least square mean differences vs. placebo in change in the Fridericia-corrected QT interval (QTcF) from baseline for CAZ-AVI at 10 time points over 24 hour were calculated with two-sided 90% confidence intervals (CIs). In order to conclude that there was no observed effect on the QTcF interval, all upper confidence bounds were required to be <10 ms. To determine assay sensitivity, the difference between moxifloxacin and placebo for the mean change from baseline in QTcF over the 1 to 4-hour post-dose interval was estimated, and the lower bound of the 90% CI was evaluated against a 5 ms threshold.

**Results:** In total, 43 subjects completed the study. Assay sensitivity was confirmed as the lower bound of the 90% CI for the difference between moxifloxacin and placebo for the mean change from baseline in QTcF over 1–4 hour was >5 ms (7.3 ms). In addition, the upper bound of the 90% CI for the difference between CAZ-AVI and placebo in the mean change from baseline in QTcF did not exceed 10 ms at any time point (Table). No QTcF interval was >450 ms nor were any QTcF interval changes from baseline >30 ms. There were no differences in change in heart rate, RR, PR, QT and QRS intervals for CAZ-AVI vs. placebo.

Time point (h)	Least square mean difference (90% CI) in change-from-baseline QTcF for CAZ-AVI vs placebo										
	0.5	1.0	1.5	2.0	3.0	4.0	6.0	8.0	12.0	24.0	
LS mean difference (90% CI) (ms)	0.7 (-1.1, 2.5)	4.1 (2.3, 5.9)	1.0 (-0.8, 2.8)	0.9 (-0.9, 2.7)	0.1 (-1.8, 1.9)	-0.5 (-2.3, 1.3)	1.1 (-0.8, 2.9)	1.2 (-0.6, 3.0)	-0.6 (-2.4, 1.3)	1.1 (-0.7, 2.9)	

**Conclusions:** This TQT study showed no observed effect on the QT interval for a supratherapeutic dose of CAZ-AVI.

**P1418** Characterisation of the mechanism of nicotinic acetylcholine receptor inhibition that is likely linked to the off-target activity by telithromycin

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**Objectives:** Previous studies carried out with telithromycin at the nicotinic acetylcholine receptors have clearly illustrated that the pyridine moiety in the side-chain of telithromycin inhibits the alpha 7 and alpha3beta4 receptors. Similarly, the visual effects of voriconazole led us to the characterization of the inhibition of alpha3beta4 nAChRs by its heterocyclic N in the pyrimidine side chain. The aim of this study was to examine the mode of action of telithromycin at the human alpha7 and alpha3beta4 nAChRs.

**Methods:** Electrophysiological studies were conducted using expression of human nAChRs in *Xenopus* oocytes. ACh dose-response curves were obtained in the absence or presence of a fixed concentration of telithromycin to determine the mode of action of telithromycin. Competitive antagonists are characterized by the fact that blockade caused by the antagonist can be surmounted by the appropriate increase in the agonist concentration. On the contrary, non-competitive antagonists are characterized by the fact that blockade is insurmountable.

**Results:** Data obtained for alpha3beta4 with 2 μM telithromycin suggests that telithromycin might have a dual action with competitive and non-competitive inhibition. The dual mode of action of telithromycin was confirmed by examining the time course of the ACh response measured at a low ACh concentration (10 μM) and at a high ACh concentration (1280 μM). Inhibition caused by telithromycin is not accompanied by a modification of the response time course at 10 μM ACh, whereas a profound modification of the decay time was observed at the high ACh-concentration. The difference in the response time course, with a faster decay time observed at ACh concentrations >160 μM, indicates that inhibition is not caused by competition only, but that telithromycin probably enters the channel pore and blocks ionic conduction by steric hindrance. Exposure of cells expressing the human alpha7 to telithromycin (20 μM) causes a shift in the concentration activation curve towards higher ACh concentrations indicative of a competitive inhibition of alpha7. Similarly to alpha3beta4 at high ACh concentrations (>600 μM), telithromycin causes an additional inhibition probably due to open channel blockade.

**Conclusions:** Mechanistic characterization of the side effects of drugs helps to optimize the side-effect profiles of drugs in development. These studies can mechanistically differentiate new macrolides/ketolides from telithromycin.

## New antibacterial agents under investigation

**P1419** GSK2251052, a novel, leucyl tRNA synthetase inhibitor, is present in the epithelial lining fluid and alveolar macrophages following intravenous dosing to healthy adult volunteers

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**Objectives:** To evaluate in healthy volunteers, the plasma and intrapulmonary pharmacokinetics (PK) of intravenous GSK2251052,

a novel, boron-containing antimicrobial in clinical development for treatment of serious Gram-negative bacterial infections.

**Methods:** In an open-label study, 30 healthy adult subjects underwent bronchoscopy and timed bronchoalveolar lavage (BAL) following either a single intravenous dose of GSK2251052 1500 mg (Cohort 1) or on the morning of days 3 after 5 intravenous doses of 1500 mg twice daily (Cohort 2). Serial PK samples and safety assessments were obtained throughout the study. Bronchoscopy was performed at a single time in each subject at 2, 6, or 12 hour after the start of the 60 minute infusion. Non-compartmental PK analysis was performed for calculation of PK parameters.

**Results:** Thirty subjects completed the study. The mean CL, V<sub>ss</sub>, and t<sub>1/2</sub> values were ~22 L/hour, 231 L, and 10.7 hour, respectively. Approximately 30% of the 1500 mg GSK2251052 dose was excreted unchanged in urine after single dose administration. GSK2251052 concentrations in epithelial lining fluid (ELF) and alveolar macrophage (AM) were ~50%, and 500–600%, respectively, compared to plasma. The most frequently reported drug-related AE was mild to moderate infusion site reactions (ISR, seven subjects) occurring primarily in the repeat dose cohort. No serious drug-related AEs occurred and no clinically significant trends in laboratory values, vital signs, or ECGs were observed.

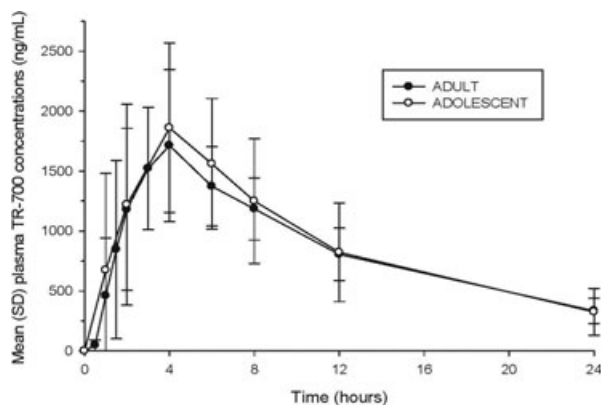
**Conclusion:** GSK2251052 given as a 1500 mg infusion was generally tolerated following single or repeat dose administration. GSK2251052 distributes into both the ELF and AM of healthy volunteers, which supports further study in patients with pneumonia.

**P1420** A phase 1, open-label, multicentre, single-dose, pharmacokinetic, safety and tolerance study of oral tedizolid phosphate in 12–17 year old patients

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**Objective:** Community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) causes significant morbidity in children. Tedizolid phosphate (TR-701 FA) is a second-generation oxazolidinone antimicrobial agent, with enhanced in-vitro activity against CA-MRSA, and preliminary evidence suggesting decreased hematopoietic toxicity. Phase 2 studies of tedizolid phosphate in adults with acute bacterial skin and skin structure infection (ABSSSI) have established a favorable antimicrobial exposure associated with efficacy and safety. Investigation of single-dose tedizolid phosphate in adolescents was designed to establish the dose that provides an exposure equivalent to that currently used in adults.

**Methods:** Medically stable adolescent patients aged 12 through 17 years, receiving concurrent systemic antibiotic treatment, were eligible for participation. A single dose of 200 mg tedizolid phosphate was administered by mouth; plasma was obtained predose (0 hour) and 1, 2, 4, 6, 8, 12, and 24 hour postdose to assess concentrations of tedizolid phosphate and its active metabolite, tedizolid (TR-700), by tandem mass spectrometry. PK parameters of tedizolid were determined by WinNonlin.



Clinical and laboratory (hematology, serum chemistry, urinalysis) safety was assessed pre- and postdose.

**Results:** The population was 90% male with a mean age of 14.8 years and mean BMI of 22.9 kg/m<sup>2</sup>. Pharmacokinetic evaluation of drug disposition in adolescents in this study (n = 10) documented similar tedizolid PK results with those seen in adults receiving 200 mg of tedizolid phosphate in a previous study. Mean (SD) AUC<sub>0-inf</sub> values of 25.2 (9.2) and 26.7 (6.0) µg hour/mL and mean C<sub>max</sub> values of 2.2 (0.5) and 1.9 (0.4) µg/mL were observed in adolescents and adults, respectively.

No drug-attributable clinical or laboratory adverse events were noted on our small cohort of adolescents.

**Conclusions:** Two hundred milligram of oral tedizolid phosphate provided an antimicrobial exposure equivalent to adults given the same dose, with no safety issues noted in these 10 adolescents. For clinical investigation of ABSSSI in adolescents, 200 mg of oral tedizolid phosphate should provide an exposure that has been linked to clinical efficacy in adults.

**P1421 Phase I (multiple ascending dose) study with the novel *Pseudomonas aeruginosa* antibiotic POL7080 in healthy volunteers**

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**Objectives:** POL7080 is a novel PEM (Protein Epitope Mimetics) antibiotic selectively targeting *Pseudomonas* species with demonstrated potent in vitro activity and in vivo efficacy in murine infection models. A multiple ascending dose (MAD) study was conducted to evaluate safety, tolerability, plasma pharmacokinetics (PK) and urinary excretion.

**Methods:** Twelve healthy male subjects, aged 18–40, were randomised and participated in a double blind, placebo-controlled study with multiple ascending doses. Each of the two dose groups consisted of six subjects which were randomised, four to receive POL7080 and two to receive placebo. POL7080 was administered as multiple 3 hour infusions of 1 mg/kg twice daily (bid) 12 hour apart (cohort 1) or as multiple 3 hour infusions of 2 mg/kg three times a day (tid) 8 hour apart (cohort 2). Plasma concentrations of the drug were determined by LC-MS/MS analysis and interim (using nominal time) PK parameters were calculated using WinNonlin®.

**Results:** The mean plasma concentration-time profiles of POL7080 both following multiple dose administrations were characterized by an increase during the 3 hour infusion period followed by a multi-phasic decline. By visual inspection of trough (pre-dose) values following multiple bid or tid administration of POL7080, steady-state was considered to have been reached on Day 2. The mean accumulation ratio based on C<sub>max</sub> (Rac, C<sub>max</sub>) or AUC<sub>tau</sub> (Rac, AUC<sub>tau</sub>) was 1.0 or 1.1 following 1.0 mg/kg bid administration and 1.2 or 1.5 following 2.0 mg/kg tid administration. Following multiple dose administration for 6 days (at steady-state), POL7080 was excreted in urine with a mean concentration of 7.57 mg/L and a mean CLR of 79.3 mL/hour. No serious adverse events (SAEs) were reported for either dose group and all AEs were mild and not prohibitive to dose increases. Blood chemistry and clinical laboratory results were normal during dosing and at follow up, indicating that POL7080 was well tolerated in both dose groups.

**Conclusions:** Multiple doses of POL7080 were well tolerated at plasma concentrations expected to meet or exceed efficacious levels and no serious adverse event was reported. The PK of POL7080 showed no accumulation following 6 days twice-daily or three times a day dose administration by intravenous infusion.

**P1422 Metabolic stability of PTK 0796 (omadacycline)**

I. Hanna, H. Sun, B. Zhu, H. Gu, L. Wang, R. Pelis, E. Heidi, D. Chun, S.K. Tanaka\* (East Hanover, Boston, US)

PTK 0796 (PTK, omadacycline) is a novel aminomethylcycline now in Phase 3 clinical development. An in vitro assessment of the potential for metabolism and/or drug-drug interactions was undertaken.

**Objectives:** The in vitro stability and interaction of PTK with human cytochrome P450 isozymes was determined to assess the potential for in vivo modification or whether there was a significant potential for drug-drug interactions.

**Methods:** Metabolism assays were conducted using either pooled human liver microsomes preparations, S9, liver cytosol, or recombinant flavin monooxygenases (FMO1, FMO3, FMO5) (BD Bioscience, Woburn, MA). Metabolism of 14C-PTK (5–50 µM) was determined with either NADPH or UDPGA or a combination of both co-factors. Binding of 14C-PTK to liver microsomes was determined by ultracentrifugation. The metabolism of 14C-PTK by human hepatocytes (Celsis, Baltimore, MD, USA) was tested at 2.5 and 12.5 µM with 2 × 10<sup>6</sup> cells/mL at 37°C for 2–24 hour. PTK and metabolites were detected by HPLC with radio-detection. CYP450 induction was determined in primary human hepatocytes (1 × 10<sup>5</sup> cells) incubated with 1–100 µM PTK and substrate probe for 24 and 48 hour. Inhibition of CYP450 isozymes was determined using pooled human microsomes (BD Biosciences, Bedford, MA, USA) with PTK (1–50 µM) and probe concentrations approximating the Km of each probe. Time-dependent inhibition was determined by preincubating microsomes with 1–50 µM. Probe metabolism was determined by LC-MS.

**Results:** There was no detectable metabolism of PTK by human microsomes, hepatocytes, S9 or cytosol, FMO1, FMO3, or FMO5. PTK did not induce activities of CYP 1A2, 2B6, 2C8, 2C9, 2C19, or 3A. There was no or minimal (<40% of maximal positive control response) induction of mRNA for CYP 1A1, 1A2, 1B1, 2B6, 2C8, 2C9, 2C19, 2J2, 3A4, or 3A5. There was no significant inhibition of CYP 1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, or 3A4/5 activity by PTK without pre-incubation nor time-dependent inhibition of CYP 1A2, 2C9, 2D6, or 3A4/5. There was no significant binding to human microsomes.

**Conclusions:** In vitro studies indicate that PTK 0796 (omadacycline) is unlikely to undergo significant metabolism in humans. Further, there was no induction or inhibition of CYP enzymes indicating little potential for drug-drug interactions based on these mechanisms.

**P1423 A single-dose study to evaluate the pharmacokinetics, safety, and tolerability of multiple formulations of PTK 0796 in healthy subjects**

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**Objectives:** PTK 0796 (omadacycline) is a first in class aminomethylcycline antibiotic with activity against Gram-positive, Gram-negative, aerobes and anaerobes, and atypical bacteria. PTK 0796 is being developed for the treatment of Acute Bacterial Skin and Skin Structure Infections (ABSSSI) and Community Acquired Bacterial Pneumonia (CABP) with once daily IV followed by oral dose administration. The bioavailability of two oral formulations (tablets) relative to the IV was investigated to select an optimal oral formulation for Phase 3 Studies. An oral solution was also included as an exploratory investigation.

**Methods:** This was an open-label, randomized, four period, complete cross-over study in healthy subjects with four treatment conditions (PTK0796 100 mg IV infusion, two 300 mg tablet formulations with different dissolution profiles, and a 300 mg oral solution for comparison to the tablets). A total of 24 subjects between the ages of 18–50 were randomized to the treatment groups. Routine safety and tolerability assessments were performed. Analysis of plasma PTK 0796 concentration was performed using a validated LC/MS/MS method.

**Results:** Twenty subjects completed all periods of the study. No SAEs were reported and only three AEs of mild intensity (dizziness, nausea, vomiting) were experienced by three subjects during the study. There

were no clinically relevant changes in laboratory tests following dose administration. Among the oral formulations studied, the oral solution had the fastest rate of absorption as evident by the earlier  $T_{max}$ . Both 300 mg tablet formulations produced equivalent total exposure relative to the 100 mg IV dose (9960 hour ng/mL) with geometric mean ratios of AUCinf (90% CI) of 1.00 (0.93, 1.07) and 0.96 (0.90, 1.03), respectively. The absolute bioavailability of the tablets was ~34%. Compared to the tablets, the oral solution yielded 19% higher total systemic exposure. The inter-subject variabilities were consistent among the oral formulation groups (~20–25%).

**Conclusions:** The two 300 mg tablet formulations of PTK 0796 produced equivalent total exposure as measured by AUC relative to the 100 mg IV dose. Single doses of PTK0796 administered orally (as different formulations) and intravenously were safe and well tolerated in the subjects studied.

#### **P1424** Lack of interaction of PTK 0796 (omadacycline) with human transporter proteins

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PTK 0796 (PTK, omadacycline) is a novel aminomethylcycline in Phase 3 development as an IV and oral therapy for bacterial skin infections and community-acquired pneumonia.

**Objectives:** The in vitro interaction of PTK with human drug transporter proteins was determined to establish the potential for drug-drug interactions based on this mechanism of drug uptake and efflux.

**Methods:** Transport of 14C-PTK by human organic ion transporters was determined in HEK293 cells stably expressing hOAT1, hOAT3, or hOCT2 compared to the parental host cell line HEK-FIP-In. Transport of PTK by organic anion transport polypeptide transporters OATP1B1 and OATP1B3 was assessed similarly in HEK cells stably expressing these transporters. Transport of 14C-PTK by P-glycoprotein (P-gp) was determined in Caco-2 cells. Induction of P-gp and multidrug resistance-associated protein-2 (MRP2) was determined in human hepatocytes by mRNA levels. Inhibition of human Breast Cancer Resistance Protein (BRCP), P-gp, and MRP2 was determined in cell lines T8, T0.3, and MDCKII cell lines.

**Results:** At 25  $\mu$ M 14C-PTK, there was no difference in intracellular concentrations with or without hOAT1 or hOAT3. Probenecid did not reduce the accumulation of PTK (8  $\mu$ M) whereas there was a significant effect on the probe substrate. Uptake of PTK into cells was rapid, reaching more than 100 pmol/mg protein within 5 minute independent of transporters. PTK appears to be a substrate for P-gp, with a Km for efflux of ~81.5  $\mu$ M and a  $J_{max}$  of 1140 pmole/hour/cm. PTK did not inhibit the function of hOAT3 and at 25  $\mu$ M inhibited hOAT1 by only 30%. Intracellular accumulation of PTK was not affected by hOATP. PTK (100  $\mu$ M) reduced transport of probes for hOATP1B1 and hOATP1B3 by  $\leq$ 10.1%. PTK did not induce P-gp or MRP-2 mRNA. PTK did not inhibit the activity of BRCP, P-gp or MRP-2 up to 50  $\mu$ M. **Conclusion:** The potential for drug-drug interactions based on PTK interactions with drug transporters appears to be minimal. PTK does not interact with the transporters tested except P-gp. It is a substrate for P-gp (Km = 81.5  $\mu$ M) but is unlikely to act as an inhibitor (no inhibition up to 50  $\mu$ M) nor an inducer of P-gp.

#### **P1425** Mouse pharmacokinetics, dose fractionation and efficacy of TP-559, a novel tetracycline with anti-Pseudomonas activity, in murine infection models challenged with *Pseudomonas aeruginosa*

J. Sutcliffe\*, X. Xiao, R. Clark, T. Grossman (Watertown, US)

**Objective:** To determine pharmacokinetics (PK) in mice and to compare the efficacy of TP-559 to comparators in murine models of lung and thigh infections with *P. aeruginosa*.

**Methods:** PK evaluation of TP-559 over 24 hour was performed in CD-1 mice after 1 mg/kg intravenous (IV) administration using LC/MS/MS quantitation and NonWinLin. For an immunocompetent lung infection model, female BALB/c mice were infected intranasally with *P. aeruginosa* PA1145. At 2 and 12 hour post-infection, mice (n = 6) were treated IV with 40 mg/kg TP-559, tigecycline or amikacin; intranasal tobramycin (40 mg/kg) was also evaluated. Compound MICs were 4, 16, 4, and 0.5  $\mu$ g/mL respectively. Further, studies with 30 mg/kg IV TP-559 fractionated over 24 hour were performed. For all studies, mice were euthanized 24 hour post-treatment initiation and colony forming units (CFUs) per gram lung were calculated. For the thigh model, female CD-1 mice (n = 5) weighing 18–20 g were made neutropenic by administration of cyclophosphamide on Days-4 and -1. On Day 0, mice were inoculated into the right thigh with *P. aeruginosa* PA694. TP-559 and meropenem (MICs, 4 and 0.13  $\mu$ g/mL, respectively) were administered at 5, 15, and 40 mg/kg IV 2 and 12 hour post-infection. At 24-hour post-infection, the muscle of the right thigh of each mouse was harvested, homogenized, serially diluted and plated on Brain Heart Infusion agar + 0.5% charcoal for CFU determination.

**Results:** TP-559 was highly efficacious in the lung infection model, providing a 3.9-log reduction in CFUs at 40 mg/kg relative to 24-hour control. Amikacin and tobramycin reduced the CFUs in the lung by 3.6 and 4.9 logs, respectively; tigecycline did not protect. There was no difference in the log CFU reduction in lung (4.42  $\pm$  0.32) when a 30 mg/kg/day TP-559 dose was given QD, BID, TID, or QID. In the neutropenic thigh model, TP-559 at 40 and 15 mg/kg provided a 4.2 and 2.5 log CFU reduction from the 24-hour control respectively. The PK of 1 mg/kg IV TP-559 in mice produced a t<sub>1/2</sub>, AUC (0-t), and C<sub>max</sub> of ~5.6 hour, 1307 ng hour/mL, and 888 ng/mL, respectively.

**Conclusions:** TP-559 (40 mg/kg IV) had efficacy comparable to amikacin/tobramycin and was superior to tigecycline in a *P. aeruginosa* murine lung infection model regardless of regimen. TP-559 was as efficacious as meropenem in a neutropenic thigh model. The AUC(0-t) and C<sub>max</sub> of TP-559 in mice were 9.8- and 1.8-fold higher than that observed with a class comparator, 1 mg/kg IV tigecycline.

#### **P1426** The intravenous pharmacokinetics and efficacy of a novel tetracycline TP-433 in murine infection models challenged with *Pseudomonas aeruginosa*

Y. Deng, T. Grossman, R. Clark, X. Xiao, J. Sutcliffe\* (Watertown, US)

**Objective:** To determine the intravenous pharmacokinetics (PK) in mice and to examine the efficacy of TP-433 in mouse lung and thigh infection models challenged with *P. aeruginosa*.

**Methods:** Lung infection model: Immunocompetent female BALB/c mice were infected with cystic fibrosis isolate *P. aeruginosa* PA1145 (TP-433 MIC = 4  $\mu$ g/mL) via intranasal administration. At 2 and 12 hour post-infection, mice (n = 6) were treated intravenously (IV) with TP-433 (5, 15, or 40 mg/kg), tigecycline (40 mg/kg), or amikacin (40 mg/kg). Mice were euthanized by CO<sub>2</sub> inhalation 24 hour post-initiation of treatment and colony forming units (CFUs) per gram of lung were calculated. Thigh model: Groups of five female CD-1 mice were made neutropenic by administration of cyclophosphamide on Days-4 and -1. On Day 0, mice were inoculated into the right thigh with *P. aeruginosa* PA694 (TP-433 MIC = 4  $\mu$ g/mL). TP-433, tigecycline, and meropenem were administered at 5, 15, and 40 mg/kg IV 2 and 12 hour post-infection. At 24 hour post-infection, the muscle of the right thigh of each mouse was harvested, homogenized, serially diluted and plated on Brain Heart Infusion agar + 0.5% charcoal for CFU determination. PK evaluation (WinNonLin) of TP-433 and tigecycline were performed in CD-1 mice after 1 mg/kg IV administration and collection of eight plasma samples over 24 hour. TP-433 levels were quantified by LC/MS/MS.

**Results:** TP-433 was efficacious in the mouse lung infection model, providing a 1, 2, or 3-log reduction in CFUs at 3, 12 and 29 mg/kg, respectively, relative to 24-hour controls. Amikacin, a positive control, reduced bacterial burden in the lung by 3.55 log CFUs while tigecycline

reduced CFUs less than one log. In the neutropenic thigh model, TP-433 at 40 mg/kg provided a 2.2 log reduction from the 24-hour control counts while meropenem reduced bacterial burden  $\geq 4$  log CFUs at doses  $\geq 15$  mg/kg. The PK of 1 mg/kg IV TP-433 administered in mice produced a  $t_{1/2}$ , AUC(0-t), and  $C_{max}$  of  $\sim 3$  hour, 348 ng hour /mL, and 487 ng/mL, respectively.

**Conclusions:** TP-433 was efficacious in both *P. aeruginosa* infection models. In the lung model TP-433 was equipotent to amikacin at 40 mg/kg, and more potent than tigecycline at all doses. TP-433 reduced bacterial burden in the thigh to a lesser extent, and this result could have been due to the impact of neutropenia on TP-433's in vivo efficacy. The AUC of TP-433 in mice was 2.6-fold higher than that observed with 1 mg/kg IV tigecycline.

**P1427 TP-834, a novel IV and oral isoindoline-containing pentacycline, is highly efficacious in rodent models of pneumonia**

J. Sutcliffe\*, P. Hogan, C. Katz, W. Zhang, Z. Zhu, T. Grossman (Watertown, US)

**Objective:** TP-834 is a novel fully synthetic pentacycline antibiotic with activity against MDR community-acquired respiratory pathogens. These studies evaluated the efficacy of TP-834 vs. comparator antibiotics in various rodent lung infection models.

**Methods:** Neutropenic (NP) mouse lung infection: BALB/c mice were challenged with either tetracycline-resistant tet(M) *Streptococcus pneumoniae* (SP) strain SP160 or tet(M) *Staphylococcus aureus* SA191. Mice were made NP by pre-treatment with cyclophosphamide and infected with either pathogen via intranasal administration. At 2 and 12 hour post-infection, mice were dosed orally (PO) and intravenously (IV) with compound. At 24 hour post-initiation of treatment, mice were euthanized and bacterial colony forming unit (CFU) reduction in lung was quantified by plating lung homogenates. Non-NP mouse lung infection: CD-1 mice were infected with SP SP514 via IN and dosed PO or IV with compound at 5, 24 and 36 hour post-infection. At 48 hour post- initiation of treatment, mice were euthanized and lung CFUs were quantified. Non-NP rat lung infection model: Sprague-Dawley rats were infected with *Haemophilus influenzae* HI551 via intratracheal administration. At 5, 24, and 48 hour rats were dosed PO with compound. At 72 hour post-initiation of treatment, rats were euthanized and lung CFUs were quantified.

**Results:** NP SA191 model: TP-834 dosed IV at 1, 5, and 10 mg/kg produced -0.45, -1.69, and -2.7 log CFU reductions in lung, respectively; linezolid (LZD) at 10 mg/kg produced a -1.29 log CFU reduction. TP-834 dosed PO at 12.5, 25, 50 and 100 mg/kg produced -1.41, -2.58, -2.58 and -2.9 log CFU reductions, respectively; LZD at 30 mg/kg produced a -2.62 log CFU reduction. NP SP160 model: TP-834 dosed IV at 1, 5, and 10 mg/kg produced -0.85, -2.26 and -3.07 log CFU reductions, respectively; LZD at 5 mg/kg produced no CFU reduction. TP-834 dosed PO at 15, 30, and 60 mg/kg produced -1.29, -1.55 and -2.34 log CFU reductions, respectively; LZD at 30 mg/kg produced a -2.5 log CFU reduction. Non-NP SP514 model: When dosed orally at 30 mg/kg, TP-834 and LZD produced -6.14 and -3.56 log CFU reductions, respectively. Non-NP HI551 model: Dosed 100 mg/kg PO and 25 mg/kg IV, TP-834 produced -2.93 and -3.4 log CFU reductions, respectively; azithromycin at 50 mg/kg PO produced a -6.24 log CFU reduction.

**Conclusion:** TP-834 is highly efficacious in rodent lung infection models using IV and PO administration.

**P1428 TP-834, an isoindoline-containing pentacycline antibiotic, is orally bioavailable, metabolically stable and has low potential for drug-drug interactions**

T. Grossman\*, M. Ronn, N. Dunwoody, J. Sutcliffe (Watertown, US)

**Objective:** The goal of these studies was to evaluate the pharmacokinetics (PK) and metabolic stability of TP-834, a novel

pentacycline antibiotic with activity against MDR community respiratory and problematic Gram-positive pathogens.

**Methods:** PK: Groups of three male Sprague Dawley rats were given TP-834 1 mg/kg IV or 10 mg/kg PO. Three non-naïve cynomolgus monkeys were administered TP-834 1 mg/kg IV or 10 mg/kg PO. Plasma was sampled over 24 hour, TP-834 levels were quantified by LC/MS/MS, and PK parameters were calculated using WinNonLin. Metabolic Stability: TP-834 (1  $\mu$ M) was incubated with cryopreserved pooled human hepatocytes for 1 hour at 37°C. The disappearance of TP-834 over time was monitored by LC/MS/MS. Cytochrome P450 (CYP) enzyme inhibition and induction: Inhibition or induction of CYP enzymes was determined using CYP-selective substrates and LC/MS/MS detection. MDR1 efflux: The apparent passive permeability (Papp A>B) and potential transport (Papp B>A) of 1  $\mu$ M TP-834 in MDCK cell cultures over-expressing Multi-Drug Resistance 1 gene (*MDR1*) was measured by adding TP-834 to apical (A) or basolateral (B) sides of the cultures. TP-834 at 1 and 2 hour was quantified by LC/MS/MS.

**Results:** PK: TP-834 given IV and PO in rats produced areas under the curve inf (AUC(inf)) of  $3746 \pm 500$  and  $18\ 079 \pm 11\ 693$  ng hour/mL, respectively. Oral bioavailability was 48.3%. In monkeys, the IV and PO AUC(inf) values were  $9310 \pm 2201$  and  $35\ 433 \pm 19\ 111$  ng hour/mL, respectively. The oral bioavailability was 33.7%. Metabolic Stability: The  $T_{1/2}$  of TP-834 was  $>145$  minute, with a CL(int) of  $<4.78$   $\mu$ L/minute/ $10^6$  cells. CYP enzyme inhibition/induction: TP-834 inhibited CYP2C8 with an IC50 value of 46  $\mu$ M and CYP3A4/5 with an IC50 ranging from 140 to  $>200$   $\mu$ M. IC50 values were  $>200$   $\mu$ M for CYP1A2, CYP2B6, CYP2C9, CYP2C19, and CYP2D6. There was no evidence of either time- or metabolism-dependent inhibition of any CYP enzyme. TP-834 up to 20  $\mu$ M, did not induce CYP1A2, CYP2B6, and CYP3A4/5. MDR1 efflux: TP-834 was classified as having a low brain penetration potential, with a mean Papp A>B of  $1.69 \times 10^{-6}$  cm/second. The mean efflux Papp B>A was  $8.63 \times 10^{-6}$  cm/seconds.

**Conclusions:** The oral bioavailability, metabolic stability, lack of CYP inhibition and induction support further studies to advance TP-834 into clinical development as an IV/oral drug with low potential for drug-drug interactions.

**P1429 In vitro pharmacodynamics of AZD5206 against *Staphylococcus aureus***

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**Objectives:** AZD5206 is a novel antimicrobial agent under investigation, which is found to have potent in-vitro activity against *Staphylococcus aureus* (SA). To facilitate clinical development of this agent, the pharmacodynamics of AZD5206 against SA was evaluated in a hollow-fiber infection model (HFIM).

**Methods:** Time-kill studies (TKS) were conducted with a standard wild-type methicillin-susceptible (ATCC 29213) and a clinical strain of methicillin-resistant SA (SA 62). TKS were performed using  $4 \times$  MIC for 24 hour, and inocula used ranged  $\sim 1 \times 10^5$ - $7$  CFU/mL. In addition, a dose ranging and fractionation study was performed in a HFIM over 72 hour, simulating projected human pharmacokinetics ( $t_{1/2}$  8-12 hour). Serial total and resistant bacterial burden was determined in duplicate by quantitative culture, in media plates with and without AZD5206 supplementation ( $>3 \times$  MIC). Serial samples were also obtained from HFIM to verify simulated drug concentrations by liquid chromatography tandem mass spectroscopy. The concentration-time profiles were characterized by a 1-compartment open model.

**Results:** MIC was 0.0625  $\mu$ g/mL for both bacteria. In both TKS and HFIM, bacterial killing against a low baseline inoculum was more remarkable. Overall, low dosing exposures and/or high baseline inoculum resulted in early reduction in bacterial burden, followed by regrowth and selective amplification of resistant population. Dose fractionating studies indicated that dosing frequency had little impact of the microbiological response (suppression vs. resistance development), suggesting that AUC/MIC was the pharmacodynamic parameter most closely linked to antimicrobial effect. To control a bacterial population

( $10^6$  CFU/mL at baseline) for up to 72 hour, an AUC/MIC of ~140–210 (equivalent to a clinical dose of 2–3 g) daily would be necessary.

**Conclusions:** The antimicrobial effect of AZD5206 was found to be most closely linked to AUC/MIC or the daily dose used. Our results could be used to set a PK/PD target for future investigations.

**P1430** [14C]-delafloxacin (RX-3341): distribution and elimination studies in the rat

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**Background:** Delafloxacin is a quinolone with antimicrobial activity against gram-positive, gram-negative organisms, atypical and anaerobic organisms. Delafloxacin has the potential to treat a variety of infections including complicated skin and skin structure infections, complicated intra-abdominal infections, and hospitalized community-acquired pneumonia. The absorption, distribution and excretion of Delafloxacin has been studied in male Lister Hooded rats following administration of a single intravenous dose of [14C]-labelled Delafloxacin (10 mg/kg).

**Methods:** In the excretion/balance phase: urine and faeces were collected up to 168 hour. After 168 hour the cages were rinsed and washings retained. The total radioactivity for each sample was determined by LSC.

Tissue distribution was determined by quantitative whole-body autoradiography (QWBA) at 6 time points up to 504 hour post dose.

**Results:** Excretion of radioactivity was predominantly via the faeces (74.21%) with urinary excretion accounting for 23.65% dose. The overall recovery was 99.79%.

Maximal radioactive concentrations for most tissues occurred at 4 hour post dose (the first timepoint) and declined steadily thereafter. By 24 hour radioactivity was generally associated with tissues of elimination (kidney and GIT).

**Conclusions:** The recovery of radioactivity was good, with the majority excreted via the faecal route. Elimination of radioactivity was fairly rapid (>90% of the administered radioactivity was recovered during the first 48 hour after dosing). As concentrations measured in the carcasses were low (0.25%), excretion was considered essentially complete during the course of the study. Radioactivity was rapidly distributed and quickly eliminated from all tissues. By 48 hour post-dose almost 90% of tissues analysed contained concentrations that were below the limit of quantification.

**P1431** Pharmacokinetics and safety of single and multiple doses of LFF571 in healthy subjects

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**Objectives:** LFF571 is a novel inhibitor of bacterial elongation factor Tu (EF-Tu), which is essential for protein synthesis. It is active against a wide range of bacteria including *C. difficile* (MIC<sub>90</sub> = 0.25 µg/mL). The antibacterial mechanism of LFF571 has the potential to reduce *C. difficile* toxin production. This first-in-human study investigated the safety and pharmacokinetics of single and multiple ascending oral doses of LFF571 in healthy subjects.

**Methods:** The study was randomized, double-blind, placebo-controlled, and time-lagged. Except in one 400 mg cohort, LFF571 was given with a high fat meal in all single dose cohorts. In the multiple dose (every 6 hour for 10 days) cohorts, LFF571 was given without regards to food. LFF571 serum and faecal concentrations were quantified, and pharmacokinetic parameters were determined where possible.

**Results:** A total of 57 subjects were enrolled and 56 completed the study, with 32 and 25 receiving single and multiple doses, respectively.

There were no deaths, serious adverse events, or subject discontinuations due to an adverse event. The most common adverse event was diarrhoea; gastrointestinal pain or distension was also noted. Diarrhoea did not develop more frequently among subjects who received LFF571.

LFF571 had limited systemic exposure and high steady-state faecal concentrations. Serum concentrations were at or slightly above the lower limit of quantitation of 0.5 ng/mL. The highest serum concentration was 3.2 ng/mL in a subject in the 200 mg multiple dose cohort 6 hour after the last dose. Higher serum concentrations were seen after fasting; the values were not considered clinically relevant.

Study cohort	Number of subjects with detectable LFF571 concentrations (out of 6)	Day 10 C <sub>max</sub> (ng/mL); Median (range)	T <sub>max</sub> (hours); Median (range)	Assumed steady-state faecal concentration (mcg/g); Median (range)
25 mg every 6 hours	2	0.63 (0.57–0.69)	1.0 (1.0–1.0)	340.5 (181–878)
100 mg every 6 hours	6	0.80 (0.52–1.33)	2.0 (2.0–5.9)	2080 (820–5890)
200 mg every 6 hours	6	2.19 (1.40–2.58)	2.0 (1.0–5.9)	5410 (2200–8720)

**Conclusion:** LFF571 was generally safe and well tolerated after single and multiple oral doses in healthy subjects. The minimal serum and high faecal concentrations suggest that LFF571 may be useful for the treatment of *C. difficile* infections.

**P1432** Mechanism of action of XF-70, a novel porphyrin antimicrobial against *Staphylococcus epidermidis*

S.A. Hassan, N. Ooi\*, W. Rhys-Williams, W.G. Love, I. Chopra (Leeds, Brighton, UK)

**Objectives:** XF-70 is a novel porphyrin antimicrobial which is being developed as a topical antibacterial drug. Potent bactericidal activity against *Staphylococcus epidermidis* has been previously demonstrated and the aim of this study was to investigate the mechanism of action of this bactericidal activity.

**Methods:** MICs for five *S. epidermidis* strains were determined by broth microdilution according to British Society for Antimicrobial Chemotherapy (BSAC) specifications. Time-kill studies using exponentially growing *S. epidermidis* ATCC35984 were undertaken at 4× MIC against XF-70 and control agents with samples taken every 30 minute. Membrane integrity after exposure to lethal concentrations of test compounds was determined using the BacLight™ assay. The leakage of intracellular potassium was determined by atomic absorption spectroscopy and a luciferin/luciferase assay was used to quantify the leakage of intracellular ATP.

**Results:** The MIC of XF-70 against the five *S. epidermidis* strains was found to be 0.5–1 mg/L, and the time-kill studies demonstrated that there was a >4 log<sub>10</sub> reduction in cell viability after 30 minute. In contrast, daptomycin, which has a similar MIC (1 mg/L) resulted in <2 log<sub>10</sub> reduction at the same timepoint. Membrane integrity was severely compromised after only 10 minute exposure to XF-70, with a >97% reduction observed. In the same experiment, results for mupirocin and daptomycin demonstrated <15% and <25% reduction in membrane integrity respectively. After 60 minute exposure to XF-70, >70% of the intracellular potassium and >90% of the intracellular ATP had leaked out of the cells. In contrast daptomycin demonstrated a 68% and 89% reduction respectively whilst for mupirocin, it was 19% and 21% respectively.

**Conclusion:** XF-70 has a very rapid bactericidal activity against *S. epidermidis* and the primary target appears to be the cytoplasmic membrane. Exposure to XF-70 results in a rapid loss of membrane integrity with leakage of the majority of the intracellular potassium and ATP after 60 minute exposure.



**P1433** Activity of XF-73 against methicillin-resistant and sensitive *Staphylococcus epidermidis*

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**Objectives:** XF-73 is undergoing clinical trials for the nasal decolonisation of *Staphylococcus aureus*. The frequency of nasal colonisation by *Staphylococcus epidermidis* is significantly higher than for *S. aureus* and recent studies have demonstrated that the presence of *S. epidermidis* inhibits colonisation by *S. aureus*. *S. epidermidis* is also a significant source of nosocomial infections. The effect of XF-73 decolonisation against nasal bacteria other than *S. aureus* is therefore an important consideration. This study aimed to investigate the activity of XF-73 against both methicillin-sensitive and methicillin-resistant *S. epidermidis* and to investigate the mechanism of action of XF-73 against *S. epidermidis*

**Methods:** Planktonic MICs for five *S. epidermidis* strains (two methicillin-sensitive; three methicillin-resistant) was determined by broth microdilution according to British Society for Antimicrobial Chemotherapy (BSAC) specifications. Time-kill studies using exponentially growing *S. epidermidis* ATCC35984 were undertaken at 4x MIC against XF-73 with samples taken every 30 minute. Membrane damage after exposure to lethal concentrations of test compounds was determined using the BacLight™ assay. The leakage of intracellular potassium was determined by atomic absorption spectroscopy and a luciferin/luciferase assay was used to quantify the leakage of intracellular ATP.

**Results:** The MIC for the five *S. epidermidis* strains tested were found to be 0.5–1 µg/mL and the presence of methicillin resistance was found to not have any effect on the MIC. Time-kill experiments demonstrated a rapid bactericidal activity against *S. epidermidis* ATCC35984 with a 99.99% reduction in viable cell numbers after only 30 minute incubation. Bacterial membrane integrity was found to be completely lost after 10 minute incubation and >78% of the internal potassium and >90% of the internal ATP was found to have leaked out of the cells after 60 minute exposure.

**Conclusions:** XF-73 demonstrated similar rapid bactericidal activity against *S. epidermidis* to that previously demonstrated for *S. aureus*. The presence of methicillin resistance had no effect on the potency of XF-73 and the mechanism of action appears to be identical to that determined against *S. aureus*. The results suggest that XF-73 has broader utility than just the potential for nasal decolonisation of *S. aureus*.

**P1434** XF-73, a novel porphyrin antimicrobial has antibacterial activity against *Staphylococcus epidermidis* irrespective of the growth state

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**Background:** The activity of many antibiotics is dependent on the growth state of the bacterial target and treatment failure is often associated with bacteria being in a non-growing phase. Bacterial biofilms are a source of clinical concern, particularly due to their resilient nature and it has been suggested that the presence of slow growing cells may contribute to the resistance of biofilms to antibiotic treatment. This study aimed to investigate the activity of XF-73, a novel porphyrin antimicrobial currently in clinical development for the nasal decolonization of *Staphylococcus aureus*, against *Staphylococcus epidermidis* cultures in exponential and stationary growth phases and within biofilms.

**Methods:** The killing kinetics of XF-73 and comparator compounds against exponential phase *S. epidermidis* ATCC35984 cells was compared to the killing kinetics against cultures determined to be in late stationary phase at 4x MIC with samples taken every 30 minute. Planktonic MICs for *S. epidermidis* ATCC35984 was determined by broth microdilution according to British Society for Antimicrobial Chemotherapy (BSAC) specifications. The Calgary biofilm device was

used to establish biofilm MICs (bMICs) and minimum biofilm eradication concentrations (MBECs).

**Results:** A growth curve for *S. epidermidis* ATCC35984 determined that the cells entered early stationary phase after 24 hour growth and exited stationary phase after 49 hour of growth. Time-kill experiments demonstrated that in exponential cultures, there was a >4 log<sub>10</sub> reduction in viable cell numbers after only 30 minute incubation. In late stationary phase, this reduction was largely unaffected, with >3.5 log<sub>10</sub> reduction observed after 30 minute. In contrast, the reduction in viable cell numbers fell from ~2 log<sub>10</sub> to <1 log<sub>10</sub> for daptomycin and >4.5 log<sub>10</sub> to <0.5 log<sub>10</sub> for CTAB in exponential and late stationary phase cultures respectively. XF-73 is highly active against planktonic cultures of *S. epidermidis* ATCC35984 (MIC 1 µg/mL) and within biofilms (bMIC 2 µg/mL, MBEC 2 µg/mL). In comparison, MBEC values for daptomycin, penicillin, mupirocin and vancomycin were >256 µg/mL.

**Conclusions:** XF-73 is rapidly bactericidal against planktonic cultures either in exponential or late stationary phase and XF-73 eradicates *S. epidermidis* ATCC35984 biofilms, supporting the concept that this compound acts on the membrane and is not dependent on the growth state of the bacteria.

## New antimicrobial agents against old and new protein targets

**P1435** Identification of novel DNA gyrase gate inhibitors from *Escherichia coli* gyrase high-throughput screens

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**Objectives:** Bacterial topoisomerases (DNA gyrase and topoisomerase IV) are clinically-validated targets for the design of new antibacterials to combat gram-negative hospital pathogens resistant to established antibiotics such as quinolones. The objective of this study was to identify novel classes of DNA gyrase especially DNA gate domain inhibitors from HTS hits in order to optimize existing inhibitors and to generate novel antibacterial leads.

**Methods:** GSK's compound collection was screened against a gyrase-dependent in vitro replication assay (using toluenised *E. coli* cells) and a DNA-dependent gyrase ATPase assay in high throughput formats. Secondary biochemical GyrB ATPase, gyrase supercoiling and DNA cleavage assays were employed to identify gyrase DNA gate inhibitors. Isogenic mutants resistant to quinolone or novel bacterial topoisomerase inhibitor (NBTI) were used to evaluate cross resistance. Spontaneous resistant mutant isolation and PCR/sequencing were utilised to map residues/domains involved in the binding of novel gyrase inhibitors. Crystallography was applied to solve co-structures of promising hits to determine exactly where they bind to gyrase.

**Results:** Screening of GSK's compound collection against DNA gyrase generated ~3700 early hits. Antibacterial mode-of-action characterization of HTS hits identified several new classes of DNA gyrase inhibitors. One example, the GW808837 series, has potent supercoiling IC<sub>50</sub> (~2–10 µM), antibacterial activity against *Staphylococcus aureus* and *Streptococcus pneumoniae* (MIC 4–16 µg/mL) and MICs against efflux mutants of key Gram-negative pathogens. Furthermore, evidence of the (i) induction of single- and double-DNA cleavage in enzyme assays, (ii) hypersensitivity to a *S. aureus* GyrA Y87F strain that carries a mutation in the DNA gate domain, and (iii) isolation of suppressor mutants to Y87F that map to GyrA gate domain, show that these novel gyrase inhibitors bind to the DNA breakage-reunion domain. Multiple hits were advanced to crystallography and, with a second chemical series GW368540, we obtained a co-crystal structure with gyrase that illustrates the potential to identify different binding modes for lead diversification.

**Conclusions:** Characterization of HTS hits has identified new gyrase inhibitors with novel pharmacophores and mechanisms of action that may help optimize existing classes of inhibitors and design novel, diverse antibacterial leads that bind to the DNA gate domain.

**P1436** **In vitro activity of delafloxacin and other agents against *S. aureus* isolates from a phase II trial for acute bacterial skin and skin structure infections**

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**Background:** Antimicrobial resistance among *S. aureus* (SA) continues to present challenges for effectively managing serious infections caused by this common pathogen. To help meet these challenges delafloxacin (DFX), a potent anti-staphylococcal fluoroquinolone, is currently under clinical development as a broad spectrum oral and intravenous compound for the treatment of acute bacterial skin and skin structure infections involving SA and other pathogens. This report documents SA resistant phenotypes encountered in an ongoing clinical trial and provides information on the activity spectrum of DFX against these resistant phenotypes.

**Methods:** The Phase 2 trial was USA based and included ~35 sites and occurred over the 2011 year. Isolates were obtained from clinical specimens using the preferred microbiology processes of the investigator microbiology sites. SA isolates were transported to Eurofins, Chantilly for confirmatory identification and antimicrobial susceptibility testing by broth microdilution according to CLSI guidelines. In addition to DFX, a range of various gram-positive drugs were also tested.

**Results:** One hundred and eighty-seven SA isolates were available for analysis; 115 (62%) were oxacillin-resistant (MRSA), 52.9% were ciprofloxacin (CP) -resistant, and 43.9% were levofloxacin (LV)-resistant. For the 187 strains the DFX MIC range was <0.001–2 µg/mL with an MIC90 of 0.12; LV and CP MIC90's were 4 and 16 µg/mL, respectively. For MSSA and MRSA the DFX MIC90's were 0.06 and 0.12 µg/mL, respectively. The MIC90's for LV against MSSA and MRSA were the same at 4 µg/mL, and for CP they were 8 and 16 µg/mL, respectively. Among LV-non-susceptible strains the LV MIC90 was 8 µg/mL and the DFX MIC90 was 0.12 µg/mL; for CP-non-susceptible strains the CP MIC90 was 16 µg/mL and the DFX MIC90 of 0.12 µg/mL.

**Conclusion:** MRSA continue to be a prevalent cause of skin infections and resistance to current fluoroquinolones remains a prominent feature among these organisms. DFX exhibited potent in vitro activity against fluoroquinolone-resistant MRSA encountered in this trial. This feature suggests that DFX can be developed as a potent new therapeutic choice for SA based infections.

**P1437** **Activity of JNJ-Q2, a new fluoroquinolone, tested against contemporary (2011) acute bacterial skin and skin-structure infection pathogens from Europe**

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**Objectives:** To determine the activity of JNJ-Q2 tested against contemporary (2011) European isolates of the most common bacterial species isolated from patients with acute bacterial skin and skin-structure infections (ABSSSI). JNJ-Q2 is a broad-spectrum bactericidal fluoroquinolone (FQ) with potent activity against Gram-positive and -negative pathogens, including methicillin-resistant (MR) *Staphylococcus aureus* (SA), and is in clinical development for the treatment of ABSSSI and community-acquired bacterial pneumonia.

**Methods:** A total of 1613 pathogens were collected from patients in 24 medical centres in 11 European countries (including Turkey and Israel) in 2011. Species/organism group (number of isolates tested) were: SA (1416) and beta-haemolytic streptococci (BHS, 197; 33.5% *S. pyogenes*). Isolates were tested for susceptibility by CLSI broth microdilution methods (M07-A8 and M100-S21). Susceptibility interpretations for comparator agents were determined using EUCAST (2011) and CLSI breakpoints.

**Results:** The table shows the cumulative percentage MIC frequency against the four species/groups tested. Against 1416 SA, JNJ-Q2

(MIC50/90, 0.008/0.25 mg/L) inhibited all isolates at a MIC ≤ 2 mg/L. Although activity was lower against MRSA (MIC50, 0.25 mg/L) compared to methicillin-susceptible (MS) SA (MIC50, 0.008 mg/L), 98.2% of MRSA were inhibited at a JNJ-Q2 MIC value of ≤0.5 mg/L. Against MRSA, JNJ-Q2 was eight- to at least 32-fold more active than moxifloxacin (MOX; MIC50/90, 2/≥8 mg/L) and at least 32-fold more active than levofloxacin (LEV; MIC50/90, ≥8/≥8 mg/L) and ciprofloxacin (CIP; MIC50/90, ≥8/≥8 mg/L). JNJ-Q2 demonstrated excellent activity (MIC50/90, 0.015/0.015 mg/L) against BHS, inhibiting 100.0% of isolates at a MIC of ≤0.12 mg/L. Using MIC90 results, JNJ-Q2 was 16-fold more active than MOX (MIC50/90, ≤0.12/0.25 mg/L) and 64-fold more active than CIP (MIC50/90, 0.5/1 mg/L) against BHS.

Organism (n)	Cum. % inhibited at JNJ-Q2 MIC (mg/L):									
	≤0.004	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2
SA (1416)	30.7	64.4	71.0	71.5	72.5	83.2	97.0	99.4	99.9	100.0
MSSA (1032)	41.2	85.7	93.9	94.6	95.1	98.1	99.8	99.9	100.0	
MRSA (384)	2.3	7.3	9.4	9.4	12.0	43.2	89.6	98.2	99.5	100.0
BHS (197)	1.0	32.5	93.4	99.0	99.5	100.0				

**Conclusions:** JNJ-Q2 demonstrated very potent activity against this collection of common ABSSSI pathogens isolated from patients in European medical centers during 2011. JNJ-Q2 exhibited eightfold or greater activity compared to CIP, LEV and MOX against these isolates. The JNJ-Q2 in vitro results remain very promising and support further clinical development of this new FQ for treatment of ABSSSI, including cases caused by MRSA.

**P1438** **Evaluation of in vitro antibacterial activity of MGB-BP-3, a new class of antibacterial**

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**Objectives:** MGB-BP-3 (MGB) is a new class of antibacterial agent discovered at the University of Strathclyde. It binds selectively to the minor grooves of DNA. We investigated the spectrum of antibacterial activity against a panel of bacteria. The antibacterial profile of MGB was investigated further in susceptible bacteria to determine MIC50/MBC50 and MIC90/MBC90, killing activity, and the potential for development of resistance.

**Methods:** The CLSI methodology was used throughout the experiments. MIC was assessed in a panel of 52 Gram-positive and 34 Gram-negative bacteria and compared to standard antibiotics. The MIC50/MBC50 and MIC90/MBC90 were determined in a panel of 210 Gram-positive isolates consisting of vancomycin susceptible and resistant enterococci, methicillin susceptible and resistant *Staphylococcus aureus* and *S. epidermidis*, and a group of streptococci. The bactericidal activity of MGB was determined measuring in vitro killing activity (time kill and bactericidal index) against vancomycin-susceptible *E. faecalis*, methicillin-susceptible *S. aureus* and *Streptococcus pyogenes*. These strains were also used to assess the propensity to develop resistance to MGB and a comparator (fusidic acid) by mutation frequency following serial passage over 14 days at sub-inhibitory antimicrobial concentrations (0.5 × MIC).

**Results:** MGB showed high activity against all Gram-positive isolates tested, including vancomycin and methicillin resistant strains. At 0.5 mg/L, MGB inhibited 86.5% of all tested Gram-positive isolates, compared to 43.4% tested with vancomycin. With Gram-negative isolates the only MGB activity observed was against *N. meningitidis* (MIC 0.25 mg/L) and two *M. catarrhalis* strains (MIC of 0.5 and 2 mg/L). The data indicate that MGB is bactericidal against staphylococci and streptococci, whilst it is bacteriostatic against enterococci. Resistance tests showed that *E. faecalis* can develop resistance against MGB, but there was no similar evidence with *S. aureus* and *S. pyogenes*.

**Conclusion:** MGB has been shown to have very strong inhibitory activity against all tested Gram-positive bacteria including vancomycin and methicillin resistant strains. Its activity against staphylococci and streptococci is bactericidal, and bacteriostatic against enterococci. The

possibility of developing resistance to MGB was confirmed with *E. faecalis* but not with *S. aureus* and *S. pyogenes*.

**P1439** Treatment of CDAD with oral CB-183 315: time to recurrence, relapse and re-infection rates compared with vancomycin

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**Objective:** The objective of this posthoc analysis of data from a Phase 2 trial was to determine if recurrences were due to relapse or re-infection based on the rates and timing of recurrence as well as the genetic relationship of the isolates.

**Methods:** In a randomized, controlled, double-blind, multi-center Phase 2 trial, 210 subjects were randomized (1:1:1) to the oral lipopeptide CB-183 315 (125 or 250 mg BID) or vancomycin 125 mg QID. Subjects were followed 28 days post treatment. *C. difficile* isolates were recovered from baseline and recurrence specimens and deemed identical based on Restriction Endonuclease Analysis (REA) type or Pulsed Field Gel Electrophoresis pattern.

**Results:** Forty-eight subjects from the Modified Intent-to-Treat population had a recurrent episode. Respective recurrence rates were 36%, 28%, and 17% for the vancomycin, CB-183 315 125 and 250 mg BID arms. Of these subjects, 41/48 had paired baseline/recurrence isolates for analysis.

Among the recurrence isolates, 33/41 (80%) were identical to the baseline isolates (putative relapses). Recurrence occurred in 27/41 (66%) subjects within the first 14 days post treatment; 22/27 (81%) isolates were identical to the baseline isolates. In the CB-183 315 250 mg BID arm, no recurrences (0/2 [0%]) at 7 days and 2/4 (50%) at 14 days post treatment were caused by an isolate identical to the baseline isolate vs. 11/12 (92%) and 13/15 (87%) in the vancomycin arm, respectively (Table 1). After 28 days post treatment, 7/10 (70%) and 15/18 (83%) recurrences were caused by an isolate identical to the baseline isolate in the CB-183 315 250 mg BID and vancomycin arms respectively.

A total of 19/41 (46%) cases were caused by a REA group BI isolate; 16/19 (84%) recurrence isolates were identical to the baseline isolate. One case of recurrence within each treatment arm was caused by an isolate unrelated to the baseline isolate and occurred more than 14 days post treatment (reinfection).

Table 1: Genetic relationship of baseline and recurrence isolates

Days post treatment	Vancomycin 125mg QID [n identical/n Total (%)]	CB-183.315 125mg/BID [n identical/n Total (%)]	CB-183.315 250mg/BID [n identical/n Total (%)]	Overall over time [n identical/n Total (%)]
1-7	11/12	4/5	0/2	15/19 (79%)
8-14	2/3	3/3	2/2	7/8 (88%)
15-21	2/3	3/4	5/6	10/13 (77%)
22-28	0/0	1/1	0/0	1/1 (100%)
Total	15/18 (83%)	11/13 (85%)	7/10 (70%)	33/41 (80%)

**Conclusions:** While the data set is small and the differences observed were not statistically significant, the majority of recurrences were relapses and this seems to be independent of timing. Differences in relapse rates and timing of recurrence trended in favor of the CB-183 315 250 mg BID arm. This study provides a hypothesis that will be further explored during the development of CB-183 315 for the treatment of CDI.

**P1440** Activity of LFF571 against 103 clinical isolates of *C. difficile*

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**Objective:** *Clostridium difficile* is the most common infectious cause of diarrhoea and colitis in hospitalized patients and can lead to colon

distension or shock associated with high mortality. LFF571 is a novel analog of the natural product GE2270 A, which inhibits bacterial growth by binding with high affinity to elongation factor Tu (EF-Tu). LFF571 is a potent inhibitor of *C. difficile* in vitro and confers better protection from relapse compared with vancomycin in an experimental hamster model of primary and relapsing *C. difficile* infection. LFF571 was generally safe and well tolerated by healthy subjects receiving single and multiple doses for up to 10 days. A phase 2 clinical study is ongoing to evaluate the safety and efficacy of LFF571 for the treatment of *C. difficile* infection. The objective of this study was to determine the activity of LFF571 compared with that of metronidazole (MET), vancomycin (VAN), and clindamycin (CLN) against recent clinical isolates of *C. difficile*.

**Methods:** One hundred and three unique, recent, geographically diverse toxin positive clinical isolates of *C. difficile* were tested using the agar dilution method according to Clinical Laboratory Standards Institute guideline M11-A7. Testing ranges for LFF571, MET, VAN and CLN were 0.03–8, 0.06–16, 0.125–16, and 0.125–64 µg/mL, respectively. *C. difficile* ATCC 700057 was used as a control.

**Results:** The genotypes of the 103 strains tested by restriction endonuclease analysis (REA) included 51 BI (ribotype 027, pulse field type NAP1), 2 BK, 3CF, 3DH, 3G, 4J, 5K, 1N, 2R, 7Y, and 21 non-typed. MIC with ranges for each compound are shown in the Table:

*C. difficile* ATCC 700057 tested within control range for both MET and VAN. There were no significant differences in activity of LFF571 against any of the genotypes.

Antimicrobial	Range (µg/mL)	MIC50 (µg/mL)	MIC90 (µg/mL)
LFF571	0.06-0.5	0.25	0.5
Metronidazole	<0.06-1	0.25	0.5
Vancomycin	0.5-4	1.0	1.0
Clindamycin	0.5->64	8.0	>64

**Conclusions:** LFF571 is highly active against all clinical isolates of *C. difficile* tested, including epidemic strain BI/NAP1/027, and is more active than VAN, and similar to MET. The activity of LFF571 against *C. difficile* is also comparable to that of the newly approved drug fidaxomicin. Continued clinical trials with LFF571 are warranted on the basis of in vitro activity.

**P1441** Decreased *Clostridium difficile* spore viability following oritavancin exposure

C.H. Chilton, J. Freeman, G.S. Crowther, M.H. Wilcox\*, S. Nicholson (Leeds, UK)

**Objectives:** We previously noted enhanced activity of oritavancin (ORI) compared to vancomycin (V) against *Clostridium difficile* (CD) in vitro. We determined whether ORI and V affected CD spore germination directly, or subsequent outgrowth.

**Methods:** (i) CD PCR ribotype 027, 001 and 078 spores (~106 CFU/mL) were incubated in Braziers broth containing no antibiotic, or 10 mg/L ORI or V. Phase contrast microscopy (PCM) was used to monitor germination and outgrowth over 48 hour, and cell suspensions were washed and plated onto Brazier's cycloserine-cefoxitin egg yolk agar with lysozyme (CCEYL) agar for total viable counts and spore viability testing.

(ii) CD germination and outgrowth were monitored by PCM for 24 hour after addition of 100 mg/L ORI or V at different stages of CD PCR ribotype 027, 001 and 106 growth (0, 1, 2 and 4 hour).

**Results:** (i) Spores incubated with ORI were able to convert from phase bright (PB; quiescent) to phase dark (PD; germinated), but were unable to outgrow into vegetative cells (VCs) unlike unchallenged controls. Germination of spores incubated with V was also reduced compared to controls (49–58% vs. 63–73% VCs respectively), but to a lesser extent than with ORI (19–23%). Fewer (~2–6 log<sub>10</sub> CFU/mL decrease) VCs and spores were recovered after washing from ORI exposed samples than from controls or V-exposed samples, although

there was inter-strain variation. For PCR ribotype 078, no spores or VCs could be recovered after ORI exposure. (ii) Effects of V and ORI addition at different stages of growth were similar. Controls showed conversion from PB to PD spores within 2 hour for all strains, and from PD to VCs within 2–4 hour incubation. CD cells exposed to ORI and V at T = 0 or 1 hour converted from PB to PD spores, but not to VCs. CD exposed to ORI and V at 2 and 4 hour following outgrowth showed reduced VCs counts thereafter. Differences were noted between control germination curves of different CD strains in both experiments.

**Conclusions:** ORI and V treated spores were able to convert from phase bright to phase dark, but were unable to form VCs. This indicates that ORI, like V, acts post-spore germination. Poor recovery of ORI-exposed spores vs. V-exposed spores, despite washing provides further supportive evidence that ORI adheres to *C. difficile* spores, preventing effective outgrowth. Inter-strain variation in recovery of ORI-exposed CD spores on CCEYL may indicate that differences in spore surface properties between strains affects ORI binding.

**P1442** Effective treatment of simulated *Clostridium difficile* infection with a shortened course (4 day) of oritavancin in a human gut model

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**Objectives:** We previously demonstrated that oritavancin (ORI) is effective as a treatment of clindamycin induced *Clostridium difficile* infection (CDI) in a human gut model, and may be more effective than vancomycin (V) due to apparent increased activity against spores, and prevention of recurrence of toxin production. We compared the efficacy of a shortened dosing period (4 day) of ORI vs. V for the treatment of CDI within the gut model.

**Methods:** A 3-stage chemostat human gut model was inoculated with pooled faeces (five healthy elderly volunteers). Clindamycin (CLIN, 33.9 mg/L qid for 7 day) was dosed to induce CDI by *C. difficile* ribotype 027 (NAPI/BI). Following CDI induction, 2x 4-day dosing regimens were used: ORI (64 mg/L) bid; or V (125 mg/L) qid. CD total viable counts (TVC), spore counts (SP), toxin titres, and gut microflora components were measured throughout

**Results:** CLIN instillation induced CD germination and high level toxin production in V and ORI models. CD TVC decreased to SP by 5 day post V, whereas both TVC and SP were undetectable by 2 day post-ORI. Toxin titres reduced to undetectable levels by 12 day post-V vs. 5 day post ORI. There was evidence of recurrence of CD germination and high level toxin production, 20 day after V instillation ceased. Conversely, low levels of toxin (titre of >2) were observed without detectable germination in the ORI model: SP remained undetectable in the ORI model for the remainder of the experiment, but TVC were detectable at the limit of detection. Lactose fermenters and enterococci decreased (~4 and ~3 log<sub>10</sub> CFU/mL reduction respectively), and *B. fragillis* group increased (~4 log<sub>10</sub> CFU/mL increase) following V treatment. Enterococci and clostridia decreased following ORI treatment (~5 and ~3 log<sub>10</sub> CFU/mL reduction respectively).

**Conclusions:** As with 7 day dosing regimens, ORI was superior to V in reducing TVC counts and SP below the limits of detection. ORI reduced SP counts whereas V did not. There was clear evidence of recurrence in the V model. These data support previous conclusions that ORI may be an effective treatment for CDI, even when administered over only four days. The confirmed observation of ORI effects on SP recovery represent a potential advantage over other CDI treatments.

**P1443** Activity of ceftaroline/avibactam tested against multidrug-resistant Enterobacteriaceae and methicillin-resistant *Staphylococcus aureus* collected from USA hospitals in 2011

H. Sader\*, R. Flamm, M. Castanheira, R. Jones (North Liberty, US)

**Objective:** To evaluate the activity of ceftaroline (CPT) combined with avibactam (formerly NXL-104) tested against resistant subsets of Enterobacteriaceae (ENT) and MRSA strains. CPT is a broad-spectrum cephalosporin with activity against Gram-negative and -positive (including MRSA and multidrug-resistant [R] *S. pneumoniae*) organisms. Avibactam is a novel non-beta lactam beta-lactamase (BL) inhibitor that inhibits Ambler class A, C, and D enzymes (e.g. ESBL, KPC, and AmpC).

**Methods:** CPT/avibactam (CPA; avibactam at fixed 4 mg/L) and various comparators were tested for susceptibility (S) by CLSI broth microdilution methods against 1502 ENT, including ESBL-phenotype *E. coli* (43) and *Klebsiella* spp. (KSP; 67), AmpC derepressed Enterobacter spp. (ESP; 60), carbapenem (CB)-non-S (most were KPC-producing) KSP (13) and ESP (2), ciprofloxacin-R ENT (224) and gentamicin-R ENT (120), among other R phenotypes. 1496 *S. aureus*, including 738 MRSA strains were also tested. The strains were consecutively collected in 2011 from 52 medical centres located in the nine USA Census Regions.

**Results:** 99.6% of ENT and 99.1% of MRSA strains were inhibited at CPA MIC of ≤1 mg/L (see Table). Highest CPA MIC was only 4 mg/L (one *S. marcescens* strain; 0.06% of ENT). The most active compounds tested against the ESBL-phenotype and CB-non-S KSP were CPA (95.5% and 76.9% inhibited at ≤0.5 mg/L [USA-FDA S breakpoint for CPT], respectively), tigecycline (95.5/85.1% and 100.0/92.3% S by CLSI/EUCAST criteria, respectively) and gentamicin (65.7/61.2% and 69.2/69.2% S by CLSI/EUCAST criteria, respectively). All MRSA strains were inhibited at ≤2 mg/L of CPA, and CPT MIC results were not affected by the addition of avibactam. Against methicillin-S *S. aureus*, CPA inhibited all at MIC ≤0.5 mg/L and was 16-fold more active than ceftriaxone.

Organism (no. tested)	No. of isolates (cumulative %) inhibited at ceftaroline/avibactam MIC (mg/L) of:							
	0.03	0.06	0.12	0.25	0.5	1	2	4
Enterobacter spp. (100)	29 (29.0)	46 (75.0)	20 (95.0)	3 (98.0)	1 (99.0)	1 (100.0)	-	-
Ceftazidime-R (11)	0 (0.0)	3 (27.3)	7 (90.9)	1 (100.0)	-	-	-	-
Enterobacter spp. (237)	56 (16.6)	117 (51.3)	91 (78.3)	49 (92.9)	15 (97.3)	6 (99.1)	3 (100.0)	-
Ceftazidime-R (60)	1 (1.7)	11 (20.0)	8 (33.3)	21 (68.3)	10 (85.0)	6 (95.0)	3 (100.0)	-
Meropenem-non-S (2)	-	-	1 (50.0)	0 (0.0)	0 (50.0)	0 (50.0)	1 (100.0)	-
<i>E. coli</i> (43)	356 (81.8)	65 (96.8)	11 (99.3)	3 (100.0)	-	-	-	-
ESBL-phenotype (43)	25 (58.1)	8 (76.7)	7 (96.0)	3 (100.0)	-	-	-	-
<i>Klebsiella</i> spp. (539)	218 (40.5)	209 (79.2)	72 (92.6)	29 (98.0)	8 (99.4)	2 (99.8)	1 (100.0)	-
ESBL-phenotype (67)	12 (17.9)	15 (40.3)	19 (68.7)	13 (88.1)	5 (95.5)	2 (98.5)	1 (100.0)	-
Meropenem-non-S (13)	0 (0.0)	1 (7.7)	2 (23.1)	6 (69.2)	1 (76.9)	2 (92.3)	1 (100.0)	-
Indole-positive Protease (91)	58 (63.7)	24 (90.1)	8 (98.9)	1 (100.0)	-	-	-	-
<i>S. marcescens</i> (148)	0 (0.0)	1 (0.7)	14 (10.1)	57 (48.7)	60 (89.2)	13 (94.0)	1 (98.7)	1 (100.0)
<i>S. aureus</i> (1496)	1 (0.1)	6 (0.5)	182 (12.6)	601 (52.8)	551 (89.6)	148 (99.5)	7 (100.0)	-
MRSA (738)	1 (0.1)	0 (0.1)	2 (0.4)	40 (5.6)	543 (79.0)	148 (99.1)	7 (100.0)	-

**Conclusions:** Avibactam can effectively lower CPT MIC values for ENT strains producing the most clinically significant BLs found in USA hospitals. CPA was highly active against ENT-producing KPC, various ESBL types, and AmpC (chromosomally derepressed or plasmid mediated), and MRSA. CPA represents a promising therapeutic option for treatment of infections caused by multidrug-R ENT and MRSA.

**P1444** Antimicrobial spectrum and potency of ceftaroline/avibactam when tested against bacterial isolates from complicated urinary tract infections in the United States

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**Objective:** To evaluate the activity of ceftaroline (CPT) combined with avibactam (CPA; avibactam at fixed 4 mg/L) against isolates from complicated urinary tract infections (cUTI) collected in USA medical centres. CPT is a broad-spectrum cephalosporin and avibactam is a novel non-beta-lactam beta-lactamase (BL) inhibitor that inhibits Ambler class A, C, and some D enzymes.

**Methods:** CPA and comparators were tested for susceptibility (S) by CLSI broth microdilution methods against 1131 strains, including *Escherichia coli* (348; 8.0% ESBL-phenotype), *Klebsiella* spp. (326; 7.7% ESBL-phenotype and 1.5% meropenem-resistant [R]), group B

streptococci (GBS; 176), *Enterococcus faecalis* (78), coagulase-negative staphylococci (CoNS; 77; 57.1% oxacillin-R), *Proteus mirabilis* (61) and *Morganella morganii* (34). Non-fermentative bacilli were not included. Isolates were collected in 2009–2010 from 65 medical centres located in all nine USA Census Regions.

**Results:** Overall, 98.4% of strains were inhibited at  $\leq 2$  mg/L of CPA and all 18 isolates with CPA MIC at  $\geq 4$  (4–16) mg/L were *E. faecalis* (Table). *E. coli* and *Klebsiella* spp. were very S to CPA with MIC50/90 of  $\leq 0.03/0.06$  and  $0.06/0.12$  mg/L, respectively. Ceftriaxone and ciprofloxacin were active against 92.0% and 73.9% of *E. coli* and 92.3% and 94.2% of *Klebsiella* spp., respectively; and 1.5% of *Klebsiella* spp. were R to meropenem. Among *P. mirabilis* and *M. morganii*, the highest CPA MIC values were only 0.5 and 0.25 mg/L, and R rates to ciprofloxacin were 29.5% and 35.3%, respectively. The highest CPA MIC value among *Enterobacter* spp. was only 0.5 mg/L (MIC50/90, 0.06/0.5 mg/L). All GBS were inhibited at CPA MIC of  $\leq 0.06$  mg/L. CPA showed activity against *E. faecalis* (MIC50/90, 2/8 mg/L) and was very active against CoNS (MIC50/90, 0.25/0.5 mg/L; 57.1% oxacillin-R).

Organism (no. tested)	No. of isolates (cumulative %) inhibited at CPA MIC (mg/L) of %:										MIC <sub>50</sub>	MIC <sub>90</sub>
	0.03	0.06	0.12	0.25	0.5	1	2	4	8	MIC <sub>50</sub>		
<i>E. coli</i> (348)	295 (84.8)	1115 (92.0)	24 (98.9)	3 (99.3)	1 (100.0)	-	-	-	-	-	40.03	0.06
<i>Klebsiella</i> spp. (330)	72 (22.1)	173 (51.5)	90 (90.5)	22 (97.2)	7 (99.4)	2 (100.0)	-	-	-	-	0.06	0.12
<i>P. mirabilis</i> (61)	2 (3.3)	16 (29.5)	34 (65.3)	7 (96.7)	2 (100.0)	-	-	-	-	-	0.12	0.25
<i>M. morganii</i> (34)	12 (35.3)	15 (44.1)	5 (94.1)	2 (100.0)	-	-	-	-	-	-	0.06	0.12
Group B strep. (176)	174 (98.9)	2 (100.0)	-	-	-	-	-	-	-	-	40.03	40.03
<i>E. faecalis</i> (78)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.3)	10 (14.1)	49 (76.9)	8 (97.2)	5 (93.6)	2 (8)	2	8
CoNS (77)	2 (2.6)	15 (22.1)	21 (40.4)	21 (76.8)	15 (96.1)	2 (99.7)	1 (100.0)	-	-	-	0.25	0.5

\*Concentrations reported in the table for ceftolozane/tazobactam (CPA) refer to the concentration of ceftolozane tested with fixed 4 mg/L tazobactam concentration.

**Conclusions:** CPA exhibited potent activity against a large collection of Enterobacteriaceae and Gram-positive organisms from patients with cUTI. Avibactam can effectively lower CPT MIC values for Enterobacteriaceae that produced the most clinically significant BLs occurring in USA hospitals.

**PI445** Activity of ceftolozane/tazobactam (CXA-201) against 270 recent isolates from the *Bacteroides* group

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**Background/Objective:** CXA-201 is the combination of ceftolozane, a new cephem antibiotic with excellent activity against *P. aeruginosa* and some Enterobacteriaceae, and tazobactam, an effective b-lactamase inhibitor with activity against most of the b-lactamases produced by the *Bacteroides fragilis* group of anaerobes. We undertook a study to evaluate the activity of CXA-201 against anaerobic pathogens with emphasis on the activity against *B. fragilis* and related species.

**Methods:** The activity of CXA-201 was compared to that of ceftolozane alone and ampicillin/sulbactam, piperacillin/tazobactam, imipenem, meropenem, moxifloxacin, tigecycline, clindamycin, metronidazole, and linezolid. The MICs were determined by agar dilution as recommended by CLSI (M11-A7).

**Results:** The addition of a fixed concentration of 4 mg/L of tazobactam to ceftolozane considerably reduced the MIC90 of CXA-201 against all the species of the *B. fragilis* group (Table 1).

Species (no)	CXA-201	Amp:Sub	Pip:Tazo	CXA-101
<i>B. fragilis</i> (201)	4	8	2	256
<i>B. ovatus</i> / <i>B. thetaalomicronn</i> (24)	32	16	4	256
<i>B. vulgatus</i> (20)	16	4	16	256
<i>P. distasonis</i> (8)	64	64	32	256
Other <i>Bacteroides</i> * (14)	16	8	4	128

\*3 *B. coccoides*, 4 *B. goldsteinii*, 2 *B. intestinalis*, 1 *B. johnsonii*, 1 *B. merdorei*, 1 *B. stercoris*, 2 *B. uniformis*

**Conclusions:** The addition of a potent beta-lactamase inhibitor, like tazobactam, to ceftolozane, a potent cephem antibiotic, gives additional activity of this combination against *Bacteroides* species. The activity against *B. fragilis* is more pronounced than the activity against the other anaerobic species tested. This activity suggests potential utility in infections with *Bacteroides fragilis*.

**PI446** Activity of the novel antimicrobial ceftolozane/tazobactam (CXA-201) tested against contemporary clinical strains from European hospitals

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**Objective:** To evaluate the in vitro activity of ceftolozane/tazobactam (CXA-201) against Gram-negative organisms isolated from patients in European (EU) hospitals in 2011. CXA-201 is a combination of the novel oxymino-aminothiazolyl cephalosporin ceftolozane and tazobactam (TAZ), and is currently under clinical development for treatment of complicated intraabdominal (cIAI) and urinary tract infections (cUTI).

**Methods:** CXA-201 and comparators were susceptibility (S) tested by CLSI broth microdilution methods against 3210 clinical strains, which included 519 *P. aeruginosa* (PSA; 25.8% ceftazidime [CAZ]-non-S and 28.5% meropenem [MER]-non-S), 1174 *E. coli* (17.2% ESBL-phenotype), 485 *Klebsiella* spp. (33.4% ESBL-phenotype and 3.3% MER-non-S), and 239 *Enterobacter* spp. (26.8% ceftriaxone-non-S), among others.

**Results:** When tested against PSA, CXA-201 was at least fourfold more active than CAZ or cefepime and inhibited 76.4% of MER-non-S strains at MIC of  $\leq 4$  mg/L. CXA-201 exhibited activity against PSA CAZ-non-S (MIC50/90, 4/>32 mg/L), MER-non-S (MIC50/90, 2/ >32 mg/L) and both CAZ and MER-non-S strains (MIC50/90, 4/ >32 mg/L). Piperacillin/TAZ (P/T; MIC50/90, 8/>64 mg/L) was active against 68.4% of PSA at S breakpoint of  $\leq 16$  mg/L. CXA-201 activity against ESBL-negative *E. coli* and *Klebsiella* spp. was similar to that of CAZ, in contrast, CXA-201 was 16- to 32-fold more active than CAZ when tested against ESBL producers. Against *Enterobacter* spp. and *Citrobacter* spp., CXA-201 was slightly more active than CAZ (Table).

Organism (no. tested)	no. of isolates (cumulative %) inhibited at ceftolozane/tazobactam MIC (mg/L) of:											
	$\leq 12$	0.25	0.5	1	2	4	8	16	32	64	128	256
<i>P. aeruginosa</i> (519)	0 (0.0)	10 (1.9)	201 (40.7)	191 (77.5)	368 (84.6)	419 (92.3)	1094 (211)	1344 (89.2)	495 (2)	-	-	-
Ceftazidime-S (98)	0 (0.0)	10 (2.6)	200 (54.5)	165 (97.4)	10 (100.0)	-	-	-	-	-	-	-
CAZ-non-S (134)	0 (0.0)	0 (0.0)	1 (0.7)	26 (20.1)	26 (98.9)	41 (70.1)	107 (77.5)	178 (4)	481 (3)	-	-	-
MER-S (31)	0 (0.0)	10 (2.7)	181 (51.5)	143 (90.0)	19 (95.2)	13 (98.7)	5 (100.0)	-	-	-	-	-
MER-non-S (148)	0 (0.0)	0 (0.0)	20 (13.5)	48 (46.6)	17 (57.4)	28 (76.4)	5 (79.7)	180 (4)	483 (3)	-	-	-
CAZ & MER-non-S (80)	0 (0.0)	0 (0.0)	0 (0.0)	7 (8.8)	10 (21.3)	28 (56.3)	5 (52.5)	163 (8)	468 (8)	-	-	-
<i>E. coli</i> (1174)	493 (42.0)	489 (91.9)	125 (92.6)	48 (96.7)	16 (98.0)	11 (99.0)	4 (99.3)	2 (99.5)	3 (99.7)	-	-	-
ESBL phenotype (202)	9 (4.5)	43 (25.7)	68 (58.4)	44 (81.2)	15 (86.9)	11 (94.1)	4 (96.0)	2 (97.0)	3 (98.5)	-	-	-
ESBL negative (972)	484 (89.8)	426 (93.6)	57 (96.5)	4 (99.6)	1 (100.0)	-	-	-	-	-	-	-
<i>Klebsiella</i> spp. (485)	104 (21.4)	159 (54.2)	77 (70.1)	53 (81.8)	22 (95.9)	21 (99.9)	10 (99.2)	6 (99.2)	9 (99.2)	-	-	-
ESBL phenotype (162)	5 (3.1)	6 (6.8)	24 (21.6)	36 (63.8)	21 (95.8)	21 (99.9)	10 (99.5)	4 (99.9)	9 (99.5)	-	-	-
ESBL negative (323)	99 (30.7)	153 (78.0)	53 (94.4)	17 (99.7)	1 (100.0)	-	-	-	-	-	-	-
<i>Enterobacter</i> spp. (239)	25 (10.9)	100 (52.7)	46 (72.8)	14 (79.7)	16 (85.4)	12 (99.4)	9 (94.1)	4 (95.8)	4 (97.5)	-	-	-
<i>Citrobacter</i> spp. (191)	18 (17.8)	49 (66.3)	9 (75.2)	9 (94.2)	1 (95.1)	3 (98.1)	3 (99.1)	7 (98.0)	1 (98.0)	-	-	-
Indole-pos. <i>Proteae</i> (109)	18 (17.4)	43 (56.3)	37 (90.8)	9 (98.2)	2 (100.0)	-	-	-	-	-	-	-
<i>P. mirabilis</i> (142)	3 (2.1)	45 (30.3)	79 (55.9)	10 (50.5)	4 (95.8)	2 (97.3)	2 (98.6)	2 (100.0)	-	-	-	-

**Conclusions:** CXA-201 demonstrated higher activity than currently available anti-PSA cephalosporins (CAZ and CPM) and P/T when tested against PSA and Enterobacteriaceae strains from EU hospitals and may represent a valuable treatment option for Gram-negative infections, including those caused by resistant organisms causing cIAI, cUTI and HAPB.

**PI447** Activity of BAL30072, alone and combined with beta-lactamase inhibitors or meropenem, against carbapenem-resistant Enterobacteriaceae

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**Background:** BAL30072 is a dihydroxypyridone-substituted mono-sulfactam active against many beta-lactamase producers, except those

with SHV ESBLs or hyperproducing AmpC enzymes. We investigated BAL30072's activity against 91 carbapenem-resistant Enterobacteriaceae, tested (i) alone, (ii) combined with BAL29980 (to inhibit AmpC enzymes) and/or clavulanate (to inhibit class A enzymes), or (iii) combined with meropenem.

**Methods:** MICs were determined by CLSI agar dilution; carbapenemases were identified by PCR and sequencing; isolates were from multiple UK hospitals to ensure epidemiological diversity.

**Results:** Overall, BAL30072 alone was active against 69% of the isolates at <4 mg/L, including 87% of isolates with OXA-48, 87% with a combination of AmpC and impermeability, 80% with IMP, 75% with NDM, 70% with ESBLs and impermeability, 67% with VIM and 40% with KPC enzymes. Nevertheless, MICs for a minority of isolates within each resistance-mechanism group ranged up to >128 mg/L. These data indicate that whilst none of the mechanisms engendered consistent resistance to BAL30072 by itself, resistance could arise in individual isolates if other mechanisms were also present. These potentially included further beta-lactamases, and 77% of the isolates became susceptible to BAL30072 at <4 mg/L if either BAL29980 or clavulanate was added at 4 mg/L whilst 89% were susceptible at <4 mg/L if both these inhibitors added, with >90% susceptibility among all the mechanism groups except those with KPC enzymes where, even with both inhibitors added, only 60% were susceptible. Many of the more resistant isolates were members of the *K. pneumoniae* ST258 clone, which typically has an SHV-11 or -12 ESBL along with KPC-2 or -3. BAL30072 + meropenem was active, at 4 + 4 mg/L against 77% of the isolates.

**Conclusions:** BAL30072 alone was active against most carbapenem-resistant Enterobacteriaceae but not some of those with multiple mechanisms, notably combinations of KPC and SHV enzymes. Its activity was expanded in the presence of beta-lactamase inhibitors (BAL29980 plus clavulanic acid), though this strategy was less successful against isolates with combinations of KPC and SHV enzymes. Adding meropenem to BAL30072 also achieved some gain in activity.

**P1448** **In vitro potency of novel tetracyclines against *Pseudomonas aeruginosa* and other major Gram-negative pathogens**

W. O'Brien, C. Fyfe, T. Grossman, C. Chen, R. Clark, Y. Deng, M. He, D. Hunt, C. Sun, X. Xiao, J. Sutcliffe\* (Watertown, US)

**Objectives:** To discover novel tetracyclines with enhanced *Pseudomonas aeruginosa* activity while maintaining in vitro activity against other important gram-negative pathogens.

**Methods:** The guidances and breakpoints of the Clinical Laboratory Standards Institute were used to determine the susceptibility of new compounds and comparators in microtiter-based cation-supplemented Mueller Hinton broth or in time-kill assays using 5 mL cultures. In the case of tigecycline, FDA breakpoints (if available) were used. In vitro potency against *Escherichia coli* DH10B strains genetically engineered to express tet(A), tet(B), tet(K), tet(M), tet(X) or blaNDM-1 was assessed. Compounds were also assessed for mechanism of action (MOA) using a coupled transcription/translation assay (TnT) fueled with S30 ribosomal extracts from either *Escherichia coli* or *P. aeruginosa*.

**Results:** Ten novel scaffolds were found that produced compounds with MICs against *P. aeruginosa* PA01 of 2–4 µg/mL and MIC90 values of 8–16 µg/mL against recent clinical isolates (n = 76). The MIC50/90 ranges against a separate panel of *P. aeruginosa* isolates from cystic fibrosis patients were 4–8/8–16 µg/mL. In vitro activity against panels of *Acinetobacter baumannii* and extended-spectrum beta-lactamase producing *Klebsiella pneumoniae* and *E. coli* was also retained by many compounds, with MIC50/90 values of ≤0.25/1 µg/mL and comparator MIC90 values of ≥32 µg/mL for tetracycline, ceftriaxone, imipenem, levofloxacin, gentamicin, tobramycin for *P. aeruginosa*, *Enterobacteriaceae* and *A. baumannii*. Two compounds were profiled in 24-hour time-kill studies using four isolates of

*P. aeruginosa* and generally found to be bactericidal at 4–8× the MIC. The new scaffolds retained activity against strains expressing genes encoding tetracycline-specific efflux pumps (Tet(A), Tet(B), Tet(K)), a ribosomal protection mechanism (Tet(M)), and a monooxygenase that inactivates tetracyclines (Tet(X)). The compounds inhibited protein synthesis in both TnT assays, with IC50 values 5–10× lower than conventional tetracyclines (1–2 µM).

**Conclusions:** This is the first report of novel tetracyclines with improved potency against contemporary *P. aeruginosa* isolates. These compounds retain activity against other major gram-negative pathogens and merit additional work to advance into development.

**P1449** **Antimicrobial activity of PTK 0796 (omadacycline) and comparator agents against contemporary pathogens commonly associated with community-acquired respiratory tract infections collected during 2011 from the European Union**

R. Flamm\*, D. Farrell, H. Sader, R. Jones (North Liberty, US)

**Objectives:** To determine the activity of PTK 0796 (omadacycline) and comparator agents against recent (2011) *Streptococcus pneumoniae* (SPN), *Haemophilus influenzae* (HI), and *Moraxella catarrhalis* (MCAT) isolated in the European Union (EU). PTK 0796 is a novel aminomethylcycline which is currently under clinical development for both intravenous and oral formulations. It has excellent activity against pathogens from the respiratory tract and overcomes tetracycline resistance.

**Methods:** Susceptibility (S) testing for omadacycline and commonly used antimicrobials was performed by Clinical and Laboratory Standards Institute (CLSI) broth microdilution methodology on a total of 1025 isolates in 2011 from medical centers in the SENTRY Antimicrobial Surveillance Program platform in the EU. S interpretations were performed using CLSI and EUCAST guidelines.

**Results:** PTK 0796 was very active against SPN independent of S to penicillin (PEN; MIC50/90, 0.06/0.06 mg/L for PEN-S and -resistant [R] strains). PTK 0796 was 16-fold more active than levofloxacin (MIC50/90, 1/1 mg/L) and ceftriaxone (MIC90, 1 mg/L) against SPN. SPN showed high R rates to erythromycin (S, 60.7%) and tetracycline (S, 69.2% CLSI/68.9% EUCAST) even though all isolates had PTK 0796 MIC values ≤0.25 mg/L. PTK 0796 against HI (13.9% beta-lactamase positive) and MCAT (98.5% beta-lactamase positive) exhibited low MIC values (Table) independent of beta-lactamase production.

	Organism (no. tested)		
	SPN (601)	HI (359)	MCAT (65)
<b>PTK 0796</b>			
MIC <sub>50/90</sub> <sup>a</sup>	0.06 / 0.06	0.5 / 1	0.12 / 0.12
<b>Ceftriaxone</b>			
MIC <sub>50/90</sub> <sup>a</sup>	≤0.06 / 1	≤0.06 / ≤0.06	0.25 / 0.5
%S <sup>b</sup>	91.9 (73.0)	100.0 (100.0)	100.0 (98.5)
<b>Erythromycin</b>			
MIC <sub>50/90</sub> <sup>a</sup>	≤0.12 / >16	NT <sup>c</sup>	NT <sup>c</sup>
%S <sup>b</sup>	60.7 (60.7)	NT <sup>c</sup>	NT <sup>c</sup>
<b>Levofloxacin</b>			
MIC <sub>50/90</sub> <sup>a</sup>	1 / 1	≤0.12 / ≤0.12	≤0.12 / ≤0.12
%S <sup>b</sup>	98.7 (98.7)	100.0 (100.0)	100.0 (100.0)
<b>Tigecycline</b>			
MIC <sub>50/90</sub> <sup>a</sup>	≤0.03/0.06	0.25/0.5	0.06/0.06
%S <sup>b</sup>	100.0 (IE) <sup>d</sup>	87.2 (IE) <sup>d</sup>	-(IE) <sup>d</sup>

a. MIC values in mg/L; b. Categorized by CLSI breakpoints and in parentheses by EUCAST breakpoints; c. categorized by USA drug package insert breakpoints and in parentheses by EUCAST breakpoints; d. IE= insufficient evidence to determine breakpoint; e. NT= not tested

**Conclusions:** PTK 0796 was very active against SPN, regardless of PEN-S status, with MIC50/90 value of 0.06/0.06 mg/L and no MIC

value >0.25 mg/L. PTK 0796 was also very active against *M. catarrhalis* (MIC50/90, 0.12/0.12 mg/L) and *H. influenzae* (MIC50/90, 0.5/1 mg/L) with activity independent of beta-lactamase status.

**P1450** Antimicrobial activity of PTK 0796 (omadacycline) tested against Gram-positive organisms isolated from European hospitals in 2011

H. Sader\*, R. Flamm, R. Jones (North Liberty, US)

**Objective:** To evaluate the activity of PTK 0796 (PTK) against Gram-positive (GP) cocci causing infections in European (EU) hospitals. PTK (7-dimethylamino, 9-(2,2-dimethyl-propyl)-aminomethylcycline) is a novel antibacterial agent of the tetracycline family, which is under clinical development (IV and oral formulations).

**Methods:** 2379 strains from 25 medical centers in 10 EU countries, Turkey and Israel were collected in 2011 and tested for susceptibility (S) against PTK, tigecycline (TIG) and many other comparators by CLSI broth microdilution methods. MIC results were interpreted according to EUCAST and CLSI breakpoint criteria. The isolates were collected mainly from skin/skin structure infections, bacteremia and pneumonia, and include *S. aureus* (1576; 27.4% oxacillin-resistant [MRSA]), coagulase-negative staphylococci (CoNS; 344, 71.5% oxacillin-resistant [R]), *E. faecalis* (EF; 270; 0.7% vancomycin [VAN]-R [MIC, ≥8 mg/L]), *E. faecium* (EFM; 156; 23.7% VAN-R), beta-haemolytic streptococci (BHS; 245) and viridans group streptococci (VGS; 132).

**Results:** PTK was very active against oxacillin-S *S. aureus* (MSSA) and MRSA with a MIC90 of 0.12 and 0.25 mg/L respectively (see Table). PTK activity against *S. aureus* was eightfold greater than linezolid and VAN, twofold greater than daptomycin and similar to TIG. MRSA rates varied from 1.0% in Sweden to 61.5% in Portugal (27.4% overall). The highest PTK MIC value among *S. aureus* was only 2 mg/L and >99% of strains were inhibited at PTK MIC of ≤0.25 mg/L. CoNS exhibited slightly higher PTK MICs (MIC50/90, 0.12/1 mg/L) compared to *S. aureus*, with a bimodal distribution. EF (MIC50/90, 0.12/0.25 mg/L) and EFM (MIC50/90, 0.06/0.12 mg/L) were very S to PTK and VAN R did not adversely affect PTK activity against enterococci. VAN-R EF was detected in 10 of 12 countries, while VAN-R EF was observed only in Germany and Italy (one strain each). BHS and VGS exhibited very low PTK MIC values (MIC50/90, 0.06/0.12 mg/L for all groups).

Organism (no. tested)	No. of isolates (cumulative %) with PTK 0796 MIC of:					
	≤0.03	0.06	0.12	0.25	0.5	1
<i>S. aureus</i> (1576)	13(0.8)	237(15.0)	1192(92.0)	121(99.7)	3(99.9)	1(100.0)
MSSA (1140)	8(0.7)	183(16.8)	878(93.8)	68(99.7)	3(100.0)	-
MRSA (432)	5(1.2)	54(13.7)	318(87.3)	53(99.5)	0(99.5)	1(100.0)
CoNS (344)	23(6.7)	117(40.7)	58(57.6)	34(67.4)	73(88.7)	2(100.0)
<i>E. faecalis</i> (270)	25(9.3)	103(47.4)	94(82.2)	46(99.3)	1(99.6)	1(100.0)
<i>E. faecium</i> (156)	21(13.5)	105(80.8)	27(98.1)	3(100.0)	-	-
VAN-S (119)	19(16.0)	81(84.0)	16(97.5)	3(100.0)	-	-
VAN-R (37)	2(5.4)	24(70.3)	11(100.0)	-	-	-
BHS (245)	56(22.9)	124(73.5)	50(93.9)	14(99.6)	1(100.0)	-
VGS (132)	32(24.2)	67(75.0)	23(92.4)	8(98.5)	1(99.2)	1(100.0)

**Conclusions:** PTK demonstrated potent activity against a large collection of contemporary (2011) GP clinical isolates. Its activity was similar to that of TIG and was not affected by R to other antimicrobial classes.

**P1451** Synthesis and antibacterial activity of isoindoline-containing pentacyclines: a novel class of tetracycline analogs with oral bioavailability

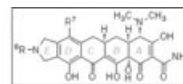
R. Clark, D. Hunt, M. He, C. Fyfe, W. O'Brien, T. Grossman, J. Sutcliffe, X. Xiao\* (Watertown, US)

**Objectives:** Antimicrobial resistance drives the increasing need for discovery and development of new antibiotics. Novel pentacyclic tetracycline analogs containing an isoindoline moiety, accessible by

total synthesis, were designed to explore their potential to achieve antibacterial potency and overcome tetracycline resistance while retaining oral bioavailability and other desirable physicochemical properties of the tetracycline class.

**Method:** Pentacyclic tetracycline analogs containing an isoindoline as the DE-ring moiety were synthesized from a DE-ring and an AB-ring precursor via a tandem Michael-Dieckmann annulation. These new isoindoline analogs were evaluated for in vitro antibacterial activity by susceptibility testing according to CLSI guidance against a panel of bacteria strains including organisms expressing tetracycline-resistant ribosomal protection tet(M) or efflux tet(K) or tet(A). In vivo efficacy was assessed in a mouse septicemia model against *Staphylococcus aureus* ATCC 13709.

**Results:** Antibacterial activity of representative isoindoline analogs are shown in the table below. Additional studies showed that compound TP-834 had 48.3% oral bioavailability in rats and ED50 values of 1.5 mg/kg (IV) and 6.2 mg/kg (PO) in the mouse septicemia model.



TP Number	MIC (µg/ml)										Survival (%)		
	SA101	SA181	SA158	SE164	EP159	SP108	SP380	HI	MAC205	IV	PO	3 mg/kg	30 mg/kg
TP-834	0.25	2	0.25	0.0313	1	0.0156	0.0625	1	0.25	100%	83%		
TP-880	0.25	2	0.0625	<0.0156	2	<0.0156	0.5	1	0.125	50%	50%		
TP-925	0.25	1	0.125	n/t	1	0.0156	0.125	n/t	n/t	50%	0%		
TP-568	0.5	2	0.125	0.0625	2	<0.0156	0.0625	0.5	0.125	100%	33%		
TP-314	1	2	0.25	0.0625	2	<0.0156	0.25	2	0.5	100%	20%		
TP-405	0.5	2	0.125	0.0625	2	<0.0156	0.125	1	0.25	100%	100%		

SA: *S. aureus*; SE: *S. epidermidis*; EF: *E. faecalis*; SP: *S. pneumoniae*; HI: *H. influenzae*; MC: *M. catarrhalis*; n/t: not tested.

**Conclusion:** Novel isoindoline-containing pentacyclines have potent in vitro activities against tetracycline-resistant, Gram-positive and Gram-negative bacterial strains, especially pathogens commonly implicated in community-acquired bacterial pneumonia (CABP). A number of the new analogs showed excellent IV and oral in vivo efficacy in a mouse septicemia model of infection. Compound TP-834 demonstrated promising oral bioavailability (%F = 48.3%, rats) and IV/oral efficacy (ED50 = 1.5 mg/kg IV, 6.2 mg/kg PO, mouse septicemia) and was selected for further pre-clinical development.

**P1452** The novel isoindoline-containing pentacycline TP-834 is active against community and biothreat respiratory pathogens, and problematic Gram-positive pathogens

T. Grossman\*, W. O'Brien, C. Fyfe, J. Sutcliffe (Watertown, US)

**Objective:** TP-834 is a novel, fully-synthetic IV/oral pentacyclic antibiotic. TP-834 was selected from over 2000 analogs on the basis of its potency and spectrum, especially against multidrug-resistant (MDR) pathogens implicated in community-acquired bacterial pneumonia (CABP) and problematic Gram-positive infections. The goal of these studies was to profile the potency and spectrum of activity of TP-834 against panels of recent clinical isolates.

**Methods:** Using standard CLSI methodology, TP-834 and clinical comparators were tested against recent clinical isolates and laboratory *Escherichia coli* strains recombinantly expressing individual tetracycline resistance genes: tet(A), tet(B), tet(M), tet(K) and tet(X). The anti-translation activity of TP-834 was confirmed in an *E. coli* in vitro coupled transcription/translation (TNT) assay in the presence and absence of purified Tet(M) protein.

**Results:** TP-834 showed good antimicrobial potency against key MDR Gram-positive and Gram-negative pathogens responsible for CABP and skin infections, with representative MIC90 values of 1, 0.5, 1, 0.5, 0.12, 1 and 0.25 µg/mL for MRSA (n = 99, including PVL+), MSSA (n = 50), *Enterococcus faecalis* (n = 157, including VRE), *Enterococcus faecium* (n = 116, including VRE), *Streptococcus*

*pneumoniae* (n = 118, including penicillin- and macrolide-resistant), *Haemophilus influenzae* (n = 64) and *Moraxella catarrhalis* (n = 64). The MIC of TP-834 (1 µg/mL) against a laboratory strain of *E. coli* was unaffected, or minimally affected, by expression of common tetracycline-resistance genes (≤fourfold shift in MIC), as compared to that of tetracycline (MIC = 2 µg/mL; ≥64-fold shift in MIC with expression of tetracycline resistance genes). The IC50 of TP-834 in the TNT assay was 0.85 µg/mL and was unaffected by the addition of purified Tet(M).

Organism	N	MIC50/MIC90 (mg/ml)						
		TP-834	TET	TGC	MACRO	LVX	LZD	VAN
<i>Streptococcus pneumoniae</i>	118	0.06/0.12	1/32	0.06/0.06	>4/4	0.5/1	1/1	0.25/0.5
<i>Streptococcus pneumoniae pen-R</i> <sup>a</sup>	58	0.06/0.12	16/32	0.06/0.06	>4/4	0.5/1	1/1	0.25/0.5
<i>S. pneumoniae mac-R</i> <sup>b</sup>	75	0.06/0.12	16/32	0.06/0.06	>4/4	1/1	1/1	0.25/0.5
<i>Streptococcus pyogenes</i>	64	0.12/0.25	0.25/32	0.06/0.06	0.12/4	0.5/1	1/1	0.5/0.5
<i>Streptococcus agalactiae</i>	50	0.5/0.5	32/32	0.12/0.12	0.06/4	0.5/1	1/1	0.5/0.5
<i>Streptococcus anginosus</i>	42	0.12/0.5	0.5/16	0.056/0.06	0.06/16	0.5/1	1/2	0.5/1
<i>Streptococcus intermedius</i>	30	0.12/0.12	0.25/4	0.03/0.12	0.06/0.5	1/2	1/1	0.5/0.5
<i>Streptococcus mitis</i>	29	0.06/0.25	0.5/4	0.03/0.12	>0.5/0.5	1/2	1/1	0.5/0.5
<i>Streptococcus sanguis</i>	18	0.06/0.12	0.25/2	0.03/0.06	0.03/0.5	0.5/2	0.5/1	0.5/1
<i>Staphylococcus aureus</i> (MRSA)	99	0.5/1	0.25/32 <sup>c</sup>	0.13/0.25	>4/64 <sup>d</sup>	4/32	2/4	1/1
<i>S. aureus</i> (MRSA) PVL <sup>e</sup>	30	0.25/0.25	0.25/0.25	0.12/0.12	>4/4	0.25/32	1/2	1/1
<i>S. aureus</i> (MSSA)	50	0.25/0.5	0.25/0.5	0.12/0.25	>4/4	0.25/1	2/4	1/1
Coagulase-negative <i>Staphylococcus</i>	88	0.25/1	0.5/1	0.12/0.25	>4/4	0.5/4	1/2	1/2
<i>Enterococcus faecalis</i>	157	0.5/1	>32/32	0.06/0.12	>4/4 <sup>f</sup>	>4/32	2/2	2/32
<i>E. faecalis</i> (VRE)	64	0.5/1	>32/32	0.06/0.12	>4/4 <sup>f</sup>	>4/32	2/2	>16/32
<i>E. faecalis</i> (VSE)	99	0.5/1	>32/32	0.06/0.12	>4/4 <sup>f</sup>	1/32	2/4	1/2
<i>Enterococcus faecium</i>	116	0.12/0.5	0.25/32	0.06/0.12	>4/4 <sup>f</sup>	>4/32	2/4	>16/32
<i>E. faecium</i> (VRE)	65	0.12/1	0.5/32	0.06/0.13	>4/4 <sup>f</sup>	>4/32	2/4	>16/32
<i>E. faecium</i> (VSE)	51	0.12/0.5	0.25/32	0.06/0.06	>4/4 <sup>f</sup>	>4/4	2/2	0.5/1
<i>Haemophilus influenzae</i>	64	0.5/1	0.5/4	0.12/0.25	1/4	0.03/0.03	ND	ND
<i>Moraxella catarrhalis</i>	64	0.12/0.25	0.25/0.5	0.06/0.06	<0.13/0.13	0.06/0.06	ND	ND

TET=tetracycline; TGC = tigecycline; MACRO=azithromycin or erythromycin; LVX=levofloxacin; LZD = linezolid; VAN=vancomycin; PE=penicillin; ND = not done; penicillin MIC ≥2 mg/ml; <sup>a</sup> mac-R=macrolide-resistant; <sup>b</sup> 82 isolates; <sup>c</sup> 94 isolates; <sup>d</sup> 101 isolates; <sup>e</sup> 48 isolates; <sup>f</sup> 53 isolates; <sup>g</sup> 100 isolates; <sup>h</sup> 50 isolates

**Conclusions:** TP-834 shows excellent potency against key MDR community respiratory and problematic Gram-positive pathogens, including those with common tetracycline-resistant mechanisms. TP-834 shows promise as an IV/oral agent for the treatment of complicated community CABP and infections due to MDR Gram-positive organisms.

#### P1453 Susceptibility to manuka honey of clinical strains of *Staphylococcus aureus* with varying sensitivity to vancomycin (VISA)

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**Objectives:** The challenge posed by *Staphylococcus aureus* as a health threat has increased by the acquisition of multiple resistance to antibiotics, including vancomycin. New antimicrobials to combat the growing number of resistant bacteria are needed. Investigations into the antimicrobial effects of manuka honey have demonstrated that it inhibits a wide range of microbes; it is also a licensed medical product in most developed countries. Currently the susceptibility of clinical strains of vancomycin sensitive/intermediate *S. aureus* is unknown and the aim of this study was to address this deficiency.

**Methods:** One hundred and thirty nine strains of *Staphylococcus aureus* were isolated from swabs collected from a range of infected wounds across the world, submitted to the Specialist Antimicrobial Chemotherapy Unit, Cardiff. Of the isolates tested here 71 were identified as hVISA, 18 as VISA and 50 as VSSA by standard bacteriological techniques and confirmed using the "gold standard technique Population analysis profile – area under curve (PAP-AUC). The minimum inhibitory concentration was determined using standard CLSI broth microdilution methodology with Mueller Hinton broth (MHB). Manuka honey was used at 1% (w/v) intervals from 10% to 1% (w/v). Inocula were prepared from direct colony suspension, and microtitre plates were inoculated with 105 CFU/mL. Plates were incubated in air at 37°C for 18 hour. The MIC was defined as the lowest concentration of the antimicrobial that prevented visible growth of a microorganism in a broth dilution susceptibility test.

**Results:** The MIC values were consistent with 139 isolates exhibiting an MIC of ≤6% (w/v) to manuka honey. Isolates of hVISA, VISA and

VSSA had a mean MIC % (w/v) of 3.9 ± 0.6, 3.6 ± 0.6 and 4.3 ± 0.6, respectively. VSSA isolates had marginally greater MIC values compared to hVISA and VISA isolates, indicating lower susceptibility. **Conclusion:** In this study all of the isolates tested were inhibited by a concentration of manuka honey ≤6% (w/v). This indicates that manuka honey at very low concentrations inhibits the growth of clinical isolates of *S. aureus* possessing varying patterns of vancomycin resistance in vitro and could potentially be used as a valid co treatment, to help decontaminate wounds infected with *S. aureus*. Many modern wound care products contain undiluted honey and the low MIC observed here suggest that it would be possible to deliver the honey at effective concentrations in a clinical setting.

#### P1454 Activity of novel peptides against planktonic and biofilm cultures of colistin-resistant Gram-negative bacilli

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**Objectives:** A number of D-confirmation alpha-helical peptides have been developed that are structurally manipulated to alter their hydrophobicity, net charge and amphipathicity properties. Charge substitutions and structure alterations on both the polar and non-polar faces of these peptides produce a unique "carpeted model" mechanism of action, broad spectrum of activity, and low level of toxicity to human cells. The peptides are membrane targeted, prokaryotic specific and have prolonged half-lives. This initial evaluation was designed to investigate the activity of five peptide constructs against a number of different gram negative bacilli with known resistance to colistin.

**Methods:** Five peptides were tested RH035, RH148, RH166, RH282 and RH 297. Colistin was also included. *Pseudomonas aeruginosa* ATCC 27853 was the quality control strain. Colistin-resistant *E. coli* (two strains isolated in New York state), one strain of *Serratia marcescens*, one strain of *P. aeruginosa*, and two strains of *Acinetobacter baumannii* complex were tested. MICs were determined by broth microdilution according to CLSI methodology, and biofilm (MBEC) MICs were measured using the Innovotech biofilm susceptibility methodology (Innovotech, Edmonton, CA). Results were read visually and compared to planktonic MICs. All strains formed biofilms in these assays.

**Results:** Colistin MICs varied from 4 to >500 mg/L (*S. marcescens* and *Acinetobacter*). The *S. marcescens* strain was resistant (>500 mg/L) to all peptides tested. For RH035, all other clinical strains had MICs of 16–32 mg/L. RH282, had similar MICs except for one *Acinetobacter* (MIC 250 mg/L). In the sessile state, all the colistin MICs were >256 mg/L. The activity of RH 035 was reduced by one dilution against the colistin-resistant *E. coli*, but sessile MICs for the other peptides were reduced by 2–3 dilutions compared to the planktonic MICs.

**Conclusions:** RH035 and RH282 were the most active peptides in these studies. Planktonic MICs were similar to those for colistin, Sessile MICs for the RH035 peptide were similar to planktonic MICs for the *E. coli*. All strains had high sessile colistin MICs. It may be possible to alter the peptide structure to maintain activity in biofilms and provide alternatives for therapy of some bacterial species existing as biofilms.

#### P1455 Mechanism of action of novel antibacterial aggregating peptides

N.G. Bednarska\*, P. Baatsen, J. Schymkowitz, F. Rousseau, J. Van Eldere (Leuven, BE)

**Objectives:** Specifically designed short interfering peptides (aggregators) can induce aggregation of proteins within bacterial cells. We have already identified several aggregators that have potent antimicrobial activity against a broad range of Gram positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE) and other clinically relevant species (1). Here we present data on the mechanism of action of the most potent of these novel antibacterial peptides.



**Methods:** MIC values were determined by the microdilution method (according to EUCAST). The antimicrobial killing kinetics were assessed by measuring changes in the viable counts of bacteria exposed to test compounds. The fluorescent dyes(DiOC2(3) and Sytox Green) were used to measure the membrane potential and permeability. Scanning (SEM) and transmission electron microscopy (TEM) were used to examine the morphological changes. Immunoelectron microscopy (IEM) was used to show peptide internalization. The early and late bacterial responses to treatment were established by 2D gel analysis. Shotgun proteomic identification was performed to study interactions between the aggregators and their target. Western blot was used for target confirmation.

**Results:** Aggregators showed strong, selective and rapid bactericidal activity towards clinically important pathogens (MIC values in the 0.5–10 µg/mL) range; 5–20 minute for 3 log reduction of viable count). Membrane integrity analysis dismissed non-specific membrano-lytic properties; this was confirmed by TEM and SEM ultrastructural examination. IEM imaging proved peptide internalization and formation of electron-dense aggregates. 2D gel analysis confirmed bacterial heat-shock responses following treatment with aggregators. Extensive proteomic analysis proved the concept of targeted protein aggregation.

**Conclusions:** We have shown that these aggregator proteins induce specific aggregation of the selected target proteins. With appropriate target proteins, the specific aggregation of target bacterial proteins leads to a strong and very rapid bactericidal effect that is independent of other antibiotic resistant mechanisms.

**Reference** 1. Bednarska NG, Schymkowitz J, Rousseau F, Van Eldere J. (2011) Targeted protein aggregation as a novel antimicrobial strategy, ICAAC Chicago, 17–20 September 2011.

**P1456 In vitro evaluation of antimicrobial spectrum and determination of optimal MIC test conditions for the novel protein epitope mimetics antibiotic POL7080**

H.S. Sader, D.J. Biedenbach, R.N. Jones, K. Dembowski, C. Zampaloni, G.E. Dale\* (North Liberty, US; Allschwil, CH)

**Objectives:** To evaluate the activity of the (protein epitope mimetics) PEM antibiotic POL7080 tested against a large collection of *P. aeruginosa* isolates and a selected group of other non-fermentative Gram-negative bacilli and Enterobacteriaceae species. The study also aimed to determine if a surfactant should be included as a standard test condition for POL7080 MIC determination by broth microdilution methods.

**Methods:** The activity of POL7080 was tested against a panel of organisms (563 strains) selected from the SENTRY Antimicrobial Surveillance Program organism collection from medical centers in Europe (EU) and the United States (USA). The strains included *P. aeruginosa* (431 strains), *Pseudomonas* spp. (56 strains), *S. maltophilia* (11 strains), *B. cepacia* (11 strains) and various Enterobacteriaceae (54 strains). The activity of POL7080 with and without the surfactant (Polysorbate-80 [P.80], 0.002%) was compared with the activity of colistin, polymyxin B, tobramycin, ceftazidime, piperacillin/tazobactam, meropenem and ciprofloxacin. CLSI M07-A8 (2009) methods were used for testing by the broth microdilution test and CLSI M100-S21 (2011) interpretive criteria were used to determine susceptibility for comparator agents.

**Results:** POL7080 MIC values for *P. aeruginosa* strains were generally one doubling dilution lower when testing with P-80 when compared to the MIC results obtained without P-80. POL7080 was very active against clinical strains of *P. aeruginosa* with MIC50/MIC90 values of 0.12/0.25 mg/L. In addition POL7080 showed good activity against *P. stutzeri*, *P. luteola*, *P. alcaligenes* and *P. mendocina*. In contrast, POL7080 showed limited activity against the other *Pseudomonas* spp. tested as well as against *S. maltophilia*, *B. cepacia* and Enterobacteriaceae.

**Conclusions:** POL7080 demonstrated potent in vitro activity when tested against a large collection of contemporary strains (2009–2010) of

*P. aeruginosa* from the USA and EU. POL7080 MIC values obtained in the presence of P-80 (0.002%) were generally twofold lower than those obtained without P-80, indicating that this surfactant should be added to the testing medium when testing POL7080 by reference broth microdilution methods.

**P1457 In vitro evaluation of MBCs, killing kinetics and inoculum effect of the novel *Pseudomonas aeruginosa* antibiotic POL7080**

F. Bernardini\*, M. Manon, G. Kunz, G.E. Dale, K. Dembowski (Allschwil, CH)

**Objectives:** To evaluate the bactericidal activity of the PEM (Protein Epitope Mimetics) antibiotic POL7080 against a panel of sensitive and MDR *Pseudomonas aeruginosa* clinical isolates, by determining MBCs. The in vitro killing kinetics were also determined against selected clinical strains. Another aim was to assess a possible inoculum effect against *Pseudomonas aeruginosa* clinical and MDR isolates.

**Methods:** The in vitro MBCs were determined against a panel of organisms (seven strains) selected from the SENTRY Antimicrobial Surveillance Program organism collection from medical centers in Europe (EU) and the United States (USA). The in vitro killing kinetics were determined at one, two, four and eightfold the corresponding MIC, against one sensitive and three MDR clinical isolates. The possible inoculum effect was determined in the range 10<sup>4</sup>–10<sup>8</sup> against one sensitive and four MDR clinical isolates. In all studies the *Pseudomonas aeruginosa* ATCC 27853 control strain was included.

**Results:** An MIC range of 0.03–1 mg/L was derived from the in vitro bactericidal activity of POL7080 against the panel of seven strains. The corresponding MBC values were in average two times the MIC against all tested strains, with a range of one to fourfold the MIC. This result was confirmed by determining the in vitro killing kinetics against a panel of clinical isolates which showed a rapid and irreversible 3-log reduction of bacterial counts at two times the MIC, within 4–6 hour. A total of six strains (including ATCC 27853) were tested for possible inoculum effects and no differences in MICs were found up to 10<sup>7</sup> CFU/mL inoculum. A fourfold increase was found at 10<sup>8</sup> CFU/mL against all strains, but POL7080 remains very potent also at a high inoculum, with a median MIC of 0.125 mg/L against MDR clinical isolates.

**Conclusions:** POL7080 has potent in vitro bactericidal activity against MDR clinical isolates which is not affected by inoculum.

**P1458 The activity of PMX-30063 against staphylococci and streptococci**

I. Morrissey, J. Dallow, E. Siegwart, A. Smith, R. Scott, B. Korczak\* (Fordham, UK; Radnor, US)

**Objective:** PMX-30063 is a novel host defense protein mimetic currently undergoing phase 2 clinical trials in patients with acute bacterial skin and skin structure infections. This study evaluated the activity of PMX-30063 against recent clinical isolates of important pathogens associated with these infections including Staphylococci and beta-haemolytic Streptococci from world-wide locations.

**Methods:** MIC was determined by CLSI broth microdilution methodology for PMX-30063 and comparator antibiotics against a total of 393 Gram-positive bacterial isolates.

**Results:** MIC distribution for PMX-30063 and MIC 50/90 data are shown in the Table. PMX-30063 MIC was distributed over a very narrow concentration range with a lower MIC against Staphylococci than against Streptococci. The activity of PMX-30063 was not affected by methicillin or multi-drug resistance (MDR; resistant to ≥3 classes) in Staphylococci or resistance to erythromycin or tetracycline in Streptococci.

**Conclusions:** PMX-30063 showed potent activity against the Gram-positive bacteria tested, particularly Staphylococci. This novel

Species (N)	% PMX-30063 MIC distribution (mg/L)							MIC (mg/L)	
	0.25	0.5	1	2	4	8	50	90	
<i>S. aureus</i> (99)		75	25				0.5	1	
MRSA (40)		70	30				0.5	1	
MSSA (59)		78	22				0.5	1	
<i>S. epidermidis</i> (57)	12	88					0.5	0.5	
MRSE (35)	14	86					0.5	0.5	
MSSE (22)	9	91					0.5	0.5	
<i>S. haemolyticus</i> (25)		100					0.5	0.5	
<i>S. hominis</i> (24)	71	29					0.25	0.25	
MDR Staphylococci (112)	15	74	11				0.5	1	
<i>Str. agalactiae</i> (78)					83	17	4	8	
<i>Str. dysgalactiae</i> (220)					82	18	4	8	
<i>Str. pyogenes</i> (88)				2	93	5	4	4	
Ery-R Streptococci (30)					67	33	4	8	
Tet-R Streptococci (72)					78	22	4	8	

antimicrobial is unaffected by resistance to existing antibiotics, including MDR Staphylococci.

#### P1459 Peptide phosphorodiamidate morpholino oligomers as antisense therapeutics for *Mycobacterium tuberculosis*

P. Iversen\*, R. Muhammad, V. Bollampalli, E. Chryssanthou, M. Maeuer, A. Rothfuchs (Bothell, US; Stockholm, SE)

**Objectives:** *Mycobacterium tuberculosis* (Mtb) is the leading cause of death worldwide due to a single microorganism. There is today an urgent clinical need to generate new therapeutics against Mtb. Peptide phosphorodiamidate morpholino oligomers (PPMOs) are DNA analogs that silence expression of specific genes. The objective of these studies was to determine if PPMO compounds directed against essential mycobacterial genes can be used to control mycobacterial replication and survival in liquid culture and when grown in macrophages. The selected PPMO candidates target known mycobacterial genes that if deleted in mycobacteria, lead to severe growth defects in 7H9 broth, macrophages and in vivo in the lung of mice.

**Methods:** To begin studying the role of PPMO compounds in blocking mycobacterial growth, log phase cultures of *M. bovis* BCG were diluted in media containing 20  $\mu$ M concentrations of PPMOs targeting expression of three different mycobacterial gene targets. BCG growth was monitored for 1–2 weeks by nephelometry (OD 600 nm), colony formation on 7H11 agar, and via the automated fluorometric MGIT 960 detection system that measures mycobacterial O<sub>2</sub> consumption in 7H9 broth (BD Biosciences). Kanamycin was used as a positive control and scramble sequence PPMO and saline vehicle were the negative controls. Bone marrow derived macrophages (BMM) were infected with BCG in the presence of PPMOs and mycobacterial colony formation enumerated at different time points after infection. IFN $\gamma$  was the positive control and scramble sequence PPMO the negative control.

**Results:** PPMO 11-mers targeting either *leuD* or *mgtC* were effective inhibitors of mycobacterial growth in 7H9 broth. The oligomers reduced OD by 20–80% in three different experiments. Colony forming numbers were reduced by up to 1 log by day 8 of incubation with the PPMOs. Growth analysis by MGIT 960 confirm activity of kanamycin and demonstrate growth inhibition by both *leuD* and *mgtC* PPMO with no significant growth inhibition in scramble control PPMO relative to saline vehicle controls. Growth of BCG in BMM was reduced by over 1 log for PPMO targeting *mgtC*, *leuD* and *pirG*.

**Conclusions:** These observations provide reproducible evidence of robust inhibition of mycobacterial growth by PPMOs and support the use of PPMO in anti-Mtb therapy. Studies are planned to evaluate these agents in multiple drug resistant (MDR) and extensively drug resistant (XDR) Mtb.

#### P1460 Entry of antisense constructs into *Streptococcus mutans*

F. McLeod\*, R. Simmonds (Dunedin, NZ)

**Objectives:** The emergence of antibiotic resistant bacteria has re-stimulated the search for new antimicrobials. Antisense Oligonucleotides (AS-ODN), target genes in a sequence specific

manner and inhibit gene function. However, barriers such as peptidoglycan and lipopolysaccharide membranes, limit uptake of the relatively large AS-ODN's. The lytic streptococcolytic enzyme zocoin A, facilitates entry of phosphorothioate oligodeoxynucleotides (PS-ODNs) into *Streptococcus mutans* and the observed degree of phenotypic response (cell growth inhibition) was sequence specific and correlated with the amount of zocoin A ( $R^2 = 0.9885$ ) and PS-ODN ( $R^2 = 0.9928$ ) (Dufour et al. 2011). The objective of the current study was to discover new agents that facilitate the entry of AS-ODN into *S. mutans*.

**Methods:** Using the method described in Dufour et al. 2011, agents such as beta-lactam antibiotics were screened to determine their ability to facilitate the entry of a PS-ODN designed to directly bind to the first 18 bp of the fructose-1, 6-bisphosphate adolase gene (*fba*) in *S. mutans*. PS-ODN's cause the degradation of the target gene mRNA by forming RNA-DNA hybrids, activating endoribonuclease Rnase H. Each agent was titrated against *S. mutans* in various growth phases and the length of time taken for the culture density to increase by 0.1 OD determined. Agents found to significantly increase this time period were retested in combination with either the targeted or a non-target PS-ODN control. The mRNA expression level of the target PS-ODN gene (*fba*) was monitored over time by use of qRT-PCR. The rate of entry non-targeted PS-ODN construct into *S. mutans* was determined using 32P-labelled PS-ODN.

**Results:** The beta-lactam antibiotic penicillin (0.8  $\mu$ g/mL) combined with 10 mM PS-ODN significantly ( $p \leq 0.001$ ) inhibited the growth of lag phase cells compared to that observed in the presence of either compound alone and qRT-PCR showed a significant decrease (650-fold) in target *fba* transcript levels of lag phase cells. Treatment of exponential phase cells with zocoin A allowed ~4000 radiolabelled PS-ODN molecules to enter the cells over a 30 minutes period. In contrast, treatment of exponential phase cells with penicillin allowed only 0.09 PS-ODN molecules to enter each cell in 30 minutes.

**Conclusions:** These results show that PS-ODN when combined with clinically useful antibiotics such as penicillin, can down-regulate target gene expression and decrease cell growth rate.

#### P1461 Bacteriophage treatment inhibits and reduces biofilm formation by *Pseudomonas aeruginosa* strains from cystic fibrosis patients

S. Morales\*, G. Mearns, R. Cole, A. Smithyman (Sydney, AU)

Chronic lung infections caused by *Pseudomonas aeruginosa* (PA) are a major cause of morbidity and mortality in cystic fibrosis (CF) patients. Of particular concern is the development of multi-drug resistant (MDR) forms of these bacteria that are proving increasingly difficult to treat with conventional antibiotics. In some cases effective antibiotic therapy is no longer available and there is, therefore, an urgent need to develop alternative means of controlling infection in these patients.

The use of lytic bacteriophages (phages) as a potential therapeutic tool provides a promising alternative/complementary option for the treatment of bacterial infections. However, questions have been raised about the ability of phages to penetrate and disrupt biofilms, such as those formed by PA in the lungs of CF patients. To examine this, biofilms formed by PA isolates from CF patients were subjected to treatment by phage cocktails specifically developed against MDR isolates from four geographical areas.

**Objective:** To evaluate the effect of phage cocktails on biofilm formation by PA strains isolated from CF patients.

**Methods:** PA strains isolated from CF patients were collected in Australia, US and the UK. The strains were either (i) exposed to phage treatment and then allowed to form biofilms over a 48 hours period in an artificial sputum medium or (ii) allowed to form biofilms and then subsequently treated with the phage cocktails. To measure any effect, colony forming units (CFU) and plaque forming units (PFU) counts were made 48 hours after treatment.

**Results:** The prophylactic use of phage cocktails resulted in a 66% to 99% inhibition of biofilm development in comparison to the controls

( $p < 0.05$ ) while phage treatment after 48 hours showed biofilm reduction of 69–98% in comparison to the controls ( $p < 0.05$ ). In both treatments, an increase in bacteriophage concentration of 76–160% was observed.

**Conclusion:** These results demonstrated that bacteriophage cocktails were capable not only of significantly reducing PA populations in existing biofilms but also preventing biofilm development itself. This dual ability of phages to both disrupt biofilms and kill MDR organisms addresses two major weaknesses of conventional antibiotic treatment and suggests that bacteriophage therapy may play an important role in the future treatment of MDR-PA in cystic fibrosis. In vivo studies are now underway to confirm the above findings.

#### P1462 Isolation of bacteriophage against currently circulating strains of *Acinetobacter baumannii*

J.F. Turton\*, C. Perry, M. Hannah (London, UK)

**Objectives:** *Acinetobacter baumannii* is an opportunistic pathogen that is usually resistant to most antibiotics. This multiresistance has sparked fears that there may shortly be no therapeutic agents to combat infections with this organism, leading to a revival in interest in use of bacteriophage as a potential therapeutic agent. However, the host range is often limited. We sought bacteriophage in activated sewage sludge against particular clinical strains to considerably increase the chance of obtaining bacteriophage to which that strain would be susceptible.

**Methods:** A fresh sample of activated sewage sludge was obtained from Thames water and cultures of representatives of clinical strains of *A. baumannii* grown in the presence of the sediment. The supernatants were filtered through 0.45  $\mu\text{m}$  filters and tested against the host strains used. Those producing plaques on a lawn of bacteria were subjected to three rounds of plaque purification, and suspensions containing  $10^5$  PFU/mL tested on a wider panel of previously characterized isolates. These had been typed by a combination of tri-locus sequence typing, pulsed-field gel electrophoresis and Variable Number Tandem Repeat analysis. Purified bacteriophage suspensions were examined by electron microscopy.

**Results:** We successfully isolated bacteriophage active against two of the four strains cultured with the activated sewage sludge sediment. Of the 35 isolates in the testing panel, which included strains belonging to each of the three international clonal lineages and sporadic and minor strains, only nine, all belonging to strains of international clone II, were susceptible to the phage. Representatives of the South East (SE) clone, in particular, were sensitive (7/8 isolates), as was a representative of the North West strain. The SE clone is one of the successful sublineages of international clone II that has affected multiple hospitals in the UK. Representatives of OXA-23 clone I, which also belongs to international clone II, and is the most prevalent type in the UK, were not susceptible (8/8), nor were isolates of four other strains belonging to international clone II.

**Conclusion:** Activated sewage sludge is a rich source of bacteriophage against currently circulating strains of *A. baumannii*. We have isolated a phage of the Myoviridae family active against most representatives of the SE clone, a sublineage of international clone II resistant to most antibiotics that has infected many patients in UK hospitals.

#### P1463 Case series: clinical experience of taurolidine use in 38 complex clinical cases including salvage therapy

B. Sangers\*, D. Puli, M. Wong, M. Qazi, A. Guleri (Blackpool, UK)

**Background:** Taurolidine ([bis(1,1-dioxoperhydro-1,2,4-thiadiazinyl-4)-methane]) is a drug with antimicrobial and anti-lipopolysaccharide properties. Derived from the amino acid taurine, its immunomodulatory action is reported to be mediated with priming and activation of macrophages and polymorphonuclear leukocytes. Its license and literature search reveal its predominant use as a venous line locking system to prevent catheter line infections. We present here clinical experience of taurolidine use in 38-complex cases over 4-years.

**Methods:** Retrospective case notes review of 38-cases over 4-years (July 2007–till date). Taurolidine is licensed as a medical device. Its use outside this indication as a salvage therapy was agreed through a treating consultant – consultant microbiologist discussion and after obtaining patient consent.

**Results:** The key results from 38-patients include: 57.8% (22/38) males; Age ranged from 15 to 84 year, with mean age of 62 years. Twenty-eight percent (7/38) patients were from haematology; followed by 23% (9/38) general surgery; 18% (7/38) cardiothoracic surgery; 15% (6/38) vascular surgery and 13% (5/38) oncology. Commonest indication for use included hickman line lock (following recurrent line infection/removal). Hickman line used for chemotherapy in 44% (17/38); followed by total parenteral nutrition and long term antibiotics in 18% (7/38) each; Unusual salvage situations included: five cases of infected femoro-popliteal vascular grafts with limb loss potential of 29–70%. Two cases of pleural irrigation for empyema thoracis with and one case of deep sternal wound infection with *Pseudomonas aeruginosa* unresponsive to long term systemic antibiotics. A complex surgical case with enterocutaneous fistula, over a dozen episodes of candidaemia, multiple central line infections had a successful outcome with taurolidine locked hickman line. Duration of taurolidine use ranged from 1 to 10 weeks with mean of 2.73 weeks. Clinical improvement and successful clearance in regular check cultures was used to define successful outcome.

S no	Pt demo	Speciality	Diagnosis	Place of infection	Use of taurolock	Duration
1	67/m	Haematology	AML	Hickman line	Line flush	3 weeks
2	75/f	Vascular	Lower limb Bypass- Dacron-infected	Long term abx line hickman- inf	Line flush	4 weeks
3	69/f	Cardio thoracic	Infected prosthetic valve	Long term abx line Hickman line inf	Line flush	2 weeks
4	60/m	Gen surgery	Leaked anastomosis	TPN line hickman	Line flush	4 weeks
5	41/m	Gen surgery	Intra abdo abscess	TPN hickman line inf	Line flush	3 weeks
6	60/m	Gen surgery	Ca colon post op chemo	Chemo line inf	Line flush	2 weeks
7	19/m	Gen surgery	Entero cutaneous fistula	T P N line infection	line flush	2 weeks
8	54/f	Haematology	AML	Chemo line infection	Line flush	2 weeks
9	27/f	Haematology		Chemo line inf	Line flush	2 weeks
10	64/f	Vascular	Graft infection	Graft flush	Graft flush	1 week
11	56/m	Haematology	AML	Chemo line inf	Line flush	2 weeks
12	77/m	Cardiothoracic	Post CABG STERNAL INF	Sterna infection	Topical flush	3 weeks
13	41/f	Breast Surgery	Ca Bhrast	Chemo line	Line Flush	6 weeks
14	54/f	Breast Surgery	Ca Breast	Chemo line	Line Flush	2 weeks
15	63/m	Haematology	High grade lymphoma	Chemo line	Line Flush	2 weeks
16	57/f	Breast Surgery	Ca Breast	Chemo line	Line flush	2 weeks
17	52/F	Breast onco	Ca br	Chemo line	Line flush	6
18	76/m	Gen surg	Ca bowel	T p n line	Line flush	2 weeks
19	71/m	Gen surg	Ca oeso	Tpn line	Line flush	2
20	68/m	Vascular	Graft inf	Topical graft	2 weeks	
21	76/m	Vascular	Graft inf	Topical	1 week	1 week
22	46/f	Onco	Ca breast	Chemo l	3 weeks	3 weeks
23	31/m	Cardiothoracic	empyema	Pleural cavity	Cavity flush	2 weeks
24	66/m	Cardiothoracic	Empyema	Pleural cav	Cavity flush	3
25	72/f	Cardiothoracic	Valve inf	Abx line	Line flush	2 weeks
26	44/f	Gen surg	Ca bowel	Tpn line	Line flush	3 weeks
27	70/f	Cardiothoracic	Valve inf	Abx line	Line f	2 weeks
28	53/m	Vascular	Graft inf	Topical	Topical	1 week
29	49/f	Haematology	Aml	Chemo line	Line flush	10 weeks
30	42/m	Gen sury	Sepsis abdo	T p n line	Line flush	4 weeks
31	53/f	Haematology	Lymphoma	line inf	Line flush	2 weeks
32	84/m	Ca rectum	Chemo line	Line inf	3 weeks	3
33	71/f	Cardiology	Vale inf	Abx line	Line flush	4 weeks
34	58/m	Vascular	Gr inf	Topical graft	Topical	1 week
35	15/f	Haematology	Aml	Chemo line	Line flush	1 week
36	52/m	Gen surg	Ca rectum	Chemo line	Line flush	6 weeks
37	63/f	Haematology	Aml	Chemo line	Line flush	2 weeks
38	44/m	Vascular	Foot inf	Abx line	Line flush	1 week

**Conclusion:** Successful outcomes with taurolidine-citrate, licensed as a medical device, finds its place in several case series and reports. However, its successful use in salvage therapy in complex clinical cases indicates its potential role beyond licensed indication and need for further large randomized controlled studies.

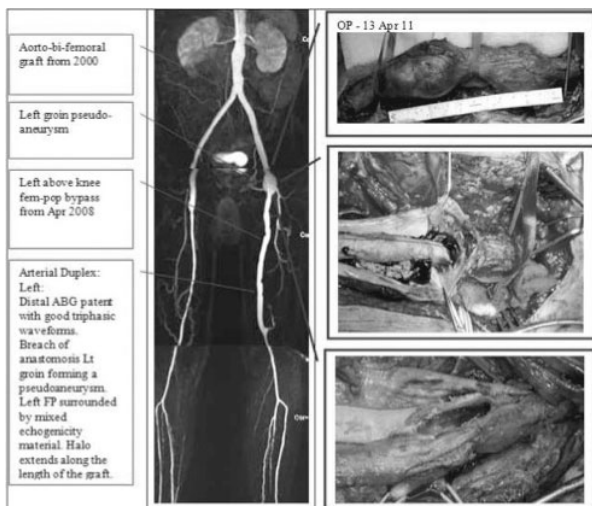
**P1464** Clinical experience and successful use of taurolidine drain-assisted irrigation as salvage therapy in three complex cases of femoro-popliteal prosthetic graft infection

B. Sangers\*, L. Barr, P. Kaur, H. Lawrence-Desmarowitz, A. Guleri (Blackpool, UK)

**Background:** An infrainguinal prosthetic graft infection is a serious complication of vascular surgery. In most cases, removal of graft is required to save the patient's life and in selected cases a reconstruction is required to save the limb. The associated morbidity and mortality of these procedures remain high. In selected cases, however, preservation of the infected patent synthetic graft is possible. Taurolidine is derived from amino-acid taurine. It has antimicrobial and anti-lipoplysaccharide properties. We present our experience of successful use of taurolidine irrigation as salvage therapy in three complicated cases of femoro-popliteal prosthetic graft infections thereby preserving graft and limb perfusion.

**Method:** Case notes review of three cases. Post procedure drain assisted Taurolidine irrigation of graft.

Summary of cases: Three patients with postoperative infrainguinal prosthetic graft infections were managed with: (i) Operative drainage of perigraft collection, (ii) Curettage of the organized bio film around graft, (iii) Lavage with saline and peroxide, (iv) Post procedure drain assisted irrigation with Taurolidine (Taurlock) (v) Simultaneous peri-operative short course of systemic antibiotics Regular, twice a week cultures from wound site were carried out. Successful outcome included clinical improvement and negative cultures. After a mean follow up of 3 months all the three patients remained infection free clinically as well as laboratory markers, and well healed wounds with patent grafts and well perfused limbs.



**Conclusion:** Surgical intervention with aggressive local clearance, coupled with post operative drain assisted local irrigation with Taurolidine appears to be effective and useful method of controlling infection as well as preserving the graft, and there by limb perfusion, in selected infrainguinal prosthetic vascular graft infections. The use of taurolidine as salvage therapy was mutually agreed between the vascular surgeon and microbiologist. Patient consent was obtained for this out of licensed indication use. The drain assisted irrigation is quite simple and with effective results. Taurolidine, with its antimicrobial properties, inhibits any microbial growth around the graft permitting the body-healing process. There is need for further large randomized, controlled studies in future. Pictures and details to be presented.

**P1465** Tolerability, safety and pharmacokinetics of single and multiple oral doses of AFN-1252 in healthy human subjects

N. Kaplan\*, B. Hafkin (Toronto, CA; Austin, US)

**Objectives:** AFN-1252 is a novel bacterial fatty acid biosynthesis inhibitor, targeting the staphylococcal FabI enzyme. AFN-1252 is in clinical development as a specific-spectrum agent for the treatment of infections due to susceptible or multi-drug resistant staphylococci. A previous single ascending dose oral Phase 1 trial of a tosylate salt form of AFN-1252, dosed as a suspension, showed excellent safety and tolerability and desired pharmacokinetic (PK) properties, but exposures were limited by reduced tolerability and bioavailability at the higher doses due to the thick and bitter suspension. Single and multiple ascending dose Phase 1 trials were conducted with a novel AFN-1252 tablet formulation, based on the free base form, to further assess safety, tolerability and PK.

**Methods:** Placebo controlled, double blind, single and multiple ascending dose, oral Phase 1 studies were conducted in healthy subjects under an IND in the USA using a 100 mg AFN-1252 oral tablet. The single ascending dose trial utilized total daily doses of 100–800 mg and in the multiple ascending dose study, doses of 200, 300 and 400 mg were given once daily for a total of 10 days. PK parameters were estimated with a WinNonLin non-compartmental model.

**Results:** AFN-1252 showed overall increased, but non-proportional plasma levels with increasing doses. Median values for T<sub>max</sub> and drug elimination half-lives were similar on Days 1 and 10 for all dose levels. C<sub>max</sub> and AUC values at Day 10 indicated no drug accumulation or metabolism. In both studies, the most frequent adverse event was mild headache, and there were no treatment-related trends in clinical laboratory results, vital sign measurements, 12 lead ECG results, or physical examination findings. AFN-1252 plasma levels with the oral tablet at daily doses of 300 and 400 mg exceeded the levels, predicted from preclinical models, for efficacy against drug susceptible and resistant staphylococci.

**Conclusions:** AFN-1252 was safe and well tolerated in healthy subjects after 10 days of dosing at 200–400 mg QD. No evidence of drug accumulation or induction of metabolism was seen after 10 days of dosing. AFN-1252 plasma levels exceeded those predicted for efficacy against all known staphylococcal drug resistant phenotypes and indicate that AFN-1252 can be dosed orally either once or twice daily. Overall, these data strongly support further clinical studies with AFN-1252 for treatment of serious staphylococcal infections.

**P1466** Antibacterial activity of nanomolecular silicon dioxide (SiO<sub>2</sub>) combined with silver ions

D. Scevola\*, V. Monzillo, P. Marone, C.A. Bignozzi (Pavia, IT)

The wide use of antibiotics in the prevention and treatment of bacterial infections has led to the emergence and spread of resistant microorganisms requiring new active molecules against bacteria. The topic use of antimicrobial substances not belonging to the group of traditional antibiotics can overcome this emerging problem. The goal of this study is the evaluation of the antibacterial activity of a nanomolecular silicon dioxide (SiO<sub>2</sub>), named SIAB, synthesized with nanotechnological method. SIAB consists of stable silica dioxide nanoparticles functionalized with ionic Ag that exhibits high bactericidal, fungicidal and virucidal action.

The substance is a new formulation presenting as an aqueous suspension of SiO<sub>2</sub> nanoparticles functionalized with silver. Silver ions, stabilized in their one electron oxidized form, are covalently bound to high surface area SiO<sub>2</sub> nanoparticles, acting in synergy with cationic species which improve the bactericidal effect of the product. Activity tests performed at our Department indicate a relevant action of the product at extreme dilutions on many bacteria of clinical impact (Table), supporting the indication of topical use in local infections of the skin and mucosae.

**Methods and results:** The activity of SIAB on bacterial species was tested by the microdilution broth method. The minimum inhibitory and bactericidal concentrations (MIC and MBC) of the compound were determined.

Microorganism	MBC: dilution %
<i>Staphylococcus aureus</i> MR	0.0007
<i>Staphylococcus aureus</i> MS	0.0015
<i>Enterococcus</i> spp.	0.0015
<i>Streptococcus pyogenes</i> 1	≤ 0.00007
<i>Streptococcus pyogenes</i> 2	≤ 0.00007
<i>Streptococcus salivarius</i>	0.0015
<i>Streptococcus mitis</i>	0.0003
<i>Pseudomonas aeruginosa</i>	0.025
<i>Escherichia coli</i>	0.006
<i>Candida albicans</i> 1	0.006
<i>Candida albicans</i> 2	0.006

**Table** - MBC (Minimal bactericidal concentration) as dilution (%) of the product Siab at which after incubation the number of living microorganisms is < 0.01 %.

**Conclusion:** Nanomolecular SiO<sub>2</sub> combined with ionic Ag shows antimicrobial activity against gram-positive and gram-negative bacteria and fungi.

**P1467** Stress induction and synergy with antibiotics of oleanolic and ursolic acids or silver nanoparticles

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**Objectives:** Due to the development of antibiotic resistance of bacterial pathogens new antibacterial agents are intensively studied. Of special interest are plant-derived compounds and nanoscale materials. Objects of this research are two pentacyclic triterpenoids: oleanolic acid (OA) and ursolic acid (UA) and silver nanoparticles (AgNPs) showing antibacterial effect. In this study we investigated the ability of OA, UA and AgNPs to induce heat shock response and their interaction with several antibiotics.

**Methods:** Bacterial strains used in the study: *Escherichia coli* PCM439, *Pseudomonas aeruginosa* PCM499, *Listeria monocytogenes* PCM2191. Compounds tested: OA, UA, AgNPs. To investigate heat shock response induction, the level of expression of bacterial heat shock proteins: DnaK, DnaJ, HtpG, IbpA, IbpB was analysed. Bacterial strains were grown in medium with subinhibitory concentrations of OA or UA or AgNPs or without compounds. Cellular bacterial lysates were then analysed by SDS-PAGE electrophoresis and Western blot with appropriate antibodies. Membranes were analysed using densitometry. To study interaction between tested compounds and selected antibiotics against bacteria grown in solution or in biofilms the checkerboard assay on microtitre dishes was applied. After 24-hours incubation bacteria was stained with 2,3,5-triphenyltetrazolium chloride (TTC). Fractional inhibitory concentration (FIC) Index was calculated.

**Results:** Differences in expression of heat shock proteins from particular bacterial strains upon addition of OA or UA or AgNPs in comparison to controls were demonstrated, OA and UA induced stress response, e.g. the level of the DnaK and DnaJ chaperones was higher for about 50% and 40% respectively than in control. Bacterial growth was inhibited in the presence of OA, UA, AgNPs combined with different antibiotics. The inhibition was 2- to 3-fold higher than in the presence of tested compounds alone, FIC Indexes was demonstrated either the additive or synergistic effect of the compound tested with antibiotics.

**Conclusion:** OA, UA and AgNPs induce heat shock response, and also act in additive or synergistic way with selected antibiotics. This result constitutes further demonstration of antibacterial potential and pleiotropic effect of tested compounds.

**P1468** Antituberculous susceptibility patterns of Mannich ketones as possible antituberculous agents and study mechanism of action

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**Objectives:** Recently we have seen an increase in number of causes caused by either multi-drug-resistant *Mycobacterium tuberculosis* strains or atypical mycobacteria. However, there are only a few effective drugs. There is a strong research activity to develop new drugs with different chemical structure and mechanism of action. To perform the screening of some Mannich ketones used as water soluble hydrochlorides against several mycobacteria, typical strains (*Mycobacterium tuberculosis*), atypical strains (*Mycobacterium goodii* and *Mycobacterium xenopi*) were tested. There was also an attempt to explore the mechanism of action.

**Methods:** We have tested the effect and efficacy some of Mannich ketones on *M. tuberculosis* (H37Rv standard strain and clinical isolates), *M. goodii* and *M. xenopi* strains (clinical isolates). We have investigated the antibacterial inhibition effect in "Sula" liquid culture medium between 200 and 3.125 µg/mL concentrations and found a good MIC value with some of the test compounds. With the use of transmission electron microscopy we investigated the morphological effects of the selected test compounds on treated and untreated culture of *M. goodii*. We examined the structure of cell wall and the changes of cell membrane and cytoplasm. As a commercial standard interfering with the biosynthesis of mycolic acid isoniazide (INH) was applied.

**Results and conclusions:** The two classes of test compounds showed variable MIC values. Their antituberculous activity did not prove to be better than that of the INH at the clinical isolates, while the standard strain showed lower or the same activity as the INH. Their efficiency toward the atypical strains was several times better than that of INH. Often a test compound exerted different activity – MIC value – against different strains. We have found clear differences between treated and untreated cultures regarding the structure of cytoplasm and bacterial cell wall in the transmission electron microscopic preparations. According to our opinion these observations refer to the inhibition of the cell wall biosynthesis by Mannich ketones. The use of Mannich ketones as antituberculous agents is very promising since they inhibit the cell wall biosynthesis in both typical and atypical mycobacteria.

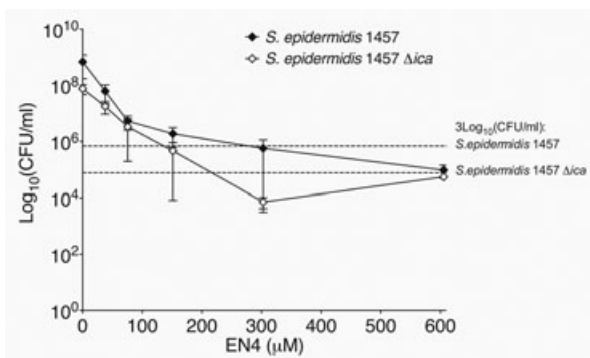
**P1469** Serrulatane EN4, a new antimicrobial compound exerts potent activity against adherent biofilm-forming bacteria in vitro

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**Objectives:** Implant-associated infections are mainly caused by biofilm-forming staphylococci. Successful treatment requires the use of bactericidal drugs active against adhering bacteria. Serrulatane EN4, a diterpene isolated from *Eremophila* plant species, was previously reported to exert antimicrobial activity. Our goal is to elucidate the activity of EN4 against these microorganisms.

**Methods:** Activity of EN4 extract was tested against different staphylococci. The minimal inhibitory and minimal bactericidal concentrations (MIC and MBC, respectively) in the logarithmic and stationary growth phase as well as the antimicrobial susceptibility of adherent bacteria to EN4 were determined. To evaluate the mode of action time-kill studies, incorporation of radiolabeled precursors of cell wall, RNA, DNA and proteins and SYTO 9/propidium iodine flow cytometry analysis were performed. Cytotoxicity was measured on mouse fibroblast L929 using LDH release. In vivo activity was tested in a previously published tissue cage mice model.

**Results:** The EN4 MIC of *S. aureus* and *S. epidermidis* in logarithmic and stationary growth phase were 25 and 50 µg/mL and MBC 50 and 100 µg/mL, respectively. EN4 was similarly effective against methicillin resistant *S. aureus* and elicited antimicrobial activity towards different Gram-positive, but not to Gram-negative bacteria. Additionally, EN4 was bactericidal against adherent bacteria independently of PIA-mediated biofilm (Fig. 1). In time-kill studies, EN4 showed rapid and concentration-dependent killing with bactericidal activity at concentrations above 50 µg/mL. In the mode of action assays no specific target could be elucidated. Cytotoxicity on L929 was time- and concentration-dependent in the range of MBC. In vivo EN4 showed neither bactericidal nor cytotoxic effect indicating an inhibition of its activity. Inhibition assays revealed that this was caused by albumin.



**Conclusion:** EN4 shows potent and similar bactericidal effects against different growing and surface-adhering staphylococci independent of PIA-mediated biofilm and may therefore be a promising antimicrobial compound for the treatment of implant-associated infections. EN4 interacts with multiple targets of Gram-positive bacteria which reduces the possibility of resistance development. However, due to in vivo inhibition structural changes of EN4 are necessary to reduce its protein affinity while retaining its bactericidal properties.

#### P1470 In vitro activity of solithromycin (CEM-101) against clinical *Neisseria gonorrhoeae* isolates displaying various types of antimicrobial resistance profiles

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**Objectives:** Resistance in *N. gonorrhoeae* to penicillins, tetracyclines, fluoroquinolones, macrolides, and early-generation cephalosporins are today common worldwide. Recently, clinical resistance to the currently recommended cefixime and ceftriaxone has been reported. Accordingly, gonorrhoea may become untreatable and the search for new treatment options for gonorrhoea is essential. In this study, we investigated the efficacy of the newly developed fluoroketolide, solithromycin (CEM-101), compared to other antimicrobials previously recommended for treatment of gonorrhoea.

**Methods:** The minimum inhibitory concentration (MIC) of solithromycin, azithromycin, erythromycin, telithromycin, spectinomycin, tetracycline, ciprofloxacin, ampicillin, cefixime, and ceftriaxone was determined using agar dilution method and Etest for a collection of 250 clinical *N. gonorrhoeae* isolates with various antimicrobial resistance genotypes and phenotypes. These included the recently described first extensively drug resistant (XDR) *N. gonorrhoeae* strain H041, other strains displaying clinical ESC resistance, and strains with other types of multi-drug clinical resistance.

**Results:** The MIC range of solithromycin was 0.001–32 mg/L (MIC<sub>50</sub>: 0.125 mg/L and MIC<sub>90</sub>: 0.5 mg/L). Ninety-three isolates had an azithromycin MIC > 0.5 mg/L. However, only six isolates had a

solithromycin MIC > 0.5 mg/L (1, 4, 4, 4, 16, and 32 mg/L). The corresponding MICs of azithromycin for these isolates were 2, 4, 8, >256, >256 and >256 mg/L, respectively. The antimicrobial activity of solithromycin was significantly superior to those of azithromycin, other macrolides, as well as other classes of antimicrobials.

**Conclusion:** The present in vitro observations regarding the efficacy of solithromycin against *N. gonorrhoeae* suggest that this new fluoroketolide could be an appropriate alternative to the currently recommended ESCs for treatment of gonorrhoea. It is of great importance to perform further in vitro studies regarding, e.g., selection of resistance and mechanisms of solithromycin resistance in *N. gonorrhoeae*. Furthermore, previous small studies have indicated that solithromycin has high potency against mycoplasmas, ureaplasmas, and *Chlamydia trachomatis*, suggesting that solithromycin might be an appropriate option for treatment of several sexual transmitted infections (STIs). Nevertheless, larger, well-designed studies examining these STI pathogens are crucial.

#### P1471 Inhibition of type III secretion system in *Chlamydia trachomatis* and *Salmonella enterica* serovar Typhimurium by small-molecule compounds in vitro and in vivo

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**Objectives:** The conservation of structural components of T3SS and their importance for virulence in many bacterial pathogens make them attractive targets for inhibition with small molecules. Such molecules – derivatives of thiohydrazides of amides of oxamic acids (DTAOA), have been synthesized and tested for their activity against T3SS in chlamydiae and salmonella.

**Methods:** Microbiological and cell culture tests, immunofluorescence microscopy, SDS-PAGE, Elispot, organic synthesis, infections models on BALB/c mice.

**Results:** Five hundred compounds of DTAOA were tested for toxicity to eukaryotic cells and T3SS inhibitory activity using cell culture and bacterial screening tests. The selected T3SS inhibitors were chemically optimized which allowed to obtain the original lead compound, characterized by low toxicity, high levels of solubility, stability, and specific efficiency. In our in vitro studies we have found that the lead compound blocked transport of early and late *C. trachomatis* T3SS effectors, which led to suppression of the intracellular pathogen development. The lead compound specifically inhibited the secretion of salmonella early effectors, with no effect on the reproduction of the pathogen. In in vivo experiments we have found that intravaginal inoculation of the compound (given three times in a dose of 0.015 g/kg per mouse) resulted in a 30-fold decrease in accumulation of the pathogen in the lower urogenital tract on the day 7 post infection (PI). In salmonella acute infection model 5 daily intraperitoneal injections of the lead compound at a dose of 0.01 g/kg resulted in a 2-fold increase in mice survival life span after infectious challenge and 500-fold reduction of bacterial load in the organs. Daily inoculation of the lead compound to infected animals at a dose of 0.05 g/kg for 12 days led to the eradication of salmonella in the peritoneal lavages and spleens; and these animals survived for more than 45 days PI. Intraperitoneal inoculation of the compound did not increase early inflammatory response mediated by neutrophils and did not suppress T cell and antibody responses.

**Conclusion:** Our data demonstrates that the selected lead compound – derivative of thiohydrazides of amides of oxamic acids affects bacterial T3SS activity in *C. trachomatis* and *S. typhimurium* in vitro and in vivo and hence could be used as a substance in the design of bacterial T3SS specific inhibitors for pharmaceutical intervention of bacterial virulence.

**P1472** Identification of chlamydial protease-like activity factor inhibitors by structure-based virtual and experimental screening

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**Objectives:** Chlamydia trachomatis is a Gram-negative obligate intracellular pathogen that causes diseases with serious complications worldwide. Chlamydial protease-like activity factor (CPAF) is one of the main chlamydial virulence factors and the identification of small molecule CPAF inhibitors could promote the development of new drug prototypes for the chlamydia treatment. Here we present the selection of CPAF inhibiting compounds by means of structure-based virtual screening and experimental testing.

**Methods:** The computational screening was performed against the active center of CPAF protein using ICM software. The cumulative size of screened libraries was over 1.5 million compounds. Selected compounds were tested for toxicity by cell viability tests (MTT, Met Blue assays). Their anti-chlamydial activity was determined in in vitro model of *C. trachomatis* infection by fluorescence microscopy, and specific anti-CPAF activity was tested on recombinant CPAF protein using Western blot assay.

**Results:** Libraries of chemical compounds were ranked according to their predicted CPAF affinity, top-scoring compounds with maximal chemical diversity were selected for testing. Experimental testing by cell-free CPAF substrates degradation assay and *C. trachomatis* infection model allowed selecting several CPAF inhibitors that were active against both acute and persistent chlamydial infection. The selected compounds were low toxic to eukaryotic cells and had a dose-dependent effect on intracellular development of *C. trachomatis*. Based on the structures of four of the identified inhibitors we were able to construct a pharmacophore model of CPAF inhibitor that was consistent with docking results. This model will be used for further optimization of lead compounds.

**Conclusion:** Our results revealed novel CPAF inhibitors identified by the combination of computational virtual screening and experimental testing as new potential drug prototypes for the treatment of *C. trachomatis* infection. Future studies will be aimed at the optimization of affinity and activity of selected compounds and the identification of new CPAF inhibitors by ligand-based approaches.

## Tigecycline activity and resistance

**P1473** The killing of high-density inocula of *E. coli* by tigecycline using the mutant prevention and maximum serum drug concentrations

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**Objective:** Tigecycline (TIG) is glycolcycline antibacterial agent with low mutant prevention concentration (MPC) values against clinical isolates of *E. coli*. We were interested in determining the rate and extent of killing by TIG using MPC and maximum serum ( $C_{max}$ ) drug concentrations against high density inocula of *E. coli*.

**Methods:** MIC testing was performed using  $10^5$  CFU/mL of *E. coli* exposed to doubling drug concentration of TIG in Mueller-Hinton (MH) broth; following incubation under ambient conditions, the MIC was the lowest concentration preventing growth. MPC testing was performed using,  $\geq 109$  CFUs exposed to doubling dilutions of TIG on drug containing MH agar plates and following incubation for 24–48 hours, the MPC was the lowest drug concentration preventing growth. For kill experiments,  $10^6$ – $10^9$  CFU/mL were exposed to the measured MPC  $C_{max}$  (1.45 mg/L) drug concentrations and the reduction in viable cells (log<sub>10</sub> and % kill) were recorded at 30 minutes, 1, 2, 3, 4, 6, 12 and 24 hours. All experiments were conducted in triplicate.

**Results:** For *E. coli* clinical isolates, MIC values were 0.063 mg/L and MPC values were 1 mg/L. When  $10^6$  or  $10^7$  CFU/mL were exposed to the MPC drug concentration, a 0.31–0.42 log<sub>10</sub> reduction (51–62%

kill) was seen by 4 hours and a 0.71–1.16 log<sub>10</sub> reduction (81–93% kill) by 12 hours; 98–99% kill by 24 hours. Exposure of  $10^8$ – $10^9$  CFU/mL to the MPC drug concentration yielded a 0.11–0.19 log<sub>10</sub> reduction (23–34% kill) by 4 hours; 30–49% kill by 24 hours. Exposure of  $10^6$  or  $10^7$  CFU/mL to the  $C_{max}$  drug concentration yielded a 0.41–0.42 log<sub>10</sub> reduction (61–62% kill) by 4 hours and 0.106–1.30 log<sub>10</sub> reduction (91–95% kill) by 12 hours; >99% kill by 24 hours. Exposure of  $10^8$ – $10^9$  CFU/mL to  $C_{max}$  drug concentration yielded a 0.18–0.31 log<sub>10</sub> reduction (34–51% kill) by 2 hours; 0.65–0.67 log<sub>10</sub> reduction (78–79% kill) by 24 hours.

**Conclusion:** TIG MIC and MPC values were low against *E. coli* – 0.031 and 1 mg/L respectively. Killing high density inocula using MPC and  $C_{max}$  drug concentrations yielded substantial reductions in viable cells by 4 hours (51–62% kill) and >98–99% kill by 24 hours for  $10^6$ – $10^7$  CFU/mL inocula; 78–79% kill for  $10^8$ – $10^9$  CFU/mL inocula exposed to the  $C_{max}$  drug concentration. The low MIC and MPC values suggest that TIG is less likely to select for resistant *E. coli* and dosing to achieve MPC results in killing of high density bacterial burdens.

**P1474** In vitro activities of tigecycline in the era of NDM-1

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**Background and Aim:** In the current era of New Delhi metallo-beta-lactamase-1 (NDM-1) infections and dry antibiotic pipeline, managing infections caused by multi-drug resistant gram-negative bacterial infections (MDR-GNBs) presents a great challenge to physicians in the developing world. Tigecycline, a broad spectrum glycolcycline is a one among the few treatment options against MDR-GNBs. But it has been studied mostly in the western world, where the prevalence of MDR-GNB infections were less when compared to developing world. We studied the in vitro susceptibility of tigecycline and compared it with cefoperazone-sulbactam (CS), imipenem and colistin.

**Methods:** Our hospital is a 600 bed tertiary care hospital located in Chennai, South India. A total of 80 isolates of *Escherichia coli* (*E. coli*-33), *Klebsiella pneumoniae* (*K. pneumoniae*-30) and *Acinetobacter baumannii* (*A. baumannii*-17) isolated using standard microbiological techniques between January 2011 and July 2011 was analyzed. Antimicrobial susceptibility testing was determined by using Vitek 2 commercial micro dilution system (bioMerieux) and CLSI breakpoints were applied in interpreting the susceptibility results.

**Results:** As shown in Table 1, tigecycline demonstrated 100% activity against *E. coli*, similar to colistin and it was higher than imipenem (93.93%) and CS (78.78%). A similar pattern was noticed for *A. baumannii*, but imipenem (35.29%) was the least susceptible drug against *A. baumannii*. Tigecycline (80%) was inferior to both colistin (100%) and imipenem (83.33%) against *K. pneumoniae* and slightly better than CS (66.66%).

Table 1 Susceptibility and comparison data of tigecycline

	Cefoperazone-sulbactam-susceptible isolates N (%)	Imipenem-susceptible isolates N (%)	Colistin-susceptible isolates N (%)	Tigecycline – susceptible isolates N (%)
<i>E. coli</i> (T-33)	26 (78.78%)	31 (93.93%)	33 (100%)	33 (100%)
<i>K. pneumoniae</i> (T-30)	20 (66.66%)	25 (83.33%)	30 (100%)	24 (80%)
<i>A. baumannii</i> (T-17)	9 (52.94%)	6 (35.29%)	17 (100%)	15 (88.23%)

**Conclusion:** In vitro tigecycline is equivalent to colistin against *E. coli*, and second best option against *Acinetobacter* after colistin. But the decline in susceptibility of tigecycline against *K. pneumoniae* is a worrisome phenomenon. Further prospective in vivo clinical studies are needed for tigecycline to explore its therapeutic potential.

**P1475** Decreased susceptibility to tigecycline in several clinical isolates of *Acinetobacter baumannii*

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**Objectives:** To explore the correlation between the decreased susceptibility to tigecycline and expression level of the *adeB* gene in several multidrug resistant *Acinetobacter baumannii* isolated from hospitalized patients.

**Methods:** In vitro activity of tigecycline against *A. baumannii* was determined by E-test method according to the manufacturer's instructions. The presence of the *adeB* gene was investigated by PCR and confirmed by sequencing. Expression level of the *adeB* gene relative to that of *rpoB* gene was quantified using real-time reverse-transcription PCR.

**Results:** The minimum inhibitory concentrations (MICs) of tigecycline for the 46 *A. baumannii* isolates ranged from 0.094 to 8 mg/L. And the MIC50 and MIC90 were 2 and 3 mg/L respectively. According to the US FDA interpretation criteria for MICs breakpoints of tigecycline against Enterobacteriaceae, the percentages of susceptible, intermediate and resistant isolates were 89.1% (41/46), 8.7% (4/46), and 2.2% (1/46), respectively. For the eight isolates with different MICs, including five non-susceptible and three susceptible isolates, the correlation between the expression level of the *adeB* gene and MICs of tigecycline was not found.

**Conclusion:** Our data suggest that the mechanism for the decreased susceptibility to tigecycline in clinical isolates of *A. baumannii* is complex and regulatory factors other than *adeB* gene remain to be determined.

**P1476** Tigecycline activity in Europe: a comparative analysis by country, 2009–2010

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**Objectives:** Development of bacterial resistance continues to cause concern worldwide, but the availability of newer agents offers clinicians options for therapy. Tigecycline has a very broad spectrum of activity, including strains resistant to other drugs. As part of the global Tigecycline Evaluation Surveillance Trial (TEST), strains collected in 25 European countries in 2009 through 2010 were evaluated for susceptibility to several commonly used antimicrobials.

**Methods:** The 15 304 strains were collected and identified at 271 sites in 25 countries. MICs were determined at each site using EUCAST guidelines on microdilution panels.

**Results:** The following table summarizes tigecycline results.

Country	Enterobacteriaceae			Acinetobacter spp			Enterococcus spp			S. aureus		
	N	MIC <sub>90</sub>	%Sus	N	MIC <sub>90</sub>	%Sus	N	MIC <sub>90</sub>	%Sus	N	MIC <sub>90</sub>	%Sus
Austria	267	1	90	4/6	1	na	53	0.25	100	77	0.25	100
Belgium	739	2	81	121	1	na	134	0.25	100	189	0.5	100
Bulgaria	637	2	84	137	2	na	111	0.25	100	198	0.5	100
Croatia	265	2	88	50	1	na	65	0.25	100	92	0.25	100
Czech Republic	251	1	97	42	1	na	45	0.25	100	66	0.25	100
Denmark	205	2	90	2/6	0.5	na	31	0.25	100	48	0.5	100
Finland	80	1	96	1/6	0.5	na	12	0.25	100	—	—	—
France	2199	2	87	393	2	na	387	0.25	99	634	0.5	100
Germany	1895	1	92	338	1	na	328	0.25	100	502	0.25	100
Greece	500	2	81	100	2	na	94	0.25	99	110	0.25	100
Hungary	413	1	92	7/4	2	na	73	0.25	100	117	0.25	100
Ireland	338	2	89	5/3	0.5	na	59	0.25	100	77	0.5	100
Italy	1410	1	91	248	2	na	249	0.25	100	353	0.5	100
Latvia	165	1	96	2/9	1	na	22	0.25	100	50	0.25	100
Lithuania	83	2	86	1/4	0.5	na	9	0.25	100	23	0.25	100
Poland	820	2	90	137	2	na	146	0.25	100	258	0.25	100
Portugal	417	2	85	6/2	1	na	73	0.25	100	115	0.25	100
Romania	328	2	82	10/9	2	na	48	0.25	100	101	0.5	100
Slovak Republic	120	1	93	1/8	1	na	27	0.25	100	19	0.5	100
Slovenia	169	2	83	3/0	2	na	30	0.25	100	47	0.25	100
Spain	1975	1	90	277	2	na	353	0.25	99	467	0.5	100
Sweden	494	1	93	8/9	0.5	na	88	0.25	100	140	0.25	100
Switzerland	83	1	98	1/6	1	na	15	0.25	100	24	0.12	100
The Netherlands	249	1	93	4/5	0.5	na	44	0.25	100	75	0.5	100
United Kingdom	267	1	96	4/1	1	na	38	0.25	100	84	0.25	100

**Conclusions:** MIC90 and %Sus values remained consistent among all European countries for tigecycline against the gram-positive enterococci and *S. aureus* with MIC90s ranging from 0.12 to 0.5 mg/L and %Sus values ranging from 99% to 100%. Half of the countries had tigecycline MIC90 values of 1 mg/L against the Enterobacteriaceae and half had values of 2 mg/L. MIC90 values were variable and ranged between 0.5 and 2 mg/L against the Acinetobacter. Tigecycline remains a potent antimicrobial agent with broad-spectrum activity throughout Europe.

**P1477** In vitro activity of tigecycline against pathogens from UK/Ireland: TEST 2008–2011

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**Background:** The rapid emergence of multi-drug resistant pathogens has undermined the efficacy of many widely used broad spectrum antibacterials and prompted the development of newer antimicrobials. Tigecycline has shown broad spectrum activity against many hospital pathogens. The purpose of this study was to examine the activity of tigecycline and comparators to nosocomial pathogens isolated in the UK and Ireland between 2008 and 2011.

**Methods:** A total of 1390 nosocomial pathogens were identified and confirmed at a reference laboratory. MICs were determined at each site utilizing supplied broth microdilution panels and interpreted according to EUCAST guidelines.

**Results:** Results are in the Table as follows: na, breakpoints not defined.\*CLSI breakpoints used if no EUCAST breakpoints determined yet.

	<i>E. coli, K. oxytoca/pneumoniae</i> n = 481		<i>Acinetobacter</i> spp n = 104		<i>P. aeruginosa</i> n = 152	
	%S	MIC <sub>90</sub>	%S	MIC <sub>90</sub>	%S	MIC <sub>90</sub>
Tigecycline	95.8	1	na	1	na	16
Amikacin	94.2	8	96.2	4	83.3	32
Cefepime	84.2	8	—	8	85.4	16
Mereopenem	99.8	<0.06	90.4	2	83.9	8
Levofloxacin	79.2	>8	88.5	2	64.9	>8
	<i>S. aureus</i> n = 202		<i>Enterococcus</i> spp n = 121		<i>S. pneumoniae</i> n = 80	
	%S	MIC <sub>90</sub>	%S	MIC <sub>90</sub>	%S	MIC <sub>90</sub>
Tigecycline	100	0.5	100	0.25	100	0.03
Levofloxacin	66.3	16	na	>32	100	1
Minocycline	89.1	1	42.2	>8	38.8	8
Vancomycin	100	1	69.4	>32	100	0.5

**Conclusions:** Mereopenem and tigecycline were the most active antimicrobial agents against Enterobacteriaceae. Tigecycline displayed the lowest MIC90 against Acinetobacter spp., but had minimal activity against *P. aeruginosa*. Against gram-positives, tigecycline had high percent susceptible rates.

**P1478** Comparison of tigecycline susceptibilities of bacteria isolated in Canada, USA, and Mexico – results from TEST programme 2008–2011

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**Background:** The Tigecycline Evaluation Surveillance Trial (TEST) monitors the activity of tigecycline and comparators against multiple pathogens from multiple infection sources collected worldwide. The current report describes the activity of tigecycline clinical isolates collected from Canada, Mexico and the United States during 2008–October 2011.

**Methods:** A total of 17 817 isolates were collected of which 1715, 3358, and 12 744 were from Canada, Mexico and the United States, respectively. Of the total, 7162 and 10 655 were gram-positive isolates or Enterobacteriaceae isolates, respectively. Susceptibility testing was performed as per CLSI guidelines and susceptibility interpreted using FDA breakpoints for tigecycline.



**Results:** Susceptibility of isolates from different countries to tigecycline were: %S, percent susceptibility; (n), number of isolates.

Species / Group	Canada	Mexico	USA
	% S (n)	% S (n)	% S (n)
<i>Staphylococcus aureus</i>	100 (265)	100 (611)	100 (1954)
<i>Streptococcus agalactiae</i>	100 (119)	100 (155)	99 (845)
<i>Streptococcus pneumoniae</i>	99 (155)	98 (141)	99 (1031)
<i>Enterococcus faecalis</i>	99 (124)	100 (291)	99 (946)
<i>Enterococcus faecium</i>	100 (52)	100 (86)	99 (387)
Enterobacteriaceae	97 (1000)	97 (2074)	97 (7581)

**Conclusions:** Tigecycline inhibited the vast majority of the isolates in this study. Susceptibility to tigecycline to the selected gram-positives ranged from 98% to 100% for all three countries and 97% for the Enterobacteriaceae. There were no important differences in susceptibility between countries.

**P1479 Activity of tigecycline and comparators against multidrug-resistant Gram-negative bacteria in Africa-Middle East: TEST 2006–2010**

M. Renteria\*, S. Bouchillon, D. Hoban, M. Hackel, B. Johnson, N. Raghuvir (Schaumburg, Collegetville, US)

**Background:** Reduced treatment options for multi-drug resistant (MDR) gram negative bacteria are a major cause of mortality for patients with nosocomial infections. The global Tigecycline Evaluation and Surveillance Trial (TEST) evaluated the activity of tigecycline and comparators against MDR *Acinetobacter baumannii*, *Enterobacter aerogenes* and *E. cloacae* from Latin America during 2006–2010.

**Methods:** A total of 851 clinical isolates of MDR *A. baumannii*, *E. aerogenes* and *E. cloacae* were collected from multiple infection sources in Africa-Middle East during 2006–2010. MICs and % Sus were determined at each site using prepared broth microdilution panels following CLSI guidelines. MDR isolates were defined as those resistant to three or more antimicrobial drug classes.

**Results:** The MIC50 and MIC90 in µg/mL, for tigecycline and selected comparators are shown in the following table of MDR species:

Drug	<i>A. baumannii</i> (N=464)			<i>E. aerogenes</i> (N=79)			<i>E. cloacae</i> (N=308)		
	MIC <sub>50</sub>	MIC <sub>90</sub>	%S	MIC <sub>50</sub>	MIC <sub>90</sub>	%S	MIC <sub>50</sub>	MIC <sub>90</sub>	%S
Tigecycline	1	2	na	1	4	84.8	1	4	89.9
Cefepime	32	> 32	8.4	=0.3	16	88.6	4	> 32	73.7
Ceftazidime	> 16	> 16	4.1	> 16	> 16	na	> 16	> 16	na
Levofloxacin	8	> 8	8.6	0.25	> 8	68.4	0.5	> 8	72.4
Meropenem	> 16	> 16	15	=0.06	1	94.7	0.12	2	89.1
Minocycline	4	16	55.6	4	> 16	51.9	8	> 16	41.2
PipTazo	> 128	> 128	1.3	64	> 128	30.4	32	> 128	37.3

**Conclusions:** In vitro susceptibility data shows tigecycline and levofloxacin with the lowest MIC90 values against MDR *A. baumannii*, with an MIC90 of 2 µg/mL for tigecycline and an MIC90 of >8 µg/mL for levofloxacin. Against *Enterobacter* spp., meropenem exhibited the lowest MIC90 (1 µg/mL for *E. aerogenes* and 2 µg/mL for *E. cloacae*), followed by tigecycline with an MIC90 of 4 mg/mL. These findings suggest that tigecycline may be a potential therapeutic option in the treatment of selected MDR gram-negative bacteria in Africa-Middle East.

**P1480 In vitro activity of tigecycline in Africa–Middle East: Test 2007–2010**

M. Renteria\*, B. Johnson, S. Bouchillon, D. Hoban, N. Raghuvir (Schaumburg, Collegetville, US)

**Background:** The global Tigecycline Evaluation Surveillance Trial (TEST) is an ongoing global surveillance study designed to evaluate the activity of tigecycline (TIG) and comparators against Gram-positive,

Gram-negative, atypical and anaerobic bacteria. This study reports tigecycline in vitro activity from 2007 to 2010 in pathogens isolated in Africa-Middle East.

**Methods:** Sixty-five investigative sites from nine countries collected clinical isolates in Africa-Middle East. MICs were determined by broth microdilution according to EUCAST guidelines using supplied Trek panels.

**Results:** Results are given by year for tigecycline and key organisms as follows: 1 Includes all phenotypes.

Organism	N	2007		2008		2009		2010	
		MIC <sub>50</sub>	MIC <sub>90</sub>	MIC <sub>50</sub>	MIC <sub>90</sub>	MIC <sub>50</sub>	MIC <sub>90</sub>	MIC <sub>50</sub>	MIC <sub>90</sub>
<i>A. baumannii</i>	511	0.5	1	1	4	1	2	1	4
<i>E. cloacae</i>	640	0.5	1	1	2	0.5	2	0.5	2
<i>E. faecalis</i>	404	0.12	0.25	0.12	0.25	0.12	0.25	0.12	0.25
<i>E. coli</i> <sup>1</sup>	951	0.25	0.5	0.25	1	0.25	0.5	0.25	1
<i>K. oxytoca</i> <sup>1</sup>	102	0.5	1	0.5	1	0.25	1	0.5	1
<i>K. pneumoniae</i> <sup>1</sup>	838	0.5	2	0.5	2	0.5	2	1	2
<i>S. aureus</i> MS	669	0.12	0.25	0.12	0.25	0.12	0.5	0.25	0.5
<i>S. aureus</i> MR	249	0.25	0.25	0.12	0.25	0.12	0.5	0.25	0.5
<i>S. pneumoniae</i> <sup>1</sup>	394	0.03	0.03	0.03	0.06	0.015	0.03	0.015	0.06
<i>P. aeruginosa</i>	739	8	>8	8	>8	8	>8	8	>8

**Conclusions:** Tigecycline retained excellent in vitro activity over time against a broad spectrum of organisms, including drug-resistant strains such as ESBL positive *E. coli*, *K. pneumoniae*, multi-resistant *Acinetobacter* spp., and methicillin-resistant *S. aureus*. Tigecycline MIC50/90 values were essentially unchanged for most pathogens studied in Africa-Middle East over the past 4 years.

**P1481 In vitro activity of tigecycline against global Gram-negative levofloxacin-resistant pathogens: Test 2009–2011**

S. Hawser\*, C. Zampaloni, F. Monti, S. Bouchillon, M. Hackel, M. Dowzicky (Epalinges, CH; Schaumburg, Collegetville, US)

**Background:** Resistance to levofloxacin has risen since its introduction in clinical use, and cross-resistance with other antibacterial classes has been reported. The global Tigecycline Evaluation and Surveillance Trial (TEST) monitors the activity of tigecycline and comparators against multiple pathogens. This report describes the activity of tigecycline against levofloxacin-resistant gram-negative pathogens collected worldwide from 2009 to October 2011.

**Methods:** A total of 32 433 gram-negative isolates were collected from various infection sources worldwide. Of those isolates, 8180 (25%) were levofloxacin-resistant. MICs were performed as specified by CLSI at each site using custom broth microdilution panels and interpreted per CLSI/FDA guidelines.

**Results:** MIC90 (mg/L) and percent susceptible (%S) of tigecycline against all isolates and levofloxacin-resistant isolates are shown below: na, breakpoint not defined; ESBL+, extended-spectrum beta-lactamase positive.

Organism	All isolates			Levofloxacin-resistant		
	n	MIC <sub>90</sub>	% S	n	MIC <sub>90</sub>	% S
<i>A. baumannii</i>	4350	2	na	2181	2	na
<i>E. aerogenes</i>	1645	2	95	193	4	81
<i>E. cloacae</i>	5987	2	95	733	4	82
<i>E. coli</i>	9037	0.5	99.9	3253	1	99.9
ESBL+	1528	0.5	99.9	1250	1	99.9
<i>K. oxytoca</i>	1606	1	99	129	2	95
ESBL+	66	2	97	30	2	97
<i>K. pneumoniae</i>	6793	2	95	1517	4	89
ESBL+	1526	2	92	909	4	90
<i>S. marcescens</i>	3015	2	93	174	4	76

**Conclusions:** Levofloxacin-resistant isolates comprised 25% of all clinical isolates, ranging from 6% in *S. marcescens* to 50% in *A. baumannii*, and up to 82% in ESBL+ *E. coli*. Tigecycline exhibited

susceptibility >90% against levofloxacin-resistant *E. coli* and *Klebsiella* spp., including ESBL+ isolates, and >80% against all other studied levofloxacin-resistant pathogens for which breakpoints exist. MIC90 values for all and levofloxacin-resistant isolates were similar.

**P1482** In vitro activity of tigecycline against vancomycin-resistant enterococci in the USA categorised by CDC regions: Test 2007–2010

S. Bouchillon\*, D. Hoban, R. Badal, M. Hackel, M. Dowzicky (Schaumburg, Collegeville, US)

**Background:** The percentage rates of vancomycin-resistant *Enterococcus* spp. (VRE) vary by country and region. Tigecycline has been shown to have potent activity against commonly encountered species, including those with resistant phenotypes. The purpose of this study was to determine regional variations, if any, and the current activity of tigecycline (TIG) against VRE in the United States.

**Methods:** A total of 2440 clinically relevant isolates of *E. faecalis* and *E. faecium* were collected from patients in 243 hospitals across the United States (US) from 2007 to 2010. MIC's were determined by broth microdilution and interpreted following CLSI guidelines. Tigecycline breakpoints were defined by the FDA. Regions are defined by the CDC.

**Results:** 555/22 544 (22%) of the enterococci were vancomycin-resistant. Results are summarized for tigecycline in the following table:

CDC Region	Total N	VRE	% VRE	mg/L		% Sus
				MIC <sub>50</sub>	MIC <sub>90</sub>	
East North Central (ENC)	600	147	25%	0.12 / 0.25		98%
East South Central (ESC)	165	20	12%	0.12 / 0.25		100%
Middle Atlantic (MA)	656	154	23%	0.12 / 0.25		99%
Mountain (M)	79	20	25%	0.06 / 0.25		100%
New England (NE)	56	7	13%	0.12 / 0.25		100%
Pacific (P)	77	18	23%	0.06 / 0.12		100%
South Atlantic (SA)	479	114	24%	0.12 / 0.25		99%
West North Central (WNC)	322	50	16%	0.12 / 0.25		100%
West South Central (WSC)	110	25	23%	0.12 / 0.25		100%
All Regions	2544	555	22%	0.06 / 0.25		99%

**Conclusions:** ESC, NE, and WNC regions have significantly lower rates of VRE ( $p < 0.05$ ) than all other regions of the US. Tigecycline demonstrated consistent potent activity against VRE in the United States, regardless of region of isolation, with an overall MIC90 of 0.25 mg/L and 99% susceptible.

**P1483** In vitro activity of tigecycline against *Streptococcus pneumoniae* in the United States: TEST 2009–2010

S. Bouchillon\*, S. Hawser, C. Zampaloni, M. Hackel, B. Johnson, D. Hoban, M. Dowzicky (Schaumburg, US; Epalinges, CH; Collegeville, US)

**Objectives:** *S. pneumoniae* (SPN) continues to be recognized as a significant respiratory and bacteremia pathogen. Resistance to both oral and parenteral antibiotics used to treat SPN infection continues to evolve and newer antibiotics are needed with anti-SPN activity. The Tigecycline Evaluation and Surveillance Trial (TEST) is an ongoing global surveillance trial designed to follow trends in antimicrobial activity for tigecycline (TIG); amoxicillin-clavulanic acid (A/C); ceftriaxone (CAX); clindamycin (CLIN); erythromycin (ERY); levofloxacin (LVX); meropenem (MER); and penicillin (PEN). This report evaluates the in vitro activity of tigecycline and comparators against SPN in the United States (US) from 2009 to 2010.

**Methods:** A total of 559 clinical isolates were collected from 78 cumulative investigator sites in the US. Clinical isolates were identified to the species level at each participating site and confirmed by the

central laboratory with the Optichin disk test. Minimum Inhibitory Concentrations (MICs) were determined both by the local laboratory and supplemented by a central laboratory using broth microdilution panels and interpreted according to current CLSI and FDA (tigecycline) guidelines.

**Results:** In vitro results are presented in the following table: MIC90 not calculated for n's <10.

\*MDR, MultiDrug-Resistant (three or more drug classes).

Phenotype	N	MIC <sub>90</sub> (mg/L) % Susceptible							
		TIG	A/C	CAX	CLIN	ERY	LVX	MER	PEN
All SPN	559	0.03/99	8/84	2/89	>64/79	64/59	1/99	1/79	4/61
Pen-Fes	104	0.03/99	8/23	2/46	>64/33	>64/13	1/99	2/3.9	4/0
Macro-Res	221	0.03/100	8/66	2/79	>64/46	>64/0	1/99	1/67	4/28
MDR*	86	0.03/100	>8/8.1	2/40	>64/15	>64/3.5	1/99	2/1.2	8/0

**Conclusions:** TIG inhibited ≥99% of all SPN at its FDA breakpoint of 0.06 mg/L for all SPN collected during the course of this study including Pen-, Levo-, Macro-Resistant, and MDR isolates. The in vitro activity of TIG was comparable to that of LVX.

**P1484** Occurrence of resistant phenotypes in the Middle East and susceptibility to tigecycline: Test 2007–2011

M. Hackel\*, S. Lob, R. Badal, S. Bouchillon, M. Dowzicky (Schaumburg, Collegeville, US)

**Objectives:** Development of bacterial resistance continues to cause concern world-wide, but availability of newer agents offers clinicians options for therapy. Tigecycline (TIG) has a very broad spectrum of activity, including strains resistant to other drugs. As part of the global Tigecycline Evaluation Surveillance Trial (TEST), strains collected in the Middle East (ME) from 2007 to 2011 were evaluated for prevalence of resistant phenotypes and susceptibility to TIG.

**Methods:** A total of 2502 pathogens were collected from nine sites in Israel, one in Jordan, one in Oman, one in Saudi Arabia, and five in Turkey from a variety of infection sources. MICs were determined at each site using custom broth microdilution panels following CLSI guidelines and interpreted according to FDA breakpoints for TIG.

**Results:** Tigecycline's in vitro activity is summarized below:

%S, % susceptible; ROW, rest of world; ESBL+, extended-spectrum beta-lactamase positive; BL+, beta-lactamase positive; MRSA, Methicillin-resistant *S. aureus*; PRSP, penicillin-resistant *S. pneumoniae*; VR, vancomycin-resistant.

Organism (% resistant phenotype ME/ROW)	n	MIC <sub>50</sub> (mg/L)	MIC <sub>90</sub> (mg/L)	% S
<i>Escherichia coli</i>	684	0.25	1	100
ESBL+ (20 ME/15 ROW)	136	0.5	1	100
<i>Klebsiella pneumoniae</i>	601	0.5	2	94
ESBL+ (24 ME/21 ROW)	144	1	4	87
<i>Haemophilus influenzae</i>	237	0.12	0.25	100
BL+ (20 ME/19 ROW)	47	0.12	0.25	100
<i>Staphylococcus aureus</i>	627	0.12	0.5	100
MRSA (27 ME/36 ROW)	172	0.25	0.5	100
<i>Streptococcus pneumoniae</i>	262	0.015	0.03	99
PRSP (26 ME/16 ROW)	68	0.03	0.03	97
<i>Enterococcus faecium</i>	91	0.12	0.25	100
VR (22 ME/31 ROW)	20	0.12	0.25	100

**Conclusion:** Significant differences exist in the proportion of resistant phenotypes of several species between ME and the rest of the world (ROW, TEST 2008–2010): ESBL+ isolates comprised 20% of all *E. coli* in the ME vs. 15% in ROW, MRSA were 27% of all *S. aureus* in ME vs. 36% in ROW, PRSP (26% vs. 16%, respectively), and VR *E. faecium* (22% vs. 31%, respectively); all  $p < 0.05$  (Chi-square with Yates correction).

Tigecycline showed excellent in vitro activity against a diverse collection of gram-negative and gram-positive pathogens from the Middle East, including ESBL+ strains, MRSA, PRSP, and VRE. Susceptibility was  $\geq 93\%$  for all tested species and their resistant phenotypes, except ESBL+ *K. pneumoniae*, which was slightly less susceptible partly due to a low %S in Israel (83%).

**P1485** In vitro activity of tigecycline against commonly-isolated pathogens of skin and skin structure infections in Europe: Test 2009–2010

S. Bouchillon\*, M. Hackel, D. Hoban, R. Badal, S. Hawser, M. Dowzicky (Schaumburg, US; Epalinges, CH; Collegenille, US)

**Background:** Tigecycline has been approved for the treatment of complicated skin and skin structure infections (SSTIs) in Europe since 2006. Since introduction, tigecycline has shown little development of resistance to common pathogens of SSTIs. The Tigecycline European Surveillance Trial (TEST) has been monitoring susceptibility of tigecycline since 2004. This study reports on the activity of tigecycline against recent clinical isolates from SSTIs in Europe.

**Methods:** A total of 6880 clinical isolates from SSTI were collected and identified in 25 countries in Europe in 2009–2010. MICs were determined by broth microdilution (aerobes) or agar dilution (anaerobes) using CLSI guidelines. Susceptibility of tigecycline was interpreted using EUCAST and FDA (anaerobes) breakpoints as available.

**Results:** Results of tigecycline activity are summarized below: na, – breakpoints not defined. FDA breakpoints used for anaerobes.

Organism	n	MIC <sub>50</sub>	MIC <sub>90</sub>	%S
<i>Acinetobacter</i> spp.	586	0.5	2	na
<i>Bacteroides</i> spp.	519	0.5	2	98
<i>Clostridium</i> spp.	149	$\leq 0.06$	0.5	99
<i>Enterobacter</i> spp.	914	0.5	2	88
<i>Enterococcus faecalis</i>	435	0.12	0.25	100
<i>Enterococcus faecium</i>	145	0.06	0.25	99
<i>Escherichia coli</i>	823	0.25	0.5	99
<i>Finexgoldia magna</i>	134	$\leq 0.06$	0.25	100
<i>Klebsiella pneumoniae</i>	492	0.5	2	86
<i>Peptoniphilus</i> spp.	46	$\leq 0.06$	0.06	100
<i>Peptostreptococcus</i> spp.	238	$\leq 0.06$	0.12	100
<i>Prevotella</i> spp.	243	0.12	0.5	100
<i>Serratia</i> spp.	368	1	2	67
<i>Staphylococcus aureus</i>	1457	0.12	0.25	100
MRSA	282	0.25	0.5	100
MSSA	1175	0.12	0.25	100
<i>Streptococcus agalactiae</i>	331	0.06	0.12	100

**Conclusions:** Tigecycline demonstrated potent in vitro activity against the vast majority of recent isolates from SSTI including gram-negative, gram-positive, aerobic and anaerobe pathogens. MIC<sub>90</sub> values ranged from  $\leq 0.06$  to 2  $\mu\text{g/mL}$ . Three species, *Enterobacter*, *K. pneumoniae*, and *Serratia*, had % susceptibility  $< 90\%$  using EUCAST breakpoints.

**P1486** Tigecycline and comparators activity in vitro against multidrug-resistant Gram-negative bacteria in Latin America collected in 2007–2010

M. Renteria\*, M. Hackel, S. Bouchillon, D. Hoban, B. Johnson, R. Badal, S. Hawser, M. Dowzicky (Schaumburg, US; Epalinges, CH; Collegenille, US)

**Background:** Reduced treatment options for multi-drug resistant (MDR) gram negative bacteria are a major cause of mortality for patients with nosocomial infections. The global Tigecycline Evaluation Surveillance Trial (TEST) evaluated the activity of tigecycline and comparators against MDR *Acinetobacter baumannii*, *Enterobacter aerogenes* and *E. cloacae* from Latin America during 2007–2010.

**Methods:** A total of 1852 clinical isolates of *A. baumannii*, *E. aerogenes* and *E. cloacae* were collected from multiple infection sources in ten countries in Latin America during 2007–2010. MICs were performed at each site using prepared broth microdilution panels following CLSI guidelines. MICs were interpreted according to CLSI/FDA guidelines. MDR isolates were defined as those resistant to three or more antimicrobial drug classes.

**Results:** MIC<sub>50</sub> and MIC<sub>90</sub> in mg/L, as well as % susceptible (%S) are shown in the following table for tigecycline and selected comparators: na: no defined breakpoint.

Drug	<i>A. baumannii</i> (n=1149)			<i>E. aerogenes</i> (n=179)			<i>E. cloacae</i> (n=903)		
	MIC <sub>50</sub>	MIC <sub>90</sub>	%S	MIC <sub>50</sub>	MIC <sub>90</sub>	%S	MIC <sub>50</sub>	MIC <sub>90</sub>	%S
Tigecycline	0.5	2	na	0.5	2	91.6	1	2	92.8
Cefepime	$> 32$	$> 32$	7.4	4	$> 32$	58.7	4	$> 32$	66.7
Levofloxacin	$> 8$	$> 8$	3.6	2	$> 8$	50.8	1	$> 8$	59.8
Meropenem	$> 16$	$> 16$	14.2	0.12	4	84.9	0.12	1	91.6
Minocycline	1	8	86.7	4	$> 16$	51.4	8	$> 16$	34.3
Pip-Tazo	$> 128$	$> 128$	1.7	32	$> 128$	40.8	32	$> 128$	44.2

**Conclusions:** In vitro susceptibility data shows tigecycline and minocycline with the lowest MIC<sub>90</sub> values against MDR *A. baumannii*, with an MIC<sub>90</sub> of 2 and 8 mg/L for tigecycline and minocycline, respectively. Against *Enterobacter* spp., tigecycline and meropenem exhibited the lowest MIC<sub>90</sub>s of 2 and  $\leq 4$  mg/L, respectively. These findings suggest that tigecycline may be a potential therapeutic option in the treatment of MDR gram-negative bacteria in patients from Latin America.

**P1487** Trends in tigecycline activity against Gram-negative respiratory pathogens: Asia/Pacific 2004–2010

M. Hackel\*, D. Hoban, S. Bouchillon, J. Johnson, S. Hawser, M. Dowzicky (Schaumburg, US; Epalinges, CH; Collegenille, US)

**Background:** Tigecycline has been shown to have potent broad spectrum activity against gram-negative pathogens except *P. aeruginosa*. This report focuses on in vitro susceptibility trends of Asia/Pacific respiratory isolates using data from the Tigecycline Evaluation Surveillance Trial (TEST).

**Methods:** Three thousand and sixty-four gram-negative clinical isolates from respiratory sources were collected from 49 sites in 11 countries in Asia/South Pacific. Minimum inhibitory concentrations (MICs) were determined by the local laboratory using supplied broth microdilution panels and interpreted according to FDA guidelines for tigecycline. Linear trends in % susceptible were assessed with the Cochran-Armitage test.

**Results:** In vitro activity for tigecycline and key respiratory species by year are as follows:

na: breakpoint not defined; ESBL+: extended-spectrum beta-lactamase positive; BL+: beta-lactamase positive

\*Statistically significant decrease in % susceptible ( $p < 0.05$ ).

**Conclusions:** Tigecycline demonstrated MIC<sub>90</sub> values of  $\leq 2$  mg/L against the majority of key gram-negative respiratory pathogens including several resistant phenotypes. In 2010, apart from

Organism n by year	MIC <sub>50</sub> (mg/L) / % Susceptible						
	2004	2005	2006	2007	2008	2009	2010
<i>A. baumannii</i> 36/27/43/143/73/15/66	1/na	1/na	2/na	2/na	2/na	2/na	2/na
<i>E. cloacae</i> 21/24/29/32/33/32/41	1/100	2/95.8	2/96.6	2/93.6	4/88.7	2/96.3	1/97.6
<i>E. coli</i> 15/9/143/4/40/46/26	0.5/100	0.5/100	0.5/100	0.25/100	1/97.5	1/100	0.5/100
<i>E. coli</i> (ESBL+) 3/3/9/10/10/21/11	0.25/100	0.25/100	0.5/100	0.25/100	1/100	0.5/100	0.5/100
<i>H. influenzae</i> 63/50/157/195/10/49/27/7	0.25/100	0.25/100	0.25/99.4	0.25/98.5	0.25/94.2	0.25/100	0.25/100
<i>H. influenzae</i> (BL+) 12/94/10/3/23/21/24	0.12/100	0.25/100	0.25/97.6	0.25/100	0.25/100	0.12/100	0.25/100
<i>K. pneumoniae</i> 45/32/50/10/9/7/11/15/53	1/97.8	2/90.6	2/96.0	2/95.4	2/90.9	2/91.3	2/90.9
<i>K. pneumoniae</i> (ESBL+) 13/13/17/23/19/34/6	1/100	4/76.9	2/100	1/95.7	1/100	4/85.3	4/83.3
<i>P. aeruginosa</i> 4/8/17/9/13/9/9/12/7/66	16/na	16/na	>16/na	16/na	>16/na	16/na	>16/na
<i>S. marcescens</i> 21/18/24/6/35/48/37	1/100	2/100	1/100	2/100	2/94.74	4/73.5	1/97.3*

*P. aeruginosa* against which tigecycline is known to have very limited activity, only ESBL+ *K. pneumoniae* showed an MIC<sub>90</sub> of 4 mg/L and a % susceptible <90%. No statistically significant decrease in susceptibility was found between 2004 and 2010 for any tested species except *S. marcescens*. Even for this species, the % susceptible appeared to be increasing again in 2010. Tigecycline is not approved for the use in HAP infections.

#### P1488 In vitro activity of tigecycline against Gram-negative species in Spain: TEST 2009–2010

M. Renteria\*, S. Hawser, S. Bouchillon, J. Johnson, C. Zampaloni, F. Monti, M. Dowzicky (Schaumburg, US; Epalinges, CH; Collejeville, US)

**Background:** The Tigecycline Evaluation and Surveillance Trial (TEST) monitors the activity of tigecycline and comparators against multiple pathogens collected worldwide. The current report describes the activity of Tigecycline against gram-negative pathogens isolated during 2009–2010 from Spain.

**Methods:** A total of 2676 gram-negative clinical isolates were collected from multiple infection sources in 33 sites in Spain. Susceptibility testing was performed as per CLSI guidelines and interpreted using EUCAST clinical breakpoints.

**Results:** Susceptibility of the 2676 isolates to tigecycline is shown below:

%S, %I, %R; percent susceptible, intermediate or resistant; ND\*: not determined as no clinical breakpoints exist for this drug / organism combination.

Species	N	MIC <sub>50</sub>	MIC <sub>90</sub>	%S	%I	%R
<i>Acinetobacter baumannii</i>	253	1	2	ND*	ND	ND
<i>Enterobacter aerogenes</i>	136	0.5	1	92.7	5.1	2.2
<i>Enterobacter cloacae</i>	410	0.5	1	90.7	5.1	4.2
<i>Escherichia coli</i>	598	0.25	0.5	99.3	0.7	0.0
<i>E. coli</i> , ESBL-positive	119	0.25	1	97.5	2.5	0.0
<i>E. coli</i> , ESBL-negative	479	0.25	0.5	99.9	0.2	0.0
<i>Haemophilus influenzae</i>	201	0.12	0.25	ND*	ND	ND
<i>Klebsiella oxytoca</i>	112	0.5	1	95.6	4.5	0.0
<i>K. oxytoca</i> , ESBL-positive	1	0.12	0.12	100	0.0	0.0
<i>Klebsiella pneumoniae</i>	482	0.5	2	86.5	9.3	4.1
<i>K. pneumoniae</i> , ESBL-positive	84	0.5	2	86.9	10.7	2.4
<i>Pseudomonas aeruginosa</i>	484	8	>8	ND*	ND	ND

**Conclusions:** Tigecycline exhibited an MIC<sub>90</sub> of 0.12 – 16 mg/L against 2209 gram-negative isolates from Spain. Susceptibility to tigecycline ranged from 86.5% to 99.9%. Although no clinical breakpoints exist for tigecycline and *Acinetobacter*, tigecycline exhibited good in vitro activity against *A. baumannii*. As expected, tigecycline has very limited activity against *P. aeruginosa*. Tigecycline continues to exhibit good activity against the majority of Spanish gram-negative isolates from TEST.

#### P1489 Comparative evaluation of Vitek 2, Etest, MIC test strip and broth microdilution for susceptibility testing of tigecycline

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**Objectives:** The increasing use of tigecycline in clinical practice necessitates the availability of simple and reliable methods for susceptibility testing. Discrepancies between methods have been reported. We compared four susceptibility methods for testing tigecycline against MDR Enterobacteriaceae and *Acinetobacter baumannii* clinical isolates.

**Methods:** A total of 241 nonduplicate clinical isolates were included in the study: ESBL-producing *E. coli* (n = 20) and *K. pneumoniae* (n = 20), KPC and/or VIM-carbapenemase-producing *K. pneumoniae* (n = 125), Enterobacter spp. (n = 20) and *A. baumannii* (n = 56). The study isolates were selected to exhibit variable tigecycline MICs according to the initial susceptibility testing by Vitek 2 (including strains with reduced susceptibility) and to possess various resistance determinants. MIC values were determined by broth microdilution (BMD) as the reference standard (Trek Diagnostics Systems, Cleveland, OH, USA), MIC Test Strip (MTS, Liofilchem, Italy), Vitek 2 and Etest (bioMérieux, France), following CLSI recommendations and manufacturers' instructions. All methods were performed simultaneously. *E. coli* ATCC 25922 was used for quality control. The FDA approved breakpoints were applied. MIC agreement between methods and error classification were based on the susceptibility testing criteria established by CLSI.

**Results:** Susceptibility rates by BMD, Vitek2, Etest and MTS were 83.4%, 42.3%, 82.2% and 94.6%, respectively. Testing by BMD, Etest and MTS resulted in an MIC<sub>50</sub> within the susceptible range (1, 2, and 0.5 mg/L), while for Vitek2 the MIC<sub>50</sub> was within the intermediate range. The MIC<sub>90</sub> values were inconsistent for Vitek2 (≥8 mg/L) and MIC Test Strip (2 mg/L) compared with BMD (4 mg/L). The highest rate of MIC agreement was noted for Etest (93.8%). MTS resulted in a relatively high rate of MIC agreement (73.9%). MIC agreement rate for Vitek2 was 59.8%. Etest exhibited the lowest error rates (Major Errors [ME] = 0.4%, Minor Errors [MiE] = 7.5%). No MEs were detected for MTS, however it yielded one Very Major Error (0.4%) and 14.5% MiE. Vitek2 generated the highest rates of both ME (9.1%) and MiE (43.2%).

**Conclusion:** There was a trend for lower MICs determined by MTS and higher MICs identified by Vitek2 than by BMD. A poor concordance of MIC results was identified for Vitek2, compared with BMD. As susceptibility testing errors may have significant therapeutic implications, confirmation of MIC results by reference methods is recommended.

#### Novel approaches in antimicrobial susceptibility testing

##### P1490 Standardised susceptibility testing with 6 or 8 hours incubation seems possible – first data from the Eurostar Rapid Disk project

E. Jonasson\*, G. Cederbrant, E. Matuschek, G. Kahlmeter, M. Sundqvist (Växjö, SE)

**Objectives:** In an era of increasing resistance 16–20 hours incubation time for antibiotic susceptibility testing (AST) is too long. We have previously shown that rapid AST on the EUCAST Mueller–Hinton Fastidious medium MH-F agar in CO<sub>2</sub> is possible. This is however not a suitable methodology for most automated systems. The aim of the Eurostar project is to develop a standardised disk diffusion test on Mueller–Hinton agar (MH) with a decidedly shorter incubation time. We present the development of first data showing the reproducibility of zone measurements after 6 and 8 hours incubation.

**Method:** The EUCAST methodology for disk diffusion testing was used with a shorter incubation time (6 and 8 hours). A set of 5–7

antibiotics were tested. Between 40 and 70 repeated tests were performed for each ATCC strain (*Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853). The target value and range were calculated.

**Results:** Zones were registered after 6 hours of incubation for *E. coli*, *E. faecalis*, and *K. pneumoniae* whereas for *S. aureus* and *P. aeruginosa* 8 hours incubation was needed. Pre-heating of the plates did not decrease the time to readable zones. A normal distribution of inhibition zones was achieved for all combinations of organisms and antimicrobials. The target value was 0–8 mm lower than the corresponding standardised EUCAST QC-target with 16–20 hours incubation. The range was typically 5 mm.

**Conclusion:** It seems possible to standardise AST for a shorter incubation time than the standard 16–20 hours, maybe as short as 6–8 hours on MH agar. All species/antibiotic combinations will not respond equally to a shorter incubation time and time related zone diameter breakpoints will be needed. A standardised rapid AST method is now developed within the Eurostar project.

#### P1491 Rapid susceptibility testing of blood culture isolates using disc diffusion and epidemiological cut-off values

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**Objectives:** Susceptibility testing (AST) using disk diffusion has been validated for 16–20 hours incubation. In an era of increasing resistance this time to result needs to be shortened. We describe a standardised method for direct susceptibility testing of blood culture isolates based on 6–8 hours incubation. Epidemiological cut off (ECOFF) values for commonly used antibiotics were used to categorize isolates as susceptible provided the wildtype were declared susceptible by EUCAST clinical breakpoints.

**Methods:** ECOFF values for commonly used antibiotics in the treatment of septicaemia were ascertained for an inoculum of approx 108 CFU/mL (corresponding to a 10-fold dilution of bacterial suspensions in BacTAlert bottles) inoculated on MH-F agar in 5% CO<sub>2</sub> in air for 6 and 8 hours. Between 45 and 100 isolates per species were used in the development of tentative ECOFFs for 6–8 hours incubation. The tentative cut-off values were then challenged with disk diffusion performed directly from positive blood cultures with a limited set of antibiotics on 100 isolates of *Enterobacteriaceae*, 4 *Pseudomonas aeruginosa*, 21 *Staphylococcus aureus*, five enterococci and seven isolates of beta haemolytic streptococci.

**Results:** Tentative ECOFFs could be calculated for all species/antibiotic combinations for 6 or 8 hours incubation. The direct ASTs from positive blood cultures could be read after 5.0–8.25 hours incubation. The normal distribution of inhibition zones derived from the clinical cultures were shifted 1–2 mm below the calculated experimental distributions. On the basis of this an adjustment of the tentative ECOFFs were made. (The adjustment is exemplified in Table 1 Cefotaxim ECOFF = 20 for Enterobacteriaceae) No errors in susceptibility categorization were seen between the blood culture direct AST results and the standard 18 hours AST. However, this required that the species were correctly identified at the time of AST result.

	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	28	29
<i>E. coli</i> experimental (n=50)																2	2	12	12	7	8	5	2
<i>E. coli</i> Blood cultures (n=66)	2															4	7	10	19	11	8	3	2

**Conclusion:** It is possible to assure susceptibility based on quantitative zone measurements after 5–8 hours incubation, the time depending on the species. The method is robust but results should, for the time being, be used as a preliminary report. The ECOFFs proposed can be used

provided that the ID to genus level is known which today can easily be obtained using MALDI-TOF.

#### P1492 Primary susceptibility testing of urine specimens: why not reduce time to laboratory report and cost?

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Clinical microbiology laboratories in Denmark have a long tradition for primary susceptibility testing (PST). Urine samples and swabs are in addition to plates for growth, enumeration and identification also inoculated on susceptibility agar with discs. The next day pathogens are identified by simple tests (CPS ID3 chromogenic agar, spot indole etc.) or MALDI-TOF, the primary susceptibility plates are read, and results are reported electronically directly to the clinician. Advantages are clear, speed and cost. However, this tradition dates long back to times before the need of documenting your practice, and also literature is sparse on this subject. Our objective was to evaluate the results of PST against a standardized susceptibility testing method.

**Methods:** During a 5 week period (April – May 2011) all culture positive urines from the routine laboratory that had a susceptibility test read day one, were included in the study. Susceptibility testing was done by the disc diffusion method using Isosensitest agar (SSI Diagnostica, Hillerød) and Oxoid discs (Oxoid, Greve) against eight antibiotics: ampicillin, cefpodoxime, gentamicin, mecillinam, nalidixic acid, sulphonamides, nitrofurantoin and trimethoprim. PST was done using a cotton swab dipped in urine, rolled against the inside of the specimen tube to remove surplus fluid, and spread directly with close streaks on the Isosensitest agar. Standardized susceptibility testing was done using a 1:100 dilution of a 0.5 McFarland suspension from a pure culture. Method and zone breakpoints were according to SRGA version 2009-04-27/28. The following FDA validation criteria for accepting a susceptibility testing systems were used; general agreement >89.9%, major errors (MaE; false resistance) ≤3.0% and very major errors (VME; false susceptibility) ≤1.5%.

**Results:** A total of 1.688 antibiotic-bacteria combinations on 219 isolates were evaluated and scored as follows: 1.591 agreements (94.4%), 52 (3.1%) minor errors (MiE), 40 (2.4%) MaE and 5 (0.3%) VME. The three most frequent errors were 28 MiE for mecillinam, 13 MaE for sulphonamides and 11 MiE for nitrofurantoin. The five VME were false susceptibility towards sulphonamides (N = 3), ampicillin (N = 1) and trimethoprim (N = 1).

**Conclusion:** Despite obvious problems as using non-standardized inoculums and potentially mixed cultures, PST of urine specimens provides acceptable results. PST is accurate, cost effective and can provide laboratory reports in 1 day.

#### P1493 Direct blood culture antimicrobial susceptibility testing in an East-Tallinn central hospital, Estonia

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**Objectives:** A rapid and accurate antimicrobial susceptibility testing (AST) is essential in the optimization of antibacterial therapy leading to successful management of patients with bloodstream infections. One potential possibility for acceleration of AST is direct susceptibility testing from positive blood cultures.

The aim of study was to evaluate the reliability of a primary direct antimicrobial susceptibility testing (DAST) results performed directly from positive blood cultures as compared to susceptibility test results performed next day on pure cultures according to classical disk diffusion method.

**Methods:** Blood cultures were performed with Bact/ALERT 3D system (Biomérieux) during period from March to October 2011.

Positive blood cultures have been tested directly to identify microorganisms and their antimicrobial susceptibility. DAST profile was selected following adapted laboratory protocol in accordance with Gram staining results using EUCAST disk diffusion with respective

breakpoints. After the final definitive identification of microorganisms additional determination of antimicrobial susceptibility on pure cultures was performed, and both susceptibility data were compared and analyzed.

**Results:** Seventy-six positive blood cultures were investigated, and 45 Gram-negative and 31 Gram-positive isolate were found. Susceptibility of isolate to antibiotics was categorized as sensitive (S) or non-sensitive (NS). Our results showed a good concordance of preliminary and final antimicrobial susceptibility testing results. Among 557 antibiotics comparisons in 76 microorganisms, only 4 (0.7%) discrepancies ("NS" on preliminary testing turned to "S" on final testing) of antibiotics testing in 4 (5.0%) microorganisms were noted. Discordance of results in these cases can be explained by prolonged incubation of plates or bottles (more than 20 hours (40–48 hours)) and by "inoculum effect" that leads to increased in-vitro resistance.

Table. Direct („preliminary") and standard („final") antimicrobial susceptibility testing results

		Preliminary susceptibility, N (%)					
		Antibiotic test			Microorganism		
		S	NS	Total	S	NS	Total
Final susceptibility, N (%)	S	476 (85,5)	4 (0,7)	480 (86,2)	48 (63,2)	4 (5,0)	52 (68,4)
	NS	0 (0,0)	77 (13,8)	77 (13,8)	0 (0,0)	24 (31,6)	24 (31,6)
	Total	476 (85,5)	81 (14,5)	557 (100,0)	48 (63,2)	28 (36,8)	76 (100,0)

S - susceptible; NS - nonsusceptible

**Conclusions:** Our study showed that direct antimicrobial susceptibility testing (DAST) is helpful tool for acceleration of AST and can be implemented in routine laboratory practice but some exceptions must be made: (i) incubation time must be 16–20 hours, otherwise only "sensitive" results can be reported, (ii) in suspicious cases additional AST on pure culture must be done, (iii) do not report result if isolate is not finally identified, and (iv) do not report result if the growth on agar is visibly light.

#### P1494 Identification of *Escherichia coli* hyperproducing chromosomal ampC using combination discs

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**Objectives:** To investigate if MAST D68C discs and Rosco ESBL + AmpC discs correctly identify plasmid-mediated ampC (pAmpC) and hyperproducing chromosomal ampC (cAmpC) in *Escherichia coli*.

**Methods:** A set of previously well-characterized strains of hyperproducing cAmpC *E. coli* (n = 16) were used to investigate the ability of MAST D68C discs and Rosco ESBL + AmpC discs to detect cAmpC. Next non-replicate clinical isolates of *E. coli* (n = 72) with reduced susceptibility to cefepodoxime were prospectively collected and classified as ESBL or AmpC producers. ESBL producers and AmpC hyperproducers were confirmed by molecular analysis and analyzed with MAST D68C discs. PCR amplification for blaCTX-M, blaSHV and blaTEM and following sequencing was used to confirm expression of ESBL. The presence of a pAmpC was investigated by multiplex PCR and the promoter of the chromosomal ampC gene was sequenced to identify promoter mutations associated with hyperproduction. Clinical strains that were found positive for an AmpC phenotype by the MAST D68C discs were also analyzed by Rosco ESBL + AmpC discs.

**Results:** All strains from a set of well-characterized strains hyperproducing cAmpC were positively identified as AmpC producing isolates by the MAST D68C discs whereas the Rosco ESBL + AmpC discs misidentified two of these strains. Of the 72 clinical isolates, 65 were categorized as ESBL producers and seven were categorized as AmpC producers by molecular methods. Two of the AmpC-producing isolates contained a blaCMY-2 gene. The remaining five isolates all had mutations or insertions in the promoter

region, which could explain increased expression of the chromosomal AmpC enzyme. All seven strains hyperproducing AmpC were positive in the AmpC disk test. Again all strains were correctly identified as ESBL or AmpC producing isolates by the MAST D68C discs whereas the Rosco ESBL + AmpC discs misidentified two of the hyperproducing cAmpC strains.

**Conclusion:** Both MAST D68C discs and Rosco ESBL + AmpC discs correctly identify ESBL and blaCMY-2 whereas only MAST D68C discs correctly identify all hyperproducing cAmpC in *E. coli*.

#### P1495 Detection of extended-spectrum beta-lactamases and carbapenemases using the MALDI-TOF mass spectrometry method

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**Objectives:** Antibiotic resistance among Gram-negative bacteria, especially Enterobacteriaceae, *Pseudomonas* spp. and *Acinetobacter* spp. is an increasing, alarming problem. Traditional susceptibility testing requires at least 18 hours for a result. MALDI-TOF mass spectrometry is a rapid method for the identification of bacteria and it has been recently used also to detect some resistance mechanisms within few hours. Objective of this study was to develop a new clinical protocol for rapid detection of ESBL- and carbapenemases-producing (KPC and VIM) isolates.

**Methods:** Vials containing 1 mL aliquots of a 1 g/L solution of four drugs (Cefotaxime, Ceftazidime, Ceftriaxone and Ertapenem in 0.85% NaCl) were inoculated with fresh, overnight cultures of the strains by a 10 mL loop. A total of 18 strains were tested, including 6 KPC-, 1 VIM-, 8 ESBL-producing isolates and three susceptible isolates.

Vials were incubated for 3 hours at 35° C and then centrifuged for 2 minutes at 13 000 rpm. One microliter of the supernatant was applied to a polished steel target and, after drying, overlaid with 1 mL HCCA (10 mg/mL in OS) and air dried again.

Mass spectra were acquired between 420 and 580 Da using a Microflex LT mass spectrometer by the flexControl 3.3 software and analyzed by the flexAnalysis 3.3 provided by the manufacturer (Bruker Daltonics GmbH, Germany).

The absence of peaks indicated the antibiotic degradation and the presence of extended-spectrum beta-lactamases or carbapenemases according to the tested drug.

**Results:** The acquired spectra of resistant strains producing KPC and VIM revealed disappearance of Ertapenem related peaks; Cefotaxime, Ceftriaxone and Ceftazidime related peaks were destroyed by the action of the different ESBL positive strains, whereas in the susceptible isolates these peaks were maintained.

**Conclusion:** MALDI-TOF seems to be a rapid, reliable method for the screening of KPC, VIM and ESBL multi-resistant clinical strains.

#### P1496 Evaluation of six different phenotypic screening tests to detect metallo-beta-lactamase production in *Pseudomonas aeruginosa* in a Belgian tertiary care hospital

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**Objectives:** To evaluate six different tests for phenotypic detection of metallo-beta-lactamases in *Pseudomonas aeruginosa* (PA) strains: three tests based on the chelating properties of ethylenediaminetetraacetic acid (EDTA) and three tests based on dipicolinic acid (DPA).

**Methods:** Six different screening tests were performed on a collection of 135 well characterized PA meropenem non-susceptible clinical isolates (58 MBL positive: 27 for bla IMP, 31 for bla VIM; four pulsotypes). For a description of the different tests: see Table. All tests were carried out by one laboratory technologist, but the tests were read individually by four other experienced technologists. Each reading was counted as one individual result. The sensitivities, specificities, positive and negative predictive values (for theoretical prevalences of 0.05 and

0.50) were determined, with presence of MBL genes (PCR) as gold standard.

**Results:** Test details are shown in the Table. Overall, none of the six different phenotypic tests evaluated yielded specificities resulting in positive predictive values above 0.60 (range: 0.07–0.53 for a theoretical and probably realistic prevalence of 0.05). The highest specificity of 0.97, with a corresponding positive predictive value of 0.53, was obtained using a test with home-made imipenem and imipenem + EDTA disks. Using the MBL-E test, a specificity and positive predictive value of respectively 0.81 and 0.20 were obtained. Other test methods resulted in positive predictive values below 0.30 (prevalence of 0.05). In contrast, the negative predictive values of all tests methods were above 0.95.

Methods	SENS % (95% CI)	SPEC % (95% CI)	PPV Prev. 0.05	PPV Prev. 0.50	NPV Prev. 0.05	NPV Prev. 0.50
ROSCO-DIP-EDTA <sup>1</sup>	95 (92-98)	39 (36-44)	0.08	0.61	0.99	0.89
ROSCO-ghost zone <sup>2</sup>	97 (95-99)	34 (31-39)	0.07	0.60	1.00	0.92
MBL E-test <sup>3</sup>	89 (84-93)	81 (77-86)	0.20	0.82	0.99	0.88
DPA- broth <sup>4</sup>	88 (83-91)	76 (73-79)	0.16	0.79	0.99	0.86
DIP-EDTA <sup>5</sup>	63 (59-66)	97 (94-99)	0.53	0.95	0.98	0.72
DIP-DPA <sup>6</sup>	93 (90-96)	46 (42-50)	0.08	0.63	0.99	0.87

<sup>1</sup>A 0.3 MDP bacterial suspension was inoculated on a MBL Biana (EAO laboratories, Bannockburn, Scotland) agar plate. DIP (10µg, Neomembra, Enzo Diagnostics) and DIP+EDTA disks (25x70 µg) were placed on the agar. Following Enzo Diagnostics interpretative criteria, a test was considered positive if the DIP+EDTA disk zone was at least 7 mm larger than the zone around the DIP disk. <sup>2</sup>A 0.3 MDP bacterial suspension was inoculated on a MDP agar plate. MBL (10µg) and DIP (10µg) were diluted around a DPA disk (25µg, Enzo) at a distance of 5 mm. Enhancement of the zone of inhibition in the area between the antiseptical agar and the antibiotic disk was considered to indicate positivity for MBL. <sup>3</sup>We performed MBL E-test (ABI Biodisk), following the manufacturer's recommendations. A test was considered positive if the DIP+DIP-EDTA zone was ≥1 and/or if an oligocephalus zone was present. <sup>4</sup>Two MBL-Biana agar plates, one blank and one with 200 µg/ml DPA (Sigma-Aldrich, Ohio), were inoculated with a 0.3 MDP bacterial suspension. MBL (10µg) and DIP (10µg) disks were placed on the agar plates. A test was considered positive if the difference between the diameters was ≥ 2 mm for the MBL and/or ≥ 2 mm for the DIP. <sup>5</sup>Minimum DIP-EDTA and EDTA disks (respectively a 10 µg DIP disk and a blank disk supplemented with a 5 µl 0.123 M EDTA solution from Sigma Aldrich) were placed on a MBL agar (which was inoculated with a 0.3 MDP suspension). An DIP disk (10µg, Enzo) was placed at a distance of 10mm from the EDTA disk. The diameters between the DIP and DIP+EDTA disk were measured and the presence of a ghost zone between the DIP and EDTA disk was recorded. The test was considered positive if the difference in diameter was ≥ 2 mm and/or if a ghost zone was present. <sup>6</sup>Identical as the DIP-EDTA test but by using DPA (Sigma-Aldrich, Ohio).

**Conclusion:** In the current epidemiological setting, the phenotypic “confirmation tests”, as evaluated in our analysis, can only be used as “screening tests” (to rule out), and are not suitable for confirmatory testing.

#### **P1497** Early detection of vancomycin resistant sub-populations in clinical methicillin-resistant *Staphylococcus aureus* with MIC ≤ 2 mg/L

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**Objectives:** Although both CLSI and EUCAST define isolates with MIC ≤ 2 mg/L as vancomycin susceptible *Staphylococcus aureus*, treatment failure has been reported even in apparently susceptible strains. This is attributed to sub-populations of vancomycin non-susceptible cells. Early identification of isolates harbouring such sub-populations soon after commencement of vancomycin therapy may benefit patients by allowing for more appropriate therapy and better clinical outcomes. This study aimed to (i) detect isolates likely to rapidly develop non-susceptibility during vancomycin therapy, (ii) determine the length of time for these isolate to progress to homogenous resistance in the presence of clinical concentrations of vancomycin, and (iii) determine their prevalence in clinical isolates in Hong Kong.

**Methods:** Vancomycin MICs were determined by spiral gradient endpoint (SGE) for consecutive methicillin-resistant *Staphylococcus aureus* (MRSA) isolates, 100 each from blood stream (BS) and soft tissue infections (ST). Strains showing endpoints of confluent growth equivalent to MIC ≤ 2 mg/L but exhibiting trailing endpoints (TE) of a resistant sub-population with MIC > 2 mg/L were incubated in brain heart infusion broth with 2 mg/L vancomycin at 37°C for 48 hours and passaged to fresh media every 48 hours for 14 day. MIC was determined weekly. Two strains with MIC < 2 mg/L without TE were included as controls. GRD Etest was performed on days 0 and 14.

**Results:** 17.5% (18% BS; 17% ST) of MRSA were non-susceptible with MIC > 2 mg/L, (MIC50/MIC90 of 2 mg/L/3 mg/L). A further

eight were heterogeneously resistant (2 BS, 6 ST), and following incubation with 2 mg/L vancomycin for 7 day, four reached MIC of 4 mg/L, the remainder 3.2 mg/L and trailing endpoints were no longer observed. By day 14 all TE isolates reached MIC ≥ 4 mg/L. Initial GRD Etest (48 hours) for all strains was 2 mg/L, but resistant sub-populations were observed in only three strains. MICs of controls did not exceed 2 mg/L at 14 day. MICs by GRD for day 14 were similar to those obtained by SGE.

**Conclusion:** Initial rates of vancomycin non-susceptibility were relatively high with a further 4% displaying heterogenous resistance. We have shown that SGE could cost-effectively (< 0.50) detect heterogenous strains within 24 hours and demonstrated that exposure of such strains to clinical concentrations of vancomycin leads to rapid development of homogeneously resistant strains. Ability to discriminate isolates with these subpopulations could have significant implications for patient management.

## Novel methods for bacterial identification and molecular detection of antimicrobial resistance – non-commercial methods

### **P1498** Will the rapid microbiological identification of positive blood cultures by PNA FISH induce the clinician to correct an empiric therapy?

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**Objectives:** Recent studies show that the use of PNA FISH can reduce the turn around time (TAT) of positive blood cultures (BC). There are, however, few European studies that evaluate how the preliminary results of the BC are used for the adjustment of antibiotic therapy. The objective of this study is the evaluation of antibiotic therapy according to PNA FISH results in case of positive blood cultures for gram-negative bacilli, enterococci and *Candida* spp.

**Methods:** Blood cultures were incubated in Bact/ALERT (Biomérieux) and after their positivity a Gram stain was done. According to the presence of gram-negative bacilli, gram positive in chain or yeasts PNA FISH (AdvanDX) was performed. The results were sent by fax to clinicians. The therapy evaluation was applied according to the local epidemiological data (ESBL producers Enterobacteriaceae more than 40%). The information of antibiotic use was collected by phone and/or control of medical records at the time of preliminary results and the next day to evaluate eventual change based on PNA FISH results.

**Results:** From September 2010 to September 2011, PNA FISH was performed in 68 positive blood cultures respectively: 16 (23.5%) *E. coli*, 31 (45.6%) enterococci and 21 (30.9%) *Candida* spp. The antibiotic empiric therapy was correct in 19 (28%) patients, in particular 2 (12.5%) *E. coli*, 8 (25.8%) in enterococci and 9 (42.8%) in *Candida* spp. The antibiotic treatment was not correct after the PNA FISH results in 23 (33.8%) patients, in particular 8 (50%) *E. coli*, 13 (41.9%) enterococci and 2 (9.5%) in *Candida* spp. The antibiotic therapy was changed, because not initially correct, after the communication of PNA FISH result in 26 (38.2%), particularly 6 (37.5%) *E. coli*, 10 (32.2%) enterococci and 10 (47.6%) in *Candida* spp. In two cases the descalation was not correctly applied: *C. albicans* shifted from fluconazole to voriconazole and *E. faecalis* maintained vancomycin.

**Conclusion:** This study shows that rapid microbiological identification of positive blood cultures for *E. coli*, enterococci and *Candida* spp. by PNA FISH is an accurate diagnostic assay and may induce the clinician to promptly correct an empiric therapy. Unfortunately in 23 (33.8%) patients the treatment was not changed and this confirms that in addition to improving TAT, the microbiologist must collaborate with clinicians to define treatment protocols and intervention policies to ensure the correct sepsis therapy.

**P1499** Rapid QuickFISH compared to standard PNA FISH for identification of *S. aureus* and CNS

L. Leerbeck\*, D. Schrøder Hansen (Hillerød, DK)

**Objectives:** Bloodstream infections with Gram positive organisms, most commonly caused by *S. aureus* (SA) in hospitalized patients, are a significant source of morbidity and mortality. Coagulase negative staphylococci (CNS) on the other hand are the most frequent contaminants in blood cultures.

As rapid identification of SA and CNS from positive blood cultures has been shown to improve patient outcomes and reduce hospital costs, high priority has been placed on the development of rapid tests allowing differentiation of staphylococcal species directly from positive blood cultures.

We evaluated the performance of *Staphylococcus* QuickFISH™, a novel fluorescence in situ hybridization (FISH) method using peptide nucleic acid (PNA) probes which stain SA green and CNS red. The method provides rapid identification of SA and CNS in 20–30 minutes.

**Methods:** Blood cultures positive for Gram positive cocci in clusters (GPCC) were tested in parallel with QuickFISH according to the manufacturer's instructions (AdvanDx, Vedbaek, DK) and with the standard PNA FISH method. The QuickFISH method involves a 3 minutes fixation step followed by 15 minutes incubation, both at 55°C; after which the smears are viewed directly on a fluorescent microscope. QuickFISH test results and turnaround time (TAT) were compared with routine clinical identification by standard PNA FISH.

**Results:** In September–November 2011, 40 GPCC blood culture samples were tested. QuickFISH identified 15 SA with 100% sensitivity, 21 CNS with 100% sensitivity, three mixed *S. aureus*/CNS with 100% sensitivity, and one sample with *Micrococcus* species the QuickFISH result were negative with 100% sensitivity. In average the TAT for QuickFISH was 25 minutes compared to 1 hour and 50 minutes from Gram stain to PNA FISH result. The trial will continue and aim is to present the results of 100 GPCC samples at ECCMID.

**Conclusions:** *Staphylococcus* QuickFISH provides accurate identification of *S. aureus* and CNS species directly from positive blood cultures. The test workflow integrates well with standard Gram stain procedure and provides results in 20–30 minutes, enabling the simultaneous reporting of Gram stain and species ID to the treating physician.

**P1500** Experience of a Detroit medical centre as a beta site for evaluation of filmarray blood culture bacterial identification system

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**Objectives:** In bacteremic patients, rapid identification of pathogens from positive blood cultures can lead to targeted antibiotic therapy, improved patient care and reduced length of stay. As a beta site, we evaluated the FilmArray Blood Culture Identification (BCID) System, currently in development. BCID is designed to identify the 25 most common etiologic agents of blood stream infection, including yeast, Gram positive and Gram negative bacteria, and to detect four antibiotic resistance markers (*mecA*, *vanA*, *vanB* and *blaKPC*).

**Methods:** Positive blood culture bottles with Gram positive bacilli were excluded, except for those morphologically consistent with *Bacillus anthracis*. Using the manufacturer's procedure, 250 mL from each positive blood culture bottle was injected into a BCID pouch. The pouch was inserted into the FilmArray instrument and testing was performed per manufacturer's recommendation. Organism identification took ~1 hour. Forty-three positive blood culture bottles were tested in this study and results were compared to phenotypic identification.

**Results:** 1 BCID provided results for 39/43 (91%) of samples in which culture identified an organism; four samples (two *Micrococcus* sp. and

two *Proteus mirabilis*) gave no results as these organisms are not included in BCID system; although the manufacturer intends to include a *Proteus* assay in a future version.

2 Hundred percent agreement (39/39 samples) occurred between phenotypic identification and BCID results for organisms included in the BCID panel.

3 Hundred percent concordance (9/9) was found between the micro-broth dilution MIC method and BCID on detection of antibiotic resistance. The *mecA* gene was correctly detected in five *Staphylococcus aureus* and three coagulase-negative *Staphylococcus*. The *vanA* gene was correctly detected in a vancomycin-resistant *Enterococcus faecium*.

4 In four cases, BCID detected two pathogens while the routine method detected only one. Review of these cases revealed that the first pathogen overgrew the second on the culture plates.

**Conclusion:** The FilmArray BCID system appears promising for the rapid identification of bacteria, yeasts and select antibiotic resistance genes from positive blood cultures as soon as they become positive. Fully automated, it requires only 2 minutes of hands-on time per sample, with no sample preparation. Results are available in ~1 hour. More studies and a larger number of specimens are needed to better determine the performance characteristics of this system.

**P1501** A novel molecular-based diagnostic screening test utilising the NanoCHIP® microarray technology for simultaneous detection of nosocomial infections: MRSA, VRE and KPC

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**Objectives:** Methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin-Resistant Enterococci (VRE) and *Klebsiella pneumoniae* carbapenemase (KPC) colonization in hospital admitted patients is the leading cause for Hospital Acquired Infections (HAI). It is now evident that HAI can be widely prevented through screening of patients before or during hospital admission and proper patient isolation and management. Savyon Diagnostics has recently finalized the development of a novel molecular-based diagnostic screening test for simultaneous detection of MRSA, VRE and KPC directly from a variety of swab sample types. The test utilizes Savyon's proprietary NC400 NanoChip® molecular electronic microarray system. The aim of this work is to demonstrate the utility of the NanoCHIP® technology for screening large number of samples for simultaneous detection of MRSA, VRE and KPC in various swab samples.

**Methods:** DNA was extracted from various types of characterized swab samples. Pathogen and antibiotic resistance specific genes were amplified through multiplex PCR and subjected to the NC400 NanoCHIP® system. The generated amplicons were electronically addressed to discrete loci on the NanoCHIP® cartridge, pre-activated with specific capture oligonucleotides. Detection was achieved through specific fluorescent reporter oligonucleotides. The output analysis of each sample was compared to the characterization of the respective original swab sample, as characterized by real-time PCR in various laboratories.

**Results:** The NanoCHIP® results were in complete accordance with the characterizations of the tested samples in terms of clinical sensitivity and specificity. The NanoCHIP® multiplex analysis provided clear results about the identity of the pathogen and its antibiotic resistance from sample to result within a working day time frame. Additional identifications of related non-pathogenic microorganisms were possible per specific requirements.

**Conclusions:** The NanoCHIP® has proven to be a useful platform for medium-high throughput screening of MRSA, VRE and KPC colonization, offering reliable diagnosis from various types of swab samples. This technology, and in particular its oligonucleotide addressing and multiplexing features, presents significant advantages, mainly in terms of minimal hands-on time, improved laboratory workflow and turn around time, enabling flexibility and saving costs.



**P1502** 16S-23S Intergenic Spacer (ITS) region sequence analysis for *Aerococcus* species identification

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**Objectives:** Bacteria belonging to the *Aerococcus* genus can cause infective endocarditis (IE), hence a rapid identification is essential for improved diagnosis.

Conventional methods are often inadequate to identify these strains to the species level. Today, molecular methods, such as 16S ribosomal RNA (rRNA) gene sequencing, are widely used for species identification. However for some genera 16S provides a weak differentiation between the species.

The purpose of this study was to investigate the possibility of using sequence analysis of the 16S-23S intergenic spacer (ITS) region for species identification of the genus *Aerococcus*.

**Methods:** Real-time polymerase chain reactions (QPCR) with specific primers for conserved regions in 16S and 23S rRNA gene were used to obtain ITS sequence of six type strains of *Aerococcus* species. The ITS sequences were edited, removing the parts belonging to the 16S and 23S gene. The edited ITS sequences were then published in GenBank. ITS sequence analysis and sequence editing were also performed for 35 clinical *Aerococcus* strains (27 *A. urinae*, 3 *A. sanguinicola*, 3 *A. christensenii* and 2 *A. viridans*) that were collected from different Danish hospitals. These ITS sequences were analysed in BLAST for species identification. An evolutionary phylogenetic tree was constructed with MEGA5. Pairwise comparison was performed to calculate the interspecies and intraspecies divergence (MEGA5, Jukes-Cantor model).

**Results:** QPCR generated one major ITS product from the six type strains and 35 clinical *Aerococcus* strains. ITS amplicon size ranged from 204 to 232 bp. By comparison of ITS sequences from the 35 clinical strains with the *Aerococcus* type strain ITS sequences submitted to GenBank, convincing identifications were obtained and identifications were in accordance with previous comprehensive phenotypic and molecular characterizations. The phylogenetic analysis of the 35 clinical *Aerococcus* strains revealed six distinct branches each containing the corresponding type strain. Pairwise comparison of the ITS sequences for the six type strains revealed high level of interspecies divergence  $d \geq 0.196$  and low level of intraspecies divergence  $d \leq 0.031$  for the 35 clinical strains.

**Conclusion:** ITS gene sequence analysis is a useful method in species identification of the genus *Aerococcus* due to exact genus identification with BLAST examination, and due to low level of intraspecies divergence ( $d \leq 0.031$ ) and high level of interspecies divergence ( $d \geq 0.196$ ).

**P1503** Microarray-based detection of intracellular pathogens including automated result evaluation which enables parallel species identification of multiple infections

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**Objectives:** We present a rapid method for identification and characterization of fastidious and difficult to cultivate intracellular pathogens from different infectious diseases, by combining PCR amplification with microarray detection.

**Methods:** For identification microbial DNA amplification and labelling is carried out using universal primers targeting a specific region of the 16S rRNA gene. The microbe panel (comprising all HACEK-group members, and among others different species of the genera *Mycoplasma*, *Chlamydia*, *Rickettsia*, *Brucella* and *Kingella*) is identified with an oligonucleotide microarray, which contains 20- to 30mer probes specific for 70 pathogens occurring in blood. The new pathogen panel was integrated into an existing microarray-based pathogen detection tool for sepsis diagnostics.

**Results:** As expected from sequence analysis of the 16S target genes, some members of the genera mycoplasma are too closely related for accurate species identification based on single species specific DNA probes. The calculation of a phylogenetic tree including all species represented on the microarray further revealed a low relatedness to the other intracellular pathogens. Thus the realisation of multiplex PCR was necessary to cover all targeted species. The automated analyses of microarray results applying statistical algorithms based on rank normalisation and nearest-centroid method allowed the analysis of typical hybridisation patterns for each species and thereby an accurate discrimination. Using this approach a correct determination of 100% of the bacteria at genus level and 98% at species level was possible. Due to centroid calculations the parallel detection of several pathogens in one sample was possible too. In case of multiple infections 82% of both genera and 79% of both species could be correctly identified. In 90% of all cases at least one species was identified correctly.

**Conclusion:** Our results show that molecular methods are highly suitable for the fast detection of pathogens which might not be detected using the currently applied methods in clinical routine. The presented approach is even suited for the parallel detection and identification of several pathogens in one sample within one reaction. Relying on our promising results we currently develop a Lab-on-a-Chip (LOC) device which also includes a software for the above mentioned automated pathogen classification.

**P1504** Development of a multiplex PCR for identification of cross-reacting *Escherichia coli* O157, *Yersinia enterocolitica* O:9 and *Brucella* spp.

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Brucellae are intracellular bacteria pathogenic for humans and animals. The laboratory diagnosis of brucellosis is generally based on serological tests. The main methods employed for years for diagnosing brucellosis are: the rose bengal test (RBT), serum agglutination test (SAT), complement fixation test (CFT) and ELISA. However, the similarity of the O-antigenic side chain of *Brucella* LPS with other microbes, has restricted the specificity of serological diagnosis resulting in false positive serological results (FPSR). Most commonly FPSR are caused by infections with *Yersinia enterocolitica* O:9, as the bacterium possesses identical O-antigen lipopolysaccharide chain (LPS) to that present in *Brucella* spp., and *Escherichia coli* O157 which was shown to cross react with *Brucella* spp. due to the presence of N-acetylated-D-perosamine.

The aim of the study was to develop a multiplex PCR which allows an identification of the universal 16S rRNA *Brucella* spp. marker and amplification of the perosamine synthetase (*per*) gene, specific for cross-reacting *Yersinia enterocolitica* O:9 and *rfbO157* gene for *E. coli* O157.

The lymph tissue samples (1 g) were artificially inoculated with various decimal dilutions of reference strains. Before inoculation the samples were tested with PCR specific for *Brucella* spp., *Y. enterocolitica* O:9 and *E. coli* O157 genes. In parallel to the PCR assays, bacteriological examination of the samples was performed according to official protocols. Moreover n = 340 rectal swabs samples were tested to validate the protocol developed.

Multiplex PCR was run in a thermocycler (T3, Biometra) under the following conditions: initial DNA denaturation at 94°C for 5 minutes, followed by 25 cycles of 94°C for 1 minutes, 56°C for 1 minute and 70°C for 1 minute. The final extension step was done at 70°C for 5 minutes.

All the strains tested generated predicted amplicon and no false positive or false negative results were observed. Moreover, the mPCR developed with direct extraction of DNA from lymph tissue or rectal swabs, allowed the identification of a very small amount of bacteria inoculated ( $10^2$  CFU/g) and avoiding a time- and labour-consuming enrichment steps. Having regard these advantages the mPCR can be a useful tool for the differentiation of infections caused by *Brucella* and cross-reacting *Y. enterocolitica* O:9 and *E. coli* O157 infections.

**P1505** An evaluation of the Luminex xTAG® gastrointestinal pathogen panel at a London teaching hospital 2011: The comparative performance of a rapid molecular multiplex assay and current standard laboratory investigations of infectious gastroenteritis

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The Luminex xTAG® Gastrointestinal Pathogen Panel (GPP) simultaneously detects 15 common causes of infectious diarrhoea in 1 day. We evaluated the performance of the GPP in comparison to our current 3 day selective gastroenteritis testing methodology involving culture, microscopy, molecular and/or immunoassay. We conducted a pilot study on 450 anonymised stools samples in February 2011. Eighty-nine percent were cultured for Salmonella, Shigella, Campylobacter, *E. coli*, 34% tested for *Cryptosporidium*, *Giardia*, *Entamoeba histolytica*, 37% tested for *Clostridium difficile* by EIA and PCR, 39% of were tested for norovirus by EIA and 2% selected for rota/adenovirus EIA. The overall detection rate of pathogens in these 450 samples increased from 8.3% with conventional selective testing to 26.2% for GPP. Detection of all pathogens increased by multiples of two to fourfold, notable was a fourfold increase in detection of norovirus by GPP. We detected two or more pathogens in 10% of positive samples. Positive GPP results for the enteric viral pathogens and *C. difficile* were evaluated using third party molecular assays. All molecular methods agreed for rotavirus and adenovirus, all non-selectively screened rotavirus positives were in children <5 years old. The 3rd party molecular test and Norovirus GPP results were in agreement and where discrepant between EIA and GPP existed confirmed in GPP's favour. GPP was compared with the GeneXpert *C. difficile* assay on an EQA proficiency panel and matched performance on all but the most dilute EQA sample (4.6E3 CFU/mL), detected at cycle threshold 38 (Ct38) showing a slight sensitivity advantage of the single target GeneXpert over the multi-target GPP. GeneXpert *C. difficile* and GPP have shown equivalent performance when compared on recent cohorts of prospective clinical positive samples. Gastroenteritis outbreaks are estimated to cost 1% of the total inpatient services NHS budget or £635 000 per 1000 beds, principally from cost of bed-days lost (Lopman et al. 2004). GPP is likely to be of benefit in using isolation beds more efficiently and aiding rapid containment of outbreaks by being a fast, sensitive comprehensive molecular diagnostic test. GSTS Infection Sciences is now working in partnership with the Directorate of Infection, Prevention and Control at St. Thomas' and Luminex to conduct a major health economics study to access that potential impact of GPP over the 2011–2012 winter season on 5000 in-patients with diarrhoeal illness.

**P1506** Evaluation of the FTD bacterial meningitis kit in comparison to in-house assays for the direct detection of *N. meningitidis*, *S. pneumoniae* and *H. influenzae* in clinical specimens

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Although relatively rare, bacterial meningitis remains a public health and health care priority. Most cases of bacterial meningitis are caused by *Neisseria meningitidis*, *Streptococcus pneumoniae* and *Haemophilus influenzae*. Classically, laboratory confirmation is achieved by microscopy (Gram stain) of CSF and blood and/or culture of the aetiological agent from these specimens. Changing clinical practice (reluctance to carry out lumbar puncture and administration of antibiotics prior to hospital admission) has led to a decline in cases ascertained by these methods. Molecular methods (PCR) for diagnosis alleviate the need for viable organisms, have increased sensitivity and allow specimens to be screened for multiple pathogens simultaneously. As disease progression can be rapid turnaround time from specimen processing to diagnosis is paramount.

Figures from England and Wales indicate that over 50% of cases of *N. meningitidis* disease are now confirmed by PCR only. At the Scottish *Haemophilus*, *Legionella*, *Pneumococcus* and *Meningococcus* Reference Laboratory (SHLMPRL) we receive over 1000 requests annually for non-culture diagnosis of bacterial meningitis.

Routinely, we have used in-house PCR assays (gel-based and more recently real-time) targeting genes specific for *N. meningitidis* (*ctrA*), *S. pneumoniae* (*lytA*) and *H. influenzae* (*bexA/hel*) for direct detection of bacterial meningitis in CSF and blood. In this study we compare our in-house assays to a commercially available one-tube real-time multiplex PCR (Fast Track diagnostics, FTD).

*S. pneumoniae* (21), *N. meningitidis* (21), *H. influenzae* (1) and negative (28) specimens were re-tested with the FTD Bacterial Meningitis kit on the Roche LC480 real-time platform. Results in both assays were comparable although Cp values would suggest that there was increased sensitivity using the FTD kit. Both real-time assays picked up an additional *H. influenzae* positive sample compared to gel-based PCR. This may be due to either increased sensitivity of the assays or specificity of the targets used (original gel-based in-house assay only detected *H. influenzae* type b).

The FTD Bacterial Kit performed well. Inclusion of positive control material and internal controls (to assess quality of the DNA extracted from clinical material) allows for easy introduction into the laboratory's repertoire of tests, both for reference facilities and the increasing number of diagnostic laboratories with molecular diagnostic capabilities.

**P1507** Analysis of *Campylobacter concisus* strains using the MALDI Biotyper

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**Objectives:** Epidemiological studies have shown that *Campylobacter concisus* can be recovered from a substantial number of human gastroenteritis cases. In this study we evaluated Matrix-Assisted Laser-Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS) for a rapid identification of *C. concisus* and strain differentiation inside the species.

**Methods:** Fourteen *C. concisus* strains, including ATCC 33237, were tested. Seven faecal and three oral strains were from diarrheic patients, and three oral strains were from healthy individuals. All strains were isolated with use of the filter method and final identification was based on a species specific real-time PCR based on the chaperonin-60 gene as well as the 23S rRNA gene. The *C. concisus* isolates were analysed using the MALDI Biotyper and the ClinProTools 2.2 software. A dendrogram of the MALDI-TOF profiles was constructed, statistical analyses of the data were performed and visual spectra comparison was made.

**Results:** The 14 strains showed a very high diversity with no real significant grouping. However, two groups could be slightly separated by principle component analysis, although inside these groups variability was also very high. The extreme variability of strain profiles was also confirmed by correlation analysis. The faecal strains showed large diversity and no real grouping among the oral isolates were found either. The three coupled sets of oral and faecal strains were also clearly different strains. Detailed visual spectra analysis revealed a unique profile for each strain. Nevertheless, in MALDI Biotyper pattern matching all strains showed medium to high log (scores) against each other and the strains grouped together in dendrogram calculation, indicating there is sufficient conserved peak information in the spectra for species identification.

**Conclusion:** The *C. concisus* strains showed a remarkable diversity based upon their mass spectra when compared against each other by visual analysis. This diversity was also confirmed by statistical analyses. The diversity of *C. concisus* seems enormous and can be compared to the closely related *Helicobacter pylori*. But in contrast to *H. pylori*, the mass spectrum of *C. concisus* contains many conserved peaks. This enables identification of the species with only a few

database entries which cover the spectral diversity. On the other hand, the very high intra-species diversity might facilitate epidemiological studies and strain tracking of *C. concisus*.

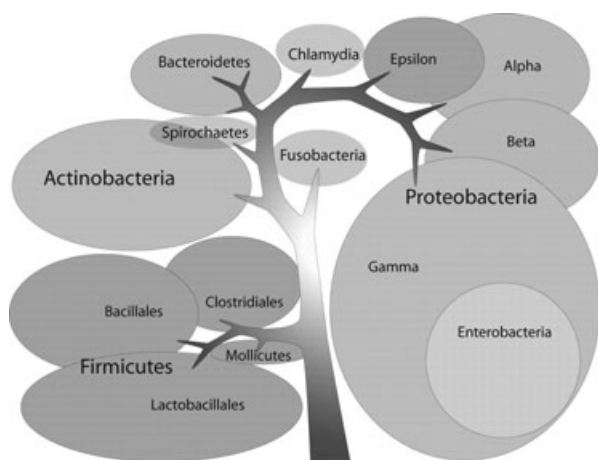
**P1508 Broad spectrum detection and identification of diverse low-titre bacteria and *Candida* in complex matrices using PCR/ESI-MS**

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**Objectives:** A sensitive assay was developed on the Ibis Biosciences PLEX-ID platform to detect diverse pathogenic bacteria and *Candida* through PCR amplification of broadly conserved genomic fragments. The assay was designed to identify 600+ clinically relevant species through mass spectrometric analysis of unfragmented amplicons and comparison of resulting mass signatures to known signatures in a reference database. In the work described here, the theoretical capabilities of this assay were tested using phenotypically characterized and culture-quantified bacterial isolates.

**Methods:** The PLEX-ID system, including automated sample lysis, DNA extraction, PCR, PCR clean-up, electrospray-ionization mass spectrometry, signal deconvolution and matching, was challenged with low titer spiked samples containing 70 diverse bacteria and *Candida* species spanning the taxonomic breadth of coverage of the assay (see Table 1). Samples were prepared in EDTA whole blood from asymptomatic donors to simulate sterile-site infection samples with high levels of background nonbacterial nucleic acids in a complex physiological matrix. Further capability of the assay for detection of both culturable and unculturable organisms was demonstrated using unquantified nucleic acid extracts from over 300 other bacterial species spiked in extracted nucleic acid derived from blood.

**Results:** The assay detected and identified all of the tested quantified bacteria (84% at the species level and the rest at the group or genus level) at low titers (1–500 CFU/mL) in whole blood. Extended breadth of coverage testing using unquantified nucleic acid spikes demonstrated high rates of correct detection and identification across all targeted bacterial groups.



**Conclusion:** The PLEX-ID broad bacterial assay is capable of detecting and identifying diverse bacteria and *Candida* occurring at low titers in otherwise sterile natural matrices such as blood using conserved-site PCR and electrospray ionization mass spectrometry to generate species-specific signatures. The limit of detection of the assay varies from 1 to 500 CFU/mL for species capable of being culture-quantified. The assay is also capable of detecting unculturable organisms, and thus could be a valuable tool in the microbiology laboratory for broad-spectrum detection of bacteria and *Candida*.

**P1509 Species identification of *Staphylococcus intermedius* group staphylococci by multiplex PCR and *gap* gene amplification and sequencing compared to MALDI-TOF**

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**Objectives:** Recently it has been demonstrated that isolates phenotypically identified as *Staphylococcus intermedius* consist of three distinct species, including *S. intermedius*, *S. pseudintermedius*, *S. delphini*, which together represent the *S. intermedius* group (SIG). It was discovered that *S. pseudintermedius*, not *S. intermedius*, is a common canine pyoderma pathogen and transmission of microorganism between infected or colonized dogs and cats and healthy people does occur. First cases of infection in human were communicated indicating that investigation of staphylococci species of animal origin may also have medical application. In routine diagnostic bacteriology *S. aureus* has long been differentiated from other species by means of a single test, most often coagulase or clumping-factor production. The biochemical properties of *S. pseudintermedius* are similar to those of *S. aureus* which readily leads to misidentification of species by commercial identification systems.

**Methods:** One hundred and five staphylococcal strains were recovered from infected dogs, among them from dogs with pyoderma (28), otitis (26), wounds (6), eyes (5), urine (2), pyometra (5) and from healthy dogs from gums and throat (33) in Belgorod region of Russia and in Germany in 2005–2010. Antimicrobial susceptibility was determined by standard methods. Species identification was carried out by routine tests, Staphy test 24 (Lachema), amplification and sequencing of *gap* gene, multiplex PCR by protocol, as described by Sasaki T. et al. (2010) and by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Reference strains *S. intermedius* DSM20373T and *S. pseudintermedius* LMG22219 were used in this study.

**Results:** 87.3; 85.5% and 88.5% tested isolates were coagulase-, mannitol-, and lecithinase positive accordingly. All isolates were identified as *S. intermedius* by Staphy test 24. Partial sequencing of *gap* gene allows differentiated all tested isolates. The most isolates were identified as by *S. pseudintermedius* (93.4%), 4 (3.8%) as by *S. schleiferyi*, 2 (1.9%), isolates as by *S. intermedius*, 1 (0.9%) as by *S. aureus*. M-PCR for amplification a portion of the *nuc* gene locus correctly identified 98, 2% isolates. Maldi-tof-MS identified correctly 68.5% isolates, 31.5% isolates were not identified.

**Conclusion:** *S. pseudintermedius* can be easily misidentified as *S. aureus* or *S. intermedius*. M-PCR for amplification a portion of the *nuc* gene locus is reliable test for identification SIG species.

**P1510 Molecular diagnosis and characterisation of bovine mastitis by PCR/ESI-MS**

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**Objectives:** Bovine mastitis (BMA), an inflammatory and infectious disorder of the udder tissue in cows, often occurs in response to bacterial invasion. Annually, the economic impact of BMA is significant; in some cases nearly ~10% of total milk production is unusable. The diagnosis of BMA is based on the appearance of abnormally appearing milk and elevated somatic cell counts (SCC). Subclinical infections are, however, more problematic since the milk may appear normal. The objectives of this study are to assess whether PCR/ESI-MS can improve the identification of the pathogens causing BMA compared to milk cultures. Our hypothesis is that earlier and more precise bacterial detection can be used to identify risk factors for BMA and aid in the development of appropriate screening and control programs.

**Methods:** Thirty-one samples of milk from cows with different mastitis symptoms and from healthy cows were prospectively collected in an aseptic fashion and frozen at  $-2^{\circ}\text{C}$ . A detailed microbiological screening of the milk was performed using standard

laboratory culture methods for analyzing bacteria, yeasts, molds and prototheca. DNA from these samples were analyzed using the PLEX-ID BAC Assay targeting most pathogenic bacteria, antibiotic resistance markers (*mecA*, *vanA*, *vanB*, *blaKpc*), and *Candida* spp.

**Results:** PCR/ESI-MS analysis using the PLEX-ID detected several bacteria and yeast from milk samples. Most samples had more than one bacteria identified. Overall, there was a 74% agreement between the two methods. Compared to culture, PCR/ESI-MS accurately detected *Streptococcus uberis* (6 vs. 3), *Staphylococcus* spp. (5 vs. 5), enterococci (4 vs. 4), *Arcanobacterium* spp. (2 vs. 2) and *Mycoplasma* spp. (2 vs. 2). *Janthinobacterium lividum*, an organism known to possess THIN-B, a B3 metallo beta-lactamase, was also detected by PCR/ESI-MS. The *mecA* gene was detected in three samples.

**Conclusions:** The major detection in each sample was consistent with standard milk culture results; although the PCR/ESI-MS found molecular signatures that suggested additional pathogens and genes were present. PCR/ESI-MS may be a robust method used to screen for pathogens in the etiology of BMA. The data suggest that pathobiology of BMA maybe even more heterogeneous than previously suspected. The broad range of bacterial DNA signatures detected by PCR/ESI-MS also suggest that milk may also be a reservoir of genetic elements that are important in establishing the microbial flora of an individual.

**P1511 Analytical and clinical validation of the 16S gene and antimicrobial resistance genes by real-time PCR in blood cultures of patients undergoing haematopoietic stem cells transplant**

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Bloodstream infections should be diagnosed and treated quickly, especially in immune compromised patients. Molecular diagnoses are time-saving and could provide an early appropriate antibiotic therapy.

**Objective:** The aim of this study was to perform analytical and clinical validation of 16S bacterial gene for bacteria detection and antimicrobial resistance genes from bottles of automated blood cultures and direct from collected blood samples of patients undergoing hematopoietic stem cells transplant.

**Methods:** Two hundred and eleven blood samples, 160 from Bactec system bottles and 51 from collected blood of 45 patients submitted to hematopoietic stem cells transplant were analyzed. The validation was performed based on CLSI documents EP-17, EP-12 and GP-10. The analysis of specificity was done by ROC curve and the sensitivity by the limit of detection (LoD) definition. The 16S rDNA gene detection with Gram-specific probes was standardized by Taqman multiplex real-time. The detection of the resistance genes *blaSHV*, *blaTEM*, *blaCTX-M*, *blaIMP*, *blaSPM*, *blaVIM*, *blaKPC*, *vanA*, *vanB* and *mecA* was done by the SYBR Green real-time technique.

**Results:** The assays were able to detect the target genes to a corresponding dilution of 15–1.5 CFU. The concordance between Gram stain of the blood culture and PCR with Gram-specific probes was 51.5%. Oxacilin susceptibility and detection of *mecA* gene was 76.93% concordant. For the ESbetaL positives isolates and *ESbetaL* gene tested the concordance was 66.6%. Vancomycin resistance and detection of *vanA* gene was 100% concordant. Of the two isolates resistant to carbapenems none were positive for carbapenemase and metallo-beta-lactamase genes.

**Conclusion:** This study suggests that multiplex PCR for detection of Gram-positive/Gram-negative bacteria and antimicrobial resistance genes could be useful for rapid diagnosis of bloodstream infection in this patient setting.

**P1512 Rapid molecular detection of ESBL gene variants with a novel ligation-dependent real-time PCR**

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**Objectives:** Extended spectrum beta-lactamases (ESBLs) are emerging worldwide. Rapid and adequate ESBL detection is desired for both infection control measures as well as for the choice of correct antimicrobial therapy. In this study we compared a new rapid ligation-dependent Real Time PCR to detect the most prevalent ESBLs TEM, SHV and CTX-M to a phenotypic confirmatory method. Moreover, we evaluated the turn-around-time (TAT) of both methods.

**Methods:** From June to October 2011 all Gram-negative ESBL positive strains from clinical specimens based on VITEK2 results were collected and tested using the phenotypic combination disk test (CDT) and ligation-dependent Real Time PCR (Checkpoints, Wageningen, The Netherlands). Per patient only the first of each putative ESBL-positive species was included. Discrepant results were analyzed using the Check-MDR CT103 assay (Checkpoints) which is able to detect most prevalent ESBLs, pAmpCs and carbapenemases.

All isolates, in the period August–October, which were selected for the Real Time PCR and tested in de CDT on the same day (Monday–Thursday starting 11:00 hour) were used to compare the TAT.

**Results:** Of the 197 putative ESBL-producing isolates 106 (53.8%) and 93 (47.2%) were positive using the CDT and Real Time PCR, respectively. Fifteen discrepancies were found of which 14 were phenotypical ESBL positive and genotypical ESBL negative.

In 12 discrepancies, the Real Time PCR results were confirmed with the CT103. In one strain the CT103 could not be interpreted because of lack of control spots. The remaining two discrepancies were repeated in the Real Time PCR and detected ESBL positive, comparable to the CDT.

The mean TAT of the CDT and Real Time PCR was comparable (24 hours 14 minutes and 25 hours 11 minutes respectively).

Of all 62 isolates of which the TAT was evaluated, 23.4% could gain results within the same day using Real Time PCR. In contrast, CDT results only were available the next day because of the overnight incubation step.

**Conclusion:** The ligation-dependent Real Time PCR can easily be executed on present instruments and reduces the TAT and contamination compared to micro-array analysis or PCR followed by electrophoresis. This assay appeared to be an extremely accurate method to detect TEM, SHV and CTX-M ESBLs rapidly. In fact, with some minor adjustments in the laboratory workflow this system will provide ESBL results within the same day, making this assay a good solution to the desire for a rapid and accurate ESBL detection method.

**P1513 Analytical and clinical validation of real-time PCR for rapid detection of KPC carbapenemase from rectal Eswab®**

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Rapid detection of KPC-producing enterobacteriaceae is of great importance in infection control and in controlling the spread of these microorganisms. The application of molecular methods in clinical samples requires analytical and clinical validation. The aim of this study was to perform the analytical and clinical validation of real time PCR for rapid detection of gene *blaKPC* from direct rectal sample collected in liquid ESwab (Copan, USA).

**Methods:** The limit of detection (LoD) and cutoff were evaluated using positive and negative control sample according to CLSI documents EP-17, EP-12. The clinical sensitivity and specificity were calculated in a ROC curve using rectal swabs samples from 156 patients hospitalized with suspected colonization by enterobacteria producing carbapenemase KPC and 30 negative control patients during a clinical outbreak in 2010 at the São Paulo University Hospital. The 16S rDNA gene was used as internal control. Bacterial DNA was extracted using 200 µL of liquid ESwab® using the QIAamp DNA Mini Kit (Qiagen, Germany) and amplification of gene *blaKPC* was analyzed by the real time PCR using the Platinum SYBR Green qPCR Kit Super Mix

(Invitrogen, CA, USA) and 7500 Real Time PCR System equipment (Applied Biosystems, CA, USA).

**Results:** The Cycle Threshold ( $C_T$ ) delimited to LoD and cutoff of molecular assay were 36.67 and 37.98 respectively. The clinical sensitivity and specificity were 100% and 87.5% respectively. A total of 156 samples analyzed 17.30% (27/156) were positive for blaKPC and one was negative for 16S rDNA PCR.

**Conclusion:** These results suggest that real time PCR for ESwab direct detection from blaKPC gene in Enterobacteria can be useful in identifying patients colonized with bacteria producing carbapenemase KPC specially for control of nosocomial outbreaks.

**PI514 Development of multiplex real-time PCR assay for detection of carbapenemase genes of VIM, IMP, NDM, KPC and OXA-48 types**

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**Objectives:** Carbapenem resistance caused by acquired carbapenemases is a growing public health concern and represents a serious problem for the treatment of related infection. Real-time PCR can be used as a rapid and effective technique for detection of acquired carbapenemases. The aim of this study was to develop a multiplex real-time PCR assay for detection of genes encoding the most common and emerging carbapenemases of VIM, IMP, NDM, KPC and OXA-48-like types.

**Methods:** The fluorogenic-probe based 5'-exonuclease technique was used to develop a new multiplex real-time PCR assay. The assay was validated using a panel of strains carrying the genes of the known carbapenemases (VIM-1, -2, -4, -10, IMP-1, -2, -12, -13, NDM-1, -2, KPC-3 and OXA-48) on natural plasmids or recombinant vectors. The ability of the assay to detect the above genes directly in blood culture was assessed using simulated spiked samples. Eighteen carbapenem non-susceptible clinical isolates that reveal positive results in the modified Hodge test (MHT) and 50 carbapenem-susceptible isolates of various Enterobacteriaceae species were also tested for primary evaluation of the assay. The presence of detected carbapenemase genes in clinical isolates was confirmed by sequencing.

**Results:** The assay composed of two multiplex real-time PCR tests, was developed for detection of genes encoding five groups of carbapenemases. The first PCR test allows the detection and discrimination of VIM, IMP and NDM metallo-beta-lactamase (MBL) genes and includes exogenous internal control (IC). The second PCR test allows differential detection of KPC and OXA-48-like serine carbapenemase genes, and an IC DNA. Both PCR-tests produced correct results for strains carrying the known carbapenemase genes. When performed on DNA extracts from spiked blood cultures the assay was able to detect all positive samples in <2 hours. In accordance with results of MHT, the assay detected blaOXA-48-like genes in 17, blaVIM in 1 carbapenem non-susceptible isolates, and no carbapenemase genes in carbapenem-susceptible isolates.

**Conclusions:** The developed real-time PCR assay allows the rapid and effective detection of VIM, IMP, NDM, KPC and OXA-48-like carbapenemase genes both in clinical isolates and in blood cultures. It therefore has a potential to improve infection control and antibiotic use practices.

**PI515 Rapid and sensitive detection of carbapenem resistance directly from urine samples using DNA microarrays**

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**Objectives:** The *Klebsiella pneumoniae* carbapenemase (KPC) is considered as a serious threat to antibiotic therapy as it belongs to a class of enzymes which confer resistance to carbapenems, a beta-lactam antibiotic group considered last resort for the treatment of infections from Extended-spectrum Beta-Lactamase (ESBL) producing bacteria.

Here we describe the development and testing of a DNA microarray for the identification and genotyping of KPC genes (blaKPC), which can identify and distinguish between all currently known KPC variants.

**Methods:** The DNA Microarray consists of specific sets of oligonucleotide probes, which are capable of detecting single point mutations (SNPs) within the blaKPC gene. The DNA extractions were carried out using kits from Qiagen (QIAprep Spin Miniprep) and Norgen (Urine Bacterial DNA Isolation) followed by fluorescent labelling of the target DNA and a subsequent hybridization to the DNA Microarray.

**Results:** The array has the potential to shorten the time of resistance testing from 24 hours of classic cultivation based minimum inhibitory concentration (MIC) measurement methods to 6 hours from sample collection. We specifically tested the whole assay procedure including preanalytical steps using several KPC reference [1] strains and spiked dilution series into human urine. We further tested and compared different commercial kits for the direct DNA extraction from urine samples. A reliable SNP typing from  $<1 \times 10^5$  CFU/mL urine was demonstrated in all cases. A detection limit of 80 CFU/mL urine from *K. pneumoniae* was possible.

**Conclusion:** The study presents interesting data on the combination of the newly developed DNA microarray and two dedicated commercial sample preparations kits directly applied on pooled urine samples. These findings demonstrate that the KPC microarray could be a useful tool for elucidating the epidemiology or clinical diagnostics of KPC in a rapid and cost-effective way directly from clinical specimen such as urine. The great multiplexing potential of microarrays makes them a suitable tool in providing detailed information quickly and reliably and for wider resistance gene detection [2].

**References:** 1. Pfeifer Y. *J Lab Med* 2010; 34(4): 205–215.

2. Leinberger DM, Grimm V, Rubtsova M, Weile J, Schröppel K, Wichelhaus TA, Knabbe C, Schmid RD, Bachmann TT. *J Clin Microbiol* 2010; 48(2): 460–471.

**PI516 Performance of the Nanosphere's Verigene BC-GP test for rapid detection of Gram-positive bacteria and resistance determinants directly from positive blood cultures**

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**Objective:** To compare the preliminary results obtained with the microarray-based Nanosphere's Verigene Blood Culture – Gram Positive (BC-GP) test, a new tool for rapid detection of gram-positive pathogens in blood stream infections (BSI) in the setting of a European evaluation study of both the assay and the Verigene® System equipment, with the conventional method performed currently in our laboratory.

**Methods:** We tested 65 positive blood cultures (BD BACTECTM). After Gram staining, we immediately performed the Verigene BC-GP assay with 350 µL of blood from the BC vial. Results were obtained in 2.5 hours. We proceeded simultaneously to quantitative subculture in common agar plates and direct disk-diffusion susceptibility testing. After overnight incubation, we got preliminary results on susceptibility and presumptive identification of bacteria, and performed automated biochemical identification and microdilution antibiotic testing with MicroScan® Gram positive Combo 32 panels (Siemens), that were again incubated overnight. Definitive results with conventional culture method took 48 hours in being released.

**Results:** Sixty-four (97%) up to 66 (one blood culture had two different isolates) initial identification Verigene results were concordant with biochemical testing. One initial negative Verigene result was retested and then coincided with the conventional method, so in 98.5% of cases results were consistent between both methods. The other initial negative Verigene result was not retested and a CoNS (*S. hominis*) was recovered in plate culture, but it had the lowest count in CFU/mL of the series, i.e.  $7.10E5$  CFU/mL vs.  $\geq 1.10E6$  CFU/mL. Two mixed infections were tested and Verigene results were concordant with

culture: one consisted of *Acinetobacter baumannii* and *S. haemolyticus*, and Verigene detected “*Staphylococcus* sp.” with no other signal; the other was composed of *S. epidermidis* and *S. mitis* and both were correctly identified by Verigene. In terms of Verigene resistance determinants’ detection, there was no failure compared to culture.

**Conclusion:** Preliminary results with the new Verigene BC-GP test are good, with 100% specificity and 98.5% sensitivity in bacterial identification and 100% specificity and sensitivity in important resistance determinants’ detection. It’s a new tool for diagnosis of gram positive’s BSI to keep under consideration because significant results can be discriminated and reported at least 24 hours before current conventional culture method.

**P1517 Phenotypic and genotypic identification of ampicillin-resistant *Haemophilus influenzae* strains and determination of their molecular mechanisms of resistance to beta-lactams**

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**Objectives:** The aim of this study was to determine the bacteriological characteristics of Ampicillin-resistant *H. influenzae* (H.i) strains isolated from children namely serotyping, biotyping and their antibiotic susceptibility specifying the mechanisms and the beta-lactams resistance genes.

**Methods:** This study concerned 62 ampicillin-resistant H.i strains selected from a collection of 138 strains isolated from January 2009 to August 2010 at the microbiological laboratory of the children’s hospital of Tunis. The bacteriological identification was made according to the conventional methods. The beta-lactamase production was performed for each strains using chromogenic cephalosporin test. Antimicrobial susceptibility for all H.i isolates was determined as recommended by the CA-SFM. We determined the beta-lactams resistance genes (*bla*TEM, *bla*ROB and *ftsI*) by PCR as well as their capsular genes (*bexA*).

**Results:** The majority of the strains were non invasive (N = 61). The biotype III was the most frequent (51.61%) followed by the biotype II (16.12%). PCR amplification of the *bexA* gene and the type b gene showed that all of the strains were identified as non-typeable. The 62 strains isolated were ampicillin-resistant, which correspond to a percentage of 44.92%. These isolates were subdivided into three groups according to the ampicillin resistance’s molecular mechanisms : The group of the type TEM-1 beta-lactamase positive ampicillin resistant isolates (BLPAR) where 31 strains (50%) were beta-lactamase positive with the presence of the *bla*TEM-1 gene and the normal *ftsI* gene. The second group is the beta-lactamase negative ampicillin resistant (BLNAR) strains where 25 isolates (40.32%) were beta-lactamase negative with neither *bla*TEM-1 gene nor the normal *ftsI* gene, finally the group of the beta-lactamase positive amoxicillin-clavulanate resistant strains (BLPACR) where six isolates (9.68%) were beta-lactamase positive and had both *bla*TEM-1 gene and a mutated *ftsI* gene.

**Conclusion:** In our country, ampicillin resistance is increasing continually mainly for the BLNAR strains which were rare in Tunisia.

**P1518 Investigation of various virulence factors among the hospital-acquired and community-acquired *Staphylococcus aureus* isolates by real-time-PCR method**

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**Objective:** *Staphylococcus aureus* is the most common cause of skin and soft tissue infection in the community and the most important cause of nosocomial infection. In this research, it was aimed to explore the presence of staphylococcal enterotoxin A (SEA), staphylococcal enterotoxin B (SEB), staphylococcal enterotoxin C (SEC), staphylococcal enterotoxin D (SED), toxic shock syndrome toxin-1 (TSST-1), Panton Valentine leukocidin (PVL) and SCCmec phenotype

in *S. aureus* strains isolated from various clinical samples from Gulhane Military Medical Academy Hospital (GMMAH) between 2007 and 2010. In addition, Multiple Locus Variable number of tandem repeat Analysis (MLVA) was used to demonstrate the genotypic association between hospital-acquired and community-acquired isolates.

**Methods:** Real-time PCR was used to determine the occurrence of genes that coding SEA, SEB, SEC, SED, PVL, TSST-1 and SCCmec types in 147 isolates of *S. aureus* (75 hospital-acquired and 72 community-acquired). Five variable numbers of tandem repeat (VNTR) loci *clfA*, *clfB*, *spa*, *ssp* and *siu-21* were analyzed for MLVA.

**Results:** Among 147 isolates, 93 (63.2%) possessed at least one toxin (77 strains carried one, and 16 strains carried more than one). The 55 (59.1%) of these 93 isolates were hospital-acquired and 38 (40.9%) were community-acquired. The SEA toxin was found in 59 isolates (40.1%), SEB in eight isolates (5.4%), SEC in 12 isolates (8.1%), SED in eight isolates (5.4%), TSST-1 in 17 isolates (11.5%), PVL toxin in six isolates (4.0%). In our study, SCCmec type III was detected in 90.9%, SCCmec type IV in 2.2% among hospital-acquired MRSA; on the other hand, SCCmec type IV was detected in 40.0% among community-acquired MRSA. The rest of the strains could not typed. Most of the strains (40/47; 85.1%) carrying SEA were hospital-acquired, and they were found as methicilline resistant. According to MLVA, hospital and community-acquired groups’ clustering rates, number of clones, number of unique profile were found as; 73.6% and 57.3%; 34 and 47; 19 and 32, respectively.

**Conclusion:** The high prevalence of SEA toxin in hospital-acquired MRSA isolates indicates that there would be an association between the presence of toxin and antimicrobial resistance.

## Resistance surveillance in defined clinical situations

**P1519 The rise of ESBL-producing *Escherichia coli* bacteraemias in Finland**

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**Objectives:** *Escherichia coli* and *Staphylococcus aureus* are the two most important bacteria causing bloodstream infections. Infections caused by ESBL-producing *E. coli* (ESBLEC) are rising in Europe; MRSA which used to be the most feared pathogen is loosing ground. In Finland, ESBLEC and MRSA prevalence has been extensively documented on a national level. Here we describe the changes seen in ESBLEC and MRSA prevalence during 2004–2010.

**Methods:** Data on clinical pathogens has been collected on a voluntary basis by the FiRe network (Finnish Study Group for Antimicrobial Resistance), which includes all large clinical laboratories in Finland. The network records >95% of all susceptibility tests done in Finland (population ca 5 million) for 15 pathogens; ESBL and MRSA positives are noted in a separate field. We looked at national ESBL and MRSA

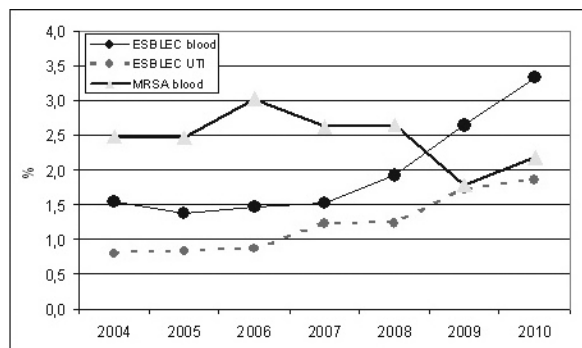


Figure. Annual percentages in Finland of MRS A/all *S. aureus* bacteremias, ESBLEC/all *E. coli* bacteremias, and ESBLEC/all *E. coli* UTI.s.

data from 2004 to 2010. *E. coli* bacteremia (about 2500/year) and urinary tract infection (UTI; about 96 000/year) isolates, and *Staphylococcus aureus* bacteremia isolates (about 1100/year) were included. Only the first isolate/patient/year is entered into the database.

**Results:** The number of MRSA infections is going down, while the rise in ESBLECs is steep and continuing, particularly from 2008 onwards.

**Bacteremias:** The annual number of diagnosed *E. coli* and *S. aureus* infections increased by ~10% per year on average. ESBLECs prevalence more than doubled between 2007 and 2010, from 1.5% to 3.3% (Fig.). In 2010, there was five times more ESBLECs than MRSA infections. MRSA infections tended to cluster into a few laboratories, indicating hospital outbreaks, while the ESBLECs were much more evenly distributed, suggesting community sources.

**ESBLEC bacteremias vs. UTI:** The increase in ESBLECs in bacteremias was steeper than in UTIs (Fig.). The age distribution of ESBLECs in UTI also changed, from being found mainly in the elderly in 2004, to being fairly evenly seen in all age groups in 2010.

**Conclusion:** The decrease in MRSA infections indicates that infection control in hospitals is effective. ESBLECs have now spread evenly in the population, and cause UTIs in all age groups. In hospitals, hygiene precautions are taken, which can stop epidemics, but the influx of ESBLECs from the community means that more and more patients are affected, even without hospital epidemics.

**P1520** Clonal group 025b-ST131 accounts for more than 89% of clinical isolates of ESBL-producing *E. coli* from hospital and nursing homes in one region of Ireland

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**Introduction:** The 025b:ST131 clonal group of *E. coli* has disseminated worldwide in both the hospital and community settings. The dissemination of this clonal group is a significant factor in the spread of CTX-M producing Extended-Spectrum beta-lactamases (ESBLs) predominately CTX-M-15. The objective of this study was to assess the extent to which 025b:ST131 accounts for ESBL dissemination in hospitals and nursing homes in Ireland and to determine if Pulsed Field Gel Electrophoresis (PFGE) can be used to determine if there is evidence of divergence of the clonal group into Nursing home related and hospital related subgroups.

**Materials and methods:** Between 1 January and 30 June 2010 96 isolates of ESBL producing *E. coli* were collected from routine clinical specimens. The collection comprised 28 isolates from residents of 25 nursing homes (NH n = 48) and 48 from hospitalised patients (HP n = 48) with no record of admission to a nursing home. All isolates were screened by PCR for blaCTX-M, blaTEM, and blaSHV. PCR was performed using primers specific to the pabB (region specific to the 025b-ST131 clone) and trpA genes as previously described. PFGE was performed using XbaI in accordance with Pulse-Net protocols.

**Results:** The blaCTX-M genes were detected in 93 (97%) isolates (blaCTX-M-group1 (n = 81); and blaCTX-M-group9 (n = 12), blaTEM (n = 33) and blaSHV (n = 3). Eighty-five (89%) isolates were members of the *E. coli* 025b:ST131 clonal group. PFGE analysis identified 73 individual pulsed field profiles (PFPs) and six major clusters (A-F) among the 96 isolates based on a similarity of >85%. The PFP's of all ST131 isolates were closely related with a similarity of >69%. The predominant PFGE cluster included both hospital and nursing home isolates (NH n = 17; HP n = 25).

**Conclusion:** We report clonal spread of the 025b:ST131 clonal group in the hospital and nursing home setting in this region. PFGE shows significant diversity within the 025b:ST131 clonal group. In general major PFGE clusters (defined as >85% similarity) within the clonal group comprise comparable proportions of NH and hospital isolates. We have not identified significant evidence of divergence into discrete populations. This is consistent with exchange of isolates between the NH and hospital sectors.

**P1521** Increased occurrence of multi-resistant *Escherichia coli* from bloodstream infections and consumption of antimicrobial agents in Danish hospitals from 2008 to 2010

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**Objectives:** The objective was to study the development of antimicrobial consumption in Danish hospitals and resistance among *Escherichia coli* from bloodstream infections in the period 2008–2010.

**Methods:** From 2008 through 2010, data on gentamicin, ciprofloxacin and 3rd gen. cephalosporin resistance in *E. coli* blood isolates from nine of 14 Departments of Clinical Microbiology (DCM) in Denmark (DCM at Rigshospitalet, Hvidovre, Hillerød, Odense, Esbjerg, Vejle, Skejby, Viborg and Aalborg Hospital), representing ~2/3 of the Danish population (3.7 million people), were collected. An average of 2300 *E. coli* blood isolates per year was tested for susceptibility towards gentamicin, ciprofloxacin and 3rd gen. cephalosporins (ceftazidime, ceftriaxone, and cefotaxime). Data on consumption of antimicrobial agents in the included hospitals were obtained from the Danish Medicines Agency. All data were collected as part of the DANMAP programme (www.danmap.org). To enable detection of changes in resistance, consumption data from 2007 were also included. Comparisons were based on the chi-square test; a p-value <0.05 was considered statistically significant.

**Results:** The level of resistance to gentamicin, ciprofloxacin and 3rd gen. cephalosporins increased significantly from 2008 to 2010. Resistance to gentamicin increased from 4.3% to 6.5%, ciprofloxacin from 9.7% to 13.6%, and 3rd gen. cephalosporins from 3.9% to 7.5%. Also the level of multi-resistance (gentamicin, ciprofloxacin, 3rd gen. cephalosporins) increased significantly from 1.3% in 2008 to 2.4% in 2010.

From 2007 to 2010, the consumption of broad spectrum antimicrobial agents also increased. This was seen especially for 2nd gen. cephalosporins (12.01 DDD/100 beddays in 2007 as compared to 15.35 DDD/100 beddays in 2010) and fluoroquinolones (8.60 DDD/100 beddays in 2007 as compared to 11.08 DDD/100 beddays in 2010). Also, the use of carbapenems increased from 2.53 DDD/100 beddays in 2007 to 4.81 DDD/100 beddays in 2010.

**Conclusion:** The increased frequency of resistance in *E. coli* blood isolates parallels the increased consumption of broad spectrum antimicrobial agents at the same hospitals. The observed rise both in antimicrobial resistance and consumption warrants surveillance of resistant enterobacteria including ESBL.

**P1522** Hierarchical clustering as a rapid tool for detection of antibiotic resistance phenotypes and outbreak in *Klebsiella pneumoniae* strains

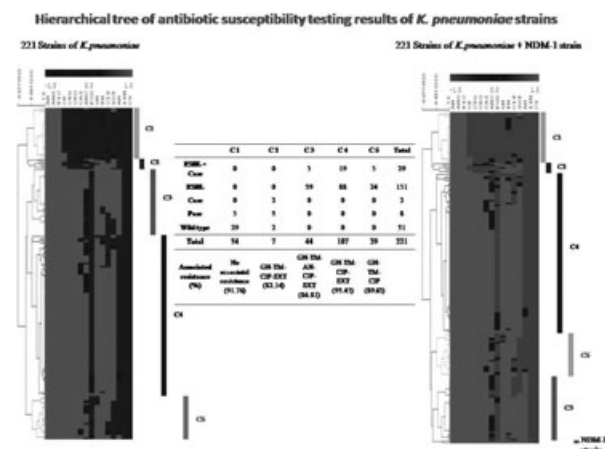
M. Berrazeg, M. Drissi, D. Raoult, J.M. Rolain\* (Marseille, FR)

**Background and objective:** Antimicrobial resistance is on the rise and its early detection is important in implementing effective control measures. The aim of this study was to detect in *Klebsiella pneumoniae* strains different antibacterial resistance phenotypes, particular profiles of antibiotic resistance and outbreaks in their early stage by using hierarchical clustering on antibiotic susceptibility testing results.

**Methods:** Two hundred and twenty-one strains of *Klebsiella pneumoniae* isolated from three different hospitals of western Algeria were identified by API 20E and MALDI-TOF mass spectrometry. Antibiograms, MICs, phenotypic and genotypic tests were determined for all isolates. Antibiograms results were introduced in MultiExperiment Viewer software to perform Hierarchical clustering so as Resistant, Intermediate and Sensitive translated to 1, 0 and -1 values, respectively.

**Result:** Phenotypic tests on the 221 *Klebsiella pneumoniae* strains showed the presence of five resistance phenotype groups: ESBL phenotype (68.32%), ESBL associated to cephalosporinase phenotype

(13.12%), cephalosporinase phenotype (0.9%), penicillinase phenotype (3.62%) and wild type phenotype (14.02%). Molecular characterisation of ESBL encoding genes showed that the majority of *K. pneumoniae* strains harboured two or three ESBL encoding genes (93.88%). All CTX-M and TEM enzymes belonged to CTX-M-15 and TEM-1 respectively. Sequencing done for blaSHV PCR products revealed the presence of SHV-1, 11, 12, 28 and 110. The two last genes were never described in Algeria. Using hierarchical clustering on MeV software as described in methods, five clusters were generated that were concordant with antibiogram susceptibility testing phenotypes. MeV software was able to survey qualitatively and quantitatively the prevalence of known and unknown phenotypes in real-time without any need of interpretation of the phenotype observed.



**Conclusion:** Our study showed high level ESBL occurrence with SHV, TEM and CTX-M as emerging type in west Algeria hospitals. SHV-28 and SHV-110 being reported for the first time in Algeria. Hierarchical clustering using MeV software is a useful and rapid tool to detect antimicrobial resistance phenotype that can be used in routine analysis for surveillance.

#### P1523 MALDI-TOF MS as a rapid and accurate tool for biotyping of *Klebsiella pneumoniae* clinical isolates from France and Algeria

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**Background and objective:** *Klebsiella pneumoniae* is often isolated in hospitals and is frequently involved in nosocomial outbreaks worldwide. Epidemiological analyses are useful in determining the extent of an outbreak and in elucidating the sources and spread of *K. pneumoniae* infections. The aim of this study was to investigate the epidemiological distribution of nosocomial *K. pneumoniae* strains using the MALDI-TOF MS and the data mining approaches.

**Methods:** Five hundred and thirty-five strains of *Klebsiella pneumoniae* isolated from Microbiology laboratories of either hospitals in France (Angers, Marseille, Nice) and Algeria (Annaba, Oran, Sidi Bel Abbes, Tlemcen) were identified by API 20E and MALDI-TOF mass spectrometry. Antibiograms and phenotypic tests were determined for all isolates. All clinical and epidemiological data were recorded as an Excel file, including clustering obtained using MSP dendrograms generated by BIOTYPYPER software and were analyzed using PASW Statistics software version 17.0.

**Result:** Susceptibility testing and phenotypic tests on the 535 *K. pneumoniae* strains showed the presence of five resistance phenotype groups: ESBL phenotype (46.4%), ESBL associated to cephalosporinase phenotype (4.5%), cephalosporinase phenotype

(0.2%), penicillinase phenotype (3.7%) and wild type phenotype (45.2%). The dendrogram generated by Biotyper software contained five distinct clusters according to an arbitrary cutoff set at the distance level of 500. Using data mining on PASW software, the analysis of the five clusters showed that *K. pneumoniae* strains isolated in Algerian hospitals were mostly associated with respiratory infections and ESBL phenotype, whereas *K. pneumoniae* strains isolated in Marseille and Angers hospitals were mostly associated with urinary tract infections and wild type phenotype. Conversely, *K. pneumoniae* strains isolated in Nice hospital were commonly associated with pus and blood culture samples and wild type phenotype.

**Conclusion:** MALDI-TOF MS along with data mining analysis were found to be an accurate tool to identify and separate *K. pneumoniae* strains into clusters according to their phenotypic properties and/or their epidemiology distribution.

#### P1524 Clinical cefoxitin-resistant *Escherichia coli* isolates in a Spanish Teaching Hospital

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**Objective:** An AmpC phenotype in *Escherichia coli* may result from overexpression of the chromosomal *ampC* gene due to mutations in its promoter/attenuator region or from acquisition of a plasmid-mediated beta-lactamase (pAmpC). The aim was to investigate resistance mechanisms in clinical cefoxitin-resistant *E. coli* isolates.

**Methods:** Clinical isolates of *E. coli* with AmpC phenotype (cefoxitin MIC > 16 mg/L and amoxicillin-clavulanic acid MIC > 4/2) were selected from June 2008 to December 2010 in a University Teaching Tertiary Hospital in the North of Spain. The presence of pAmpC was carried out by a multiplex PCR. For the ampC mutation analysis, a 271-bp fragment, which contains the ampC promoter enclosing the -35 box, the -10 box and the ampC attenuator was amplified, sequenced and compared with *E. coli* K12 *ampC* gene.

**Results:** One hundred and five *E. coli* fulfilled the screening criteria. Seventy-four isolates harboured a pAmpC gene (70.4%) including 70 blaCMY-2 and 4 blaDHA-1. No enzymes belonging to the ACC, FOX, MOX or EBC families were detected. The 74 isolates were recovered from urine (74.3%), fluids and tissue (22.9%) and other samples (2.7%). Several mutations in the ampC promoter/attenuator region were identified in the 31 pAmpC-negative isolates. Twenty of the 31 isolates had mutations at positions -42 and -18 rising to a stronger promoter. Fifteen of these 20 also showed mutations at the -1 and +58 positions and five had changes at -42, -18, -1. Five of the 31 isolates had a mutation at the -32 position. The mutations at positions -42 or -32 were mutually exclusive. Six isolates had attenuator mutations and also had changes that created a stronger promoter. None of them presented mutations that increased the spacer from the usual 16 bp. Two isolates exhibited single-base-pair insertion/deletion at positions +21 and +34, respectively. Twenty strains showed mutations at position +58, the codon immediately preceding the start codon of the *ampC* gene.

**Conclusions:** In this study, pAmpC was the most prevalent mechanism in cefoxitin-resistant *E. coli*, observing a dominance of CMY-2 type pAmpC. Several mutations were found in pAmpC-negative isolates and positions -42, -18, -1, +58 appeared to be more subject to mutation.

#### P1525 Assessment of haematology patients for duration of carriage and changes in antibiotic susceptibility of a CTX-M producing *Klebsiella pneumoniae* with a carbapenem-resistant phenotype

C. Dunn\*, L. Mitchell, P. Paterson, D. Inverarity (Lanarkshire, UK)

**Objectives:** To investigate the length of bowel carriage (continuous or intermittent) of a CTX-M producing *K. pneumoniae* (resistant to aminoglycosides, quinolones, piperacillin-tazobactam and occasionally



ertapenem) and changes in antibiotic Minimum Inhibitory Concentrations (MICs) affecting a population of haematology patients. **Methods:** Following investigation of carbapenem resistant, CTX-M producing, *K. pneumoniae*, manifesting as neutropenic sepsis and asymptomatic carriage, we began screening haematology patients. We cultured rectal swabs on chromogenic Brilliance™ UTI Agar (Oxoid) and assessed MICs using a Vitek 2 analyser (BioMerieux) on all ward inpatients once per week and at day case follow up of patients known to be carriers. Surveillance data was assessed to calculate duration of carriage (either continuous or intermittent) and to monitor antibiotic MICs.

**Results:** Twenty two carriers were assessed. Reasons for cessation of screening included death, transfer to another hospital or transfer to outpatient care. Isolates from 13 patients revealed continuous carriage until cessation of screening with three patients demonstrating intermittent carriage and four patients demonstrating initially continuous then intermittent carriage. Two patients only had one positive isolate throughout their screening period. The median length of continuous carriage was 22 days (range 2–188 days) and of patients with intermittent carriage was 7 days (range 6–132 days). Two patients appeared to lose carriage. Transformation from carbapenem susceptible to carbapenem resistant phenotypes and vice versa occurred during screening. We also observed resistance to colistin, fosfomicin, tigecycline and temocillin sometimes while using these antibiotics but sometimes without any exposure. Aminoglycoside MICs varied greatly, including between individual isolates from the same person.

**Conclusions:** There is wide variation in length of continuous and intermittent gastrointestinal carriage of this, potentially carbapenem resistant, *K. pneumoniae*. During carriage, MICs to some antibiotics e.g. carbapenems and aminoglycosides can vary. Resistance to so many antibiotic classes conventionally used to treat neutropenic sepsis and prolonged carriage has implications for the management of sepsis in this patient population.

#### P1526 Risk factors for KPC-producing *Klebsiella pneumoniae* faecal carriage of patients admitted at an intensive care unit

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**Objective:** KPC-producing *Klebsiella pneumoniae* (KPC-Kp) poses a serious threat for the Health Care Systems by provoking serious infections especially in critically ill patients. Therefore, the objective is to study the epidemiology, the risk factors and the outcome of Intensive Care Unit (ICU) patients for KPC-Kp faecal carriage at ICU admission.

**Methods:** During a 2-year period rectal samples were taken from each patient at ICU admission at the University Hospital of Patras, Greece. Rectal swabs were inoculated in chromogenic agar (Oxoid). *Klebsiella pneumoniae* isolates were identified by standards methods (Enterotube II, BD, BBL). Antibiotic susceptibility test was performed by the agar disk diffusion method according to CLSI guidelines. MIC was determined by the Etest (AB Biodisk). Isolates were tested applying Hodge Test for the presence of carbapenemases according to CDC and by meropenem-Boronic acid synergy disk test, for the presence of KPC. The presence of *blaKPC* gene was confirmed by PCR. Molecular typing was performed by PFGE of XbaI restricted genomic DNA. Epidemiologic data were collected from the ICU computerized database and patient chart reviews. Univariate statistical analysis was performed by SPSS ver. 17.0.

**Results:** From the 392 patients admitted, 48 (12.2%) were colonized at admission. Resistance rate among standard antibiotics was 100%, while 19 (39.6%) of KPC-Kp isolates were resistant to gentamicin, 17 (35.4%) to colistin and six (12.5%) to tigecycline. Three (6.3%) were pan-resistant. *blaKPC2* gene was found in all KPC-Kp isolates. The majority of KPC-Kp isolates belonged to Pulsotype A. The variables studied and the results of the univariate analysis are shown in Table 1.

Table 1: Univariate analysis comparing KPC-Kp-positive and -negative patients admitted at a general ICU.

	KPC-Kp NEGATIVES (n=344)	KPC-Kp POSITIVES (n=48)	Significance (p)
MALE PATIENTS	230 (66.9%)	28 (58.3%)	ns
MEAN AGE (days)	55.4 ± 19.3	65.1 ± 18.5	ns
EM	27.3 ± 5.4	28.1 ± 6.3	ns
APACHE II SCORE	14.4 ± 7.4	17.4 ± 8.1	*
SAPS II SCORE	34.2 ± 12.8	40.6 ± 14.3	**
PREVIOUS HOSPITALIZATION (days)	2.4 ± 4.5	44.5 ± 50.3	***
PREVIOUS ICU HOSPITALIZATION (days)	0.1 ± 0.3	21 ± 39.9	***
CO-MORBIDITIES			
- CHRONIC DISEASES (number)*	0.8 ± 0.9	1.73 ± 1.18	***
- INSULIN-DEPENDENT DIABETES MELITUS	10 (2.9%)	11 (22.9%)	***
- CHRONIC OBSTRUCTIVE PULMONARY DISEASE	33 (9.6%)	25 (52.1%)	***
- MALIGNANCIES	16 (4.7%)	13 (27.1%)	***
DURATION OF ANTIBIOTICS ADMINISTERED (days)	2 ± 4.8	42.8 ± 45.2	***
NUMBER OF ANTIBIOTICS ADMINISTERED	0.7 ± 1.2	5.7 ± 3.1	***
CARBAPENEM ADMINISTRATION (days)	0.7 ± 2.7	26.6 ± 35.3	***
GLUCOPEPTIDE ADMINISTRATION (days)	1 ± 2.9	25.9 ± 26.7	***
QUINOLONE ADMINISTRATION (days)	0.5 ± 1.8	10 ± 9.3	***
PIPERACILIN-TAZOBACTAM ADMINISTRATION (days)	0.4 ± 1.5	3.5 ± 5	***
MORTALITY	91 (26.8%)	20 (41.3%)	**
ICU LENGTH OF STAY (days)	15 (15.2)	32.4 (14.1)	ns

Data are in number (%). ns = not significant, \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001

\*Chronic diseases: Congestive Heart Failure, Chronic Renal Failure, Hepatic insufficiency, Obesity, Chronic Obstructive Pulmonary Disease, Diabetes Mellitus (insulin dependent or not dependent).

**Conclusion:** There was no patient positive for KPC-Kp without prior hospitalization or antibiotic use before ICU admission. Patients colonized with KPC-Kp had higher mortality, higher APACHE II and SAPS II scores at admission, while ICU length of stay (LOS) was unaffected. Therefore, the effect of KPC-Kp colonization might not solely contribute to ICU mortality. The high level of resistance of the KPC-Kp isolates of the patients admitted at ICU mandates the implementation of surveillance measures and isolation of these patients in order to reduce further contamination of other patients.

#### P1527 Clonal outbreak of carbapenem- non-susceptible *Acinetobacter baumannii* in a Croatian Hospital

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**Objectives:** The aim of the study was to characterize the mechanism of reduced susceptibility to carbapenems in *A. baumannii*.

**Methods:** Sixty-nine *A. baumannii* isolates were collected in last three months of 2009 in the hospital in Pula, Croatia. The antimicrobial susceptibility to a wide range of antibiotics was determined by broth microdilution according to CLSI guidelines. E-test MBL strips were used for detection of metallo-carbapenemases following the manufacturer's instructions. PCR was used to detect the presence of the genes encoding the MBLs of IMP, VIM and SIM series and, blaOXA (blaOXA-51, blaOXA-23, blaOXA-40, blaOXA-58 and blaOXA-143) genes as previously described. The genetic context of blaOXA-51 genes was determined by PCR mapping with the primers for ISAbal1 combined with forward and reverse primers for blaOXA-51. Sequence groups (1–3) corresponding to EU clones I-III were determined by multiplex PCR. Genetic relatedness of the isolates was investigated by PFGE. The presence of genes encoding CarO porin was investigated by PCR.

**Results:** More than 90% of the strains were resistant to cefotaxime, ceftriaxone, piperacillin, piperacillin/tazobactam, ciprofloxacin and gentamicin. The strains showed variable degrees of susceptibility/resistance to imipenem and meropenem. Most strains were intermediate susceptible to both carbapenems with MICs around 8 mg/L. All strains were susceptible to colistin. Colistin was the most potent antibiotic with MIC90 of 2 mg/L.

Acquired oxacillinases were not found. Isolates were positive only for naturally occurring OXA-51 beta-lactamase which was associated with ISAbal1 insertion sequence located upstream of blaOXA-51. No MBLs were found. Chromosomal AmpC beta-lactamases did not affect the susceptibility to carbapenems. Sodium chloride did not decrease carbapenem MICs. MICs of meropenem were not significantly reduced by CCCP (proton-gradient pump inhibitor). The strains were found to belong to EU clone I (sequence group 2). PFGE confirmed the clonality of the isolates. All strains were found to possess gene encoding CarO porin.

**Conclusions:** This study demonstrated clonal outbreak of multiresistant *A. baumannii* in Pula county hospital. The presence of

ISAbal insertion sequence is thought to upregulate the expression of *blaOXA-51* gene and therefore may be responsible for elevated carbapenem MICs. In conclusion, this study highlights the need to establish an antimicrobial surveillance network for *A. baumannii*.

**P1528** **Outbreak of carbapenemase-producing *Klebsiella pneumoniae* and *Enterobacter cloacae* strains in a Greek University Hospital**

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**Objective:** To report an outbreak of carbapenem-resistant *Klebsiella pneumoniae* and *Enterobacter cloacae* in our institution and focus on the mechanisms of resistance using phenotypic and molecular methods.

**Methods:** In the period from February to October 2011, 599 strains of *K. pneumoniae* and 116 strains of *E. cloacae* were isolated. Identification and susceptibility testing was performed using the Vitek 2 automated system (Biomérieux, France). Resistance to carbapenems was confirmed through in vitro susceptibility tests determined by the E-test method (AB Biodisk, Solna, Sweden). MIC results for carbapenems were interpreted according to the breakpoint tables for interpretation of MICs as specified by EUCAST, 2011. All resistant isolates were phenotypically screened for MBL-type and KPC-type carbapenemases using the imipenem/EDTA, double disk synergy test (DDST) and imipenem/boronic acid combined disk test, respectively. Susceptibility testing to tigecycline and colistin was performed using the E-test method and testing to chloramphenicol using the disk diffusion method according to EUCAST breakpoints tables. Detection of carbapenemase and  $\beta$ -lactamase genes was identified by PCR using primers for blaVIM, blaIMP, blaKPC and blaSHV.

**Results:** From 599 *K. pneumoniae* isolates, 58 (9.68%) were resistant to carbapenems and were recovered from cultures of blood (n = 16), urine (n = 18), wounds (n = 7), bronchial fluid (n = 7), central venous catheter tips (n = 6), ulcers (n = 4). Two strains were resistant to colistin. PCR analysis revealed that 53 strains were carrying the blaKPC-2 gene, three strains the blaVIM-1 gene and two strains were carrying blaVIM-1 and blaSHV-5 genes.

From 116 *E. cloacae* isolates, 12 (10.34%) were resistant to carbapenems and were recovered from urine (n = 3), wounds (n = 3), blood (n = 2), CVC tips (n = 2), sputum (n = 2). All strains were sensitive to colistin. Eight strains were carrying the blaVIM-1 gene and four the blaKPC-2 gene.

The outbreak began on February 2011 with four cases and reached the maximum on July 2011 with 21 cases. Despite control measures including isolation, cohort and contact precautions, today the average of isolated cases is around four new cases monthly, becoming endemic in our hospital. All cases came from internal medicine and surgical units and mainly from ICU.

**Conclusion:** Such outbreaks are very frequent in Greek hospitals. The isolation of resistant Enterobacteriaceae is appropriate for scientific goals and mainly for the control of their spread.

**P1529** **Resistance to carbapenems in *Klebsiella pneumoniae* isolates is related to CTX-M-15 or DHA-1 and loss of OmpK35 and/or OmpK36**

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**Objectives:** The aims of this study was to characterized molecular mechanism of carbapenem resistant in clinical *Klebsiella pneumoniae* isolates with resistant or reduce susceptibility to carbapenems, in university hospital in Lisboa, Portugal.

**Methods:** Carbapenem-resistant *K. pneumoniae* isolates (n = 5), recovered between March and April 2010 from five patients hospitalized in different wards at university hospital, were examined for minimal inhibitory concentration (MIC) by Etest according to Clinical and Laboratory Standards Institute guidelines. Presence of genes encoding metallo- $\beta$ -lactamases, extended-spectrum  $\beta$ -

lactamase (ESBLs) and plasmid-mediated AmpC enzymes were screened by PCR using specific primers. Outer membrane protein (OMP) genes were analyzed by sequencing of *ompk35* and *ompk36* genes. Molecular typing was performed by M13-PCR fingerprinting and Multilocus Sequence Typing (MLST). Replicon typing was used to define plasmid incompatibility groups (Inc).

**Results:** The five isolates showed resistant to amoxicillin/clavulanic acid, cefotaxime, cefoxitin, ceftazidime, aztreonam, ciprofloxacin and gentamicin, and showed MICs ranging between 8 and 24 mg/L to imipenem and meropenem. PCR and sequencing experiments detected in two isolates the bla(CTX-M-15) gene and in three isolates the bla(DHA-1) gene. OMP gene analysis revealed numerous deletions and insertions in *ompk35* and *ompk36* porin genes. M13-PCR fingerprinting analysis demonstrated that there were no major clonal relationships among *K. pneumoniae* isolates. However, MLST experiments showed that all isolates shared the same sequence type (ST), ST14. The predominant plasmid was included in IncFIA (n = 5) and was found plasmids belong to different replicons IncA/C (n = 4) and IncF (n = 1).

**Conclusions:** In conclusion, reduced susceptibility among *K. pneumoniae* clinical isolates at University hospital was largely co-mediated by production of plasmid-mediated CTX-M-15 ESBLs or DHA-1 AmpC  $\beta$ -lactamases coupled with the loss of OmpK35 and/or OmpK36.

**P1530** **Surfing on the large sea of carbapenemases**

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**Objectives:** The spread of carbapenemase-producing (CP) Enterobacteriaceae (CPE) is matter of concern worldwide. Even if different genera of Enterobacteriaceae can harbour carbapenemase genes, this resistance trait has become more common in *Klebsiella pneumoniae* (KP) isolates, which can express different resistance mechanisms such as KPC, IMP, VIM, NDM, OXA48 etc. Recently different KPC+ KP outbreaks were described in different hospitals of our region. Guidelines for preventing the diffusion of CP-KP, based on the evidences of literature, were developed by the Infectious Risk Unit of the Agenzia Sanitaria e Sociale Regionale and were applied from last August. After having discovered that in a healthcare facility two patients were colonized by CP-KP (index cases), a surveillance protocol based on rectal screening was performed on the patients that had been in contact with these two index cases.

**Methods and results:** CP-KP were isolated from three different patients other than the two index cases; the confirmatory test was initially based on the modified Hodge test. When in a later time the laboratory implemented the confirmatory tests using the disk diffusion synergy test, one of the two index cases appeared to be colonized by a KPC+-KP, whereas the other one was colonized by a metallo- $\beta$ -lactamase (MBL) producer strain. The phenotypic data were confirmed by molecular methods for the KPC producer strains, whereas the MBL genes are currently under definition through molecular tests. The three patients colonized by CP-KP had all MBL+ strains. In this case, only the staff that had in charge the MBL+ patient was responsible of the spread (although contained) of the microorganism. After this small outbreak, we documented single cases of colonization and to date we have recognized five patients with KPC+-KP and 10 patients harbouring MBL+-KP. Strain typing using the ERIC-PCR technique is currently in progress.

**Conclusion:** Although the small number of cases, our experience demonstrates that the problem of the spread of CPE can be quite complex. The possible diffusion of different resistance genes should be always considered. To correctly evaluate the epidemiology of the strains and to better manage the outbreaks, it is mandatory that the microbiology Lab performs immediately the appropriate phenotypic tests to detect the type of carbapenemases involved. Strain typing could be also encouraged to define the epidemiology even in small clusters.

**P1531 Epidemiology and prognostic determinants of bacteraemic biliary tract infection**

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**Objectives:** To know epidemiology in bacteremia due to biliary tract infections (BTI) and to identify independent predictors of mortality.

**Design:** This study was part of a bloodstream infections surveillance study that prospectively collected consecutive patients with bacteremia in our institution from 1991 to 2010. BTI was the confirmed source of 1373 of the 21 006 (7%) bacteremias and the independent prognostic factors of 30-days mortality were determined.

**Results:** The mean age of patients with biliary sepsis was 71 years ( $\pm 14$ ). The most frequent comorbidity was biliary lithiasis and solid organ cancer (35% and 26%). The BTI was nosocomially-acquired in 33% patients. Shock and mortality accounted for 209 and 126 cases respectively (15% and 9%). The more frequent microorganisms isolated were: *E. coli* (749, 55%), *Klebsiella* spp. (240, 17%), *Enterococcus* spp. (171, 12%), *P. aeruginosa* (86, 6%) and *Enterobacter* spp. (63, 5%). There were 47 (3%) cefotaxime resistant (CTX-R) *E. coli* or *Klebsiella* spp. Inappropriate empirical antibiotic treatment was an independent factor associated with mortality (OR:1.4; 95%CI: 1.1–1.7). Inappropriate empirical treatment was more frequent in *P. aeruginosa* and CTX-R Enterobacteriaceae bacteremia. These microorganisms were significantly more common in patients with previous antibiotic therapy, solid-organ cancer or transplantation and in nosocomially-acquired bacteremia.

**Conclusions:** In patients with bacteremic BTI, inappropriate empirical therapy was more frequent in *P. aeruginosa* and CTX-R Enterobacteriaceae infection and it was associated with a higher mortality rate. In patients with bacteremia due to BTI and solid organ cancer or transplantation, nosocomially-acquired infection or previous antibiotic treatment, initial therapy should include piperacillin-tazobactam or a carbapenem.

**P1532 Emergence of colistin-resistant Enterobacteriaceae strains after previous exposure to colistin**

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**Objectives:** the aim of this study was to report the emergence of colistin-resistant (CR) Enterobacteriaceae strains in patients previously harbouring colistin-susceptible (CS) strains and treated with colistin in Sfax university hospital (Tunisia).

**Methods:** This retrospective study was conducted between 2005 and 2009. Five patients with CR Enterobacteriaceae urinary tract infection were included in this study. The history and the duration of colistin treatment were obtained from patients' medical records. Twenty Strains isolated from these patients before and after treatment with colistin were studied. MICs for colistin were obtained by agar dilution method. Resistance to colistin was defined as MICs > 2 mg/L. Strain typing was performed using pulsed-field gel electrophoresis (PFGE).

**Results:** Of the five patients included in this study, three have urinary tract infection caused by *Klebsiella pneumoniae*, one by *Escherichia coli* and one by *Enterobacter cloacae*. All of these patients received suboptimal dosages of colistin (1–2 MU/day) as monotherapy. The time interval between the isolation of susceptible and resistant strains varied from 5 days to many weeks.

Twenty strains of Enterobacteriaceae were studied: 14 *K. pneumoniae* (nine CR and five CS), four *E. coli* (three CR and one CS) and two *E. cloacae* (one CR and one CS). All of these strains produced extended spectrum  $\beta$  lactamase and were multidrug resistant.

For three patients, molecular typing by PFGE showed that CR and CS strains were clonally related. Three clones (one clone of *E. cloacae* and two clones of *K. pneumoniae*) were identified suggesting a selection during treatment with colistin of CR strain from a CS strain. For the two remaining patients, reinfection with CR strains under colistin selective pressure has been confirmed by PFGE. Different clones: four clones of

*E. coli* in one patient and five clones of *K. pneumoniae* in the other patient were observed.

**Conclusion:** These observations showed that selective pressure due to inadequate colistin use may lead to the emergence of resistance in the same strain or to the reinfection with a resistant strain. Thus, to treat infections caused by multidrug resistance Gram-negative bacteria, appropriate dosage regimens for colistin should be determined and a combination therapy is necessary in order to prevent colistin resistance.

**P1533 Occurrence of resistance to fosfomycin in urine isolates of *E. coli* collected from outpatients in Germany, 2010**

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**Objectives:** Fosfomycin (FOS) exhibits activity against a broad spectrum of aerobic bacteria including *Escherichia coli* (ECO), the leading pathogen responsible for uncomplicated and complicated urinary tract infections (UTI). The use of FOS for first-line therapy of uncomplicated UTI has attracted increasing attention as there is a lack of cross-resistance (R) between FOS and other classes of antibiotic, including those associated with high or increasing rates of R. The objective of this study was to evaluate the susceptibilities of ECO urine isolates to FOS and other oral drugs commonly used for the treatment of UTI in outpatients.

**Methods:** In a surveillance study conducted by the Paul-Ehrlich-Society between October and December 2010, 25 laboratories across Germany were requested each to collect 20 non-duplicate ESC urine isolates. Isolates were identified by standardized methods. MICs of FOS, amoxicillin (AMX), amoxicillin-clavulanic acid (A/C), cefuroxime (CXM), cefixime (CFM), cefpodoxime (CPD), ciprofloxacin (CIP), levofloxacin (LVX), ofloxacin (OFX), norfloxacin (NOR), trimethoprim (TMP), trimethoprim-sulfamethoxazole (SXT) and nitrofurantoin (NIT) were determined by the broth microdilution procedure according to the standard DIN ISO in a central laboratory. MICs were interpreted by EUCAST criteria. Breakpoints of FOS were  $\leq 32$  mg/L (susceptible, S) and  $> 32$  mg/L (R).

**Results:** Four hundred and ninety-nine isolates were collected. Isolates were obtained from 428 (85.8%) female and 71 (14.2%) male patients. Patients ranged in age from 1 to 93 years (median 59 years). MICs 50/90 obtained with the antimicrobial agents as well as the rates of S, intermediate (I) and R isolates are shown in the Table. Six strains were R to FOS and another 19 had elevated FOS MICs (16–32 mg/L), though rated S. Of these 25 isolates, 17 (68%), 14 (56%), 10 (40%), 7 (28%) and 3 (12%) were R to AMX, A/C, SXT, CIP, and CPD. R to A/C and/or AMX was more frequently distributed among isolates with FOS MICs of  $\geq 16$  mg/L than among isolates with FOS MICs of  $\leq 8$  mg/L (AMX 68% vs. 41.6%,  $p < 0.01$ ; A/C 56% vs. 31.4%,  $p < 0.05$ ). One FOS-R strain and two strains with elevated FOS MICs were R to all drug classes, except nitrofurantoin.

	MHK-50 (mg/l)	MHK-90 (mg/l)	%S	%I	%R
FOS	$\leq 1$	4	98.8	-	1.2
AMX	4	$\geq 64$	57.1	-	42.9
A/C	4	64	67.3	-	32.7
CXM	4	8	90.0	-	10.0
CFM	0.25	1	91.8	-	8.2
CPD	0.25	1	91.4	-	8.6
CIP	$\leq 0.063$	$\geq 16$	79.4	0.8	19.8
LVX	$\leq 0.063$	8	80.4	0	19.6
OFX	$\leq 0.125$	$\geq 16$	76.0	3.8	20.2
NOR	$\leq 0.125$	$\geq 16$	78.8	1.0	20.2
TMP	$\leq 0.25$	$\geq 32$	66.9	0.4	32.7
SXT	$\leq 0.25$	$\geq 32$	68.7	0.4	30.9
NIT	$\leq 16$	32	99.2	-	0.8

**Conclusion:** Overall, S to FOS seems to be very high among ECO urine isolates from outpatients in Germany. FOS trometamol may thus be considered as an important option for first-line therapy of uncomplicated UTI. Prudent use of FOS, however, is mandatory in order to restrict the spread of R.

**P1534** Factors associated with carriage of multi-resistant commensal *Escherichia coli* among post-menopausal women in Ujjain, India

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**Objective:** To determine factors associated with carriage of multi-resistant *E. coli* by healthy women of age 45 and above.

**Methods:** This cross-sectional study was conducted over a period of 15 months between 2007 and 2009. A structured questionnaire was completed for healthy women attending outpatient clinics of department of Obstetrics and Gynaecology at RD Gardi Medical College, Ujjain. Identification of *E. coli* and antibiotic susceptibility was done using standard guidelines. The multi-drug resistance (MDR) was defined as those isolates having co-resistance to three or more antibiotics belonging to different classes. The relationship between patient characteristics and the outcome (carriage of MDR *E. coli*) was explored using odds ratios (OR). Crude ORs were calculated from two by two tables. Multi-variable logistic regression models were used to calculate adjusted OR in the final model. In the final model a variable was entered only if the uni-variate analysis yielded a p-value <0.1 for that variable.

**Results:** The study showed that 28% of healthy women carried MDR *E. coli* in the peri-anal region. The factors significantly associated with carriage of MDR *E. coli* were "family size more than 10" (OR 8.18; 95% CI 3.13–21.3;  $p < 0.001$ ), "antibiotic use in the past 2 weeks" (OR 7.83; 95% CI 3.81–16.01;  $p < 0.001$ ), "hospitalization in the past 2 weeks" (OR 5.68; 95% CI 2.82–8.24). Among MDR isolates higher proportion of resistance was noted for nalidixic acid (85%), tetracycline (76%), ampicillin (66%), and co-trimoxazole (53%). Resistance to ciprofloxacin (43%), norfloxacin (45%) and third generation cephalosporin's, cefotaxime (38%) and ceftriaxone (29%) is a cause of concern as they are an important therapeutic armament in *E. coli* infections. No resistance to imipenem was noted.

**Conclusions:** Overcrowding, past antibiotic use and hospitalization history were identified as risk factors for carriage of MDR *E. coli* in this first study healthy women above 45 years of age.

**P1535** Increasing annual prevalences of third-generation cephalosporins resistant *Escherichia coli* in urine in the community predominantly due to chronic or recurrent urinary tract infections

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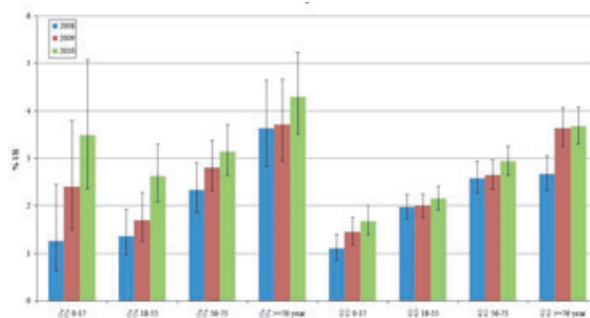
**Objectives:** To determine whether annual prevalences of urine cultures with third-generation cephalosporins (3gCEP) resistant *Escherichia coli* (ECO) in the community is increasing, and if so, to what extent this increase is determined by patients with chronic or recurrent UTI with 3gCEP resistant ECO.

**Methods:** All ECO isolates (first isolate per patient per year) from GP populations submitted from 2008 to 2010 by 20 non-academic medical microbiology laboratories to the Dutch Infectious Diseases Surveillance Information System on Antimicrobial Resistance (ISIS-AR) were included.

The MIC values in combination with EUCAST breakpoints were used to calculate 3gCEP resistance (I + R) percentages per age-group and gender. To account for chronic or recurrent UTI, the same analysis was repeated excluding patients in the years 2009 and 2010 with a 3gCEP resistant ECO isolate in 2008 or 2009.

**Results:** A total of 137 911 isolates of 26 303 males and 111 608 females were included. The annual prevalence per gender and age-group of non-susceptible 3gCEP ECO, including confidence intervals is shown in Fig. 1. The annual prevalence of 3gCEP resistant ECO significantly increased for both male and females from 2008 onwards for all age-groups (overall resistance increased from 2.1% in 2008 to 2.8% in 2010,  $p < 0.0001$ ).

However, after excluding those 20% of the patients with a 3gCEP resistant ECO detected in a previous year the annual prevalence remained stable (2.1% in 2009 and to 2.2% in 2010). Only for males aged 0–17 years a significant increase ( $p = 0.006$ ) was found after excluding in the previous year positive patients.



**Conclusion:** The significant increase in prevalence of urine cultures with 3gCEP resistant ECO in the community in 2009 and 2010 was predominantly due to patients with chronic or recurrent UTI with 3gCEP resistant ECO. Only for males aged 0–17 years the increase could not be explained by this phenomenon, a worrisome finding that needs further study.

**P1536** Antimicrobial resistance of *Escherichia coli* isolates from primary care patients and nursing home residents in the Netherlands and Germany

C. van der Donk\*, E. Stobberingh on behalf of the EurSafety Health-net EMR group

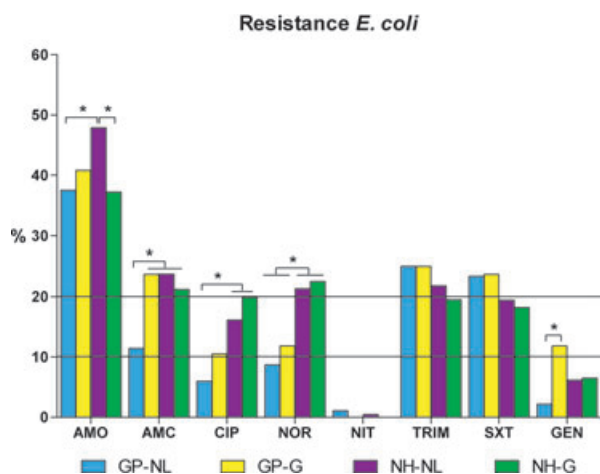
**Objectives:** We assessed the antibiotic resistance of *Escherichia coli* isolated from primary care (GP) patients and nursing home (NH) residents in the Netherlands (NL) and Germany (G) in the Euregion Meuse-Rhine. High resistance rates could have implications on empiric treatment.

**Methods:** A total of 184 and 76 *E. coli* isolates were collected from urine samples from Dutch and German PC patients and 231 and 211 from Dutch and German NH residents, respectively. Quantitative susceptibility testing was performed with the micro broth dilution method according to EUCAST guidelines. Putative extended beta-lactamase (ESBL) producing isolates were tested for ESBL production with a combination disk diffusion test.

**Results:** Between isolates from the NHs and GPs significant differences were found for amoxicillin (with clavulanic acid) ( $p = 0.037$  and  $p < 0.013$ ) and the fluoroquinolones ( $p < 0.05$ ). Concerning is the high resistance rates for the quinolones among the NH isolates (>15%) compared with GP isolates ( $\leq 10\%$ ). Between the NH isolates from NL and G significant differences were only observed for amoxicillin ( $p = 0.023$ ). Between the GP isolates from NL and G significant differences were demonstrated for amoxicillin-clavulanic acid ( $p = 0.022$ ) and gentamicin ( $p = 0.002$ ).

No significant differences in resistance were demonstrated for nitrofurantoin, since resistance was <2%. For the folate antagonist resistance varied from 18% to 25%. ESBL prevalence was low (<2%). Considering the 20% and 10% cut-off for empiric treatment for uncomplicated and complicated urinary tract infections (UTI),

respectively, nitrofurantoin is appropriate for all groups. Moreover, for complicated UTIs only the quinolones are appropriate only in NL for GP patients. For uncomplicated UTIs the quinolones can be used among GP patients and possibly among NH residents. Also amoxicillin-clavulanic acid is suitable for Dutch GP patients and co-trimoxazole for NH residents.



**Conclusions:** There are differences in resistance between GP and NH isolates but also between isolates from two countries. This could be related to high use of these agents. Especially the high resistance for the quinolones among NH isolates is a point of concern. Also with a high resistance to amoxicillin-clavulanic acid and the folate antagonists, options for empiric treatment are limited. However, for empiric therapy of uncomplicated cystitis nitrofurantoin remains the agent of first choice.

**P1537** Susceptibility of Gram-negative urinary tract isolates to mecillinam in a large Glasgow teaching hospital

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Mecillinam (pro-drug Pivmecillinam) is a beta-lactam antibiotic which inhibits cell wall synthesis in gram negative bacteria. Mecillinam has been used to treat uncomplicated urinary tract infection (UTI) in Scandinavia for many years. Surveillance in Scandic countries shows that mecillinam resistance in *Escherichia coli* is low and has remained stable for the past 20 years. In the UK mecillinam has become an attractive option to treat UTI secondary to extended spectrum beta-lactamase (ESBL) producing organisms where oral options are limited. European surveillance data previously indicated that mecillinam sensitivity for uropathogens is high at 95.9% with little variation between participating countries. There is however little UK-specific data available for mecillinam. Our objectives were therefore to determine local resistance data for mecillinam in resistant gram negative organisms and ESBLs and to assess whether mecillinam is an attractive agent of the management of uncomplicated UTI secondary to these organisms. We also wished to assess variation in susceptibility according to organism.

**Methods:** We extracted and analysed six months of susceptibility data (January–June 2011) from our laboratory system, telepath. In our laboratory, mecillinam susceptibility is performed using the automated system, VITEK 2, for isolates that are ESBL producers (identified by a combination disc method and VITEK 2) or those testing resistant to all or limited oral options. A sensitive result is an MIC  $\leq 8$   $\mu\text{g}/\text{mL}$  and resistant MIC  $\geq 32$   $\mu\text{g}/\text{mL}$ . Results were broken down by organism and by whether or not the isolates were ESBL producers.

**Results:** Four hundred and ninety-nine samples were tested, all from different patients, during the sampling period. 71/499 (14.2%) were

resistant to mecillinam. Interestingly, 11/46 (23.9%) of *Klebsiella pneumoniae* tested mecillinam resistant. 35/388 (9%) of *Escherichia coli* isolates were also resistant. 336/499 (67%) of isolates were ESBL producers, and mecillinam sensitivity did not appear to be associated with ESBL production ( $p = 0.075$ , Chi-squared test).

**Conclusions:** Although an element of selection bias may contribute to our results, we have observed that a significant proportion of isolates in our dataset appear mecillinam resistant, and up to 25% of *Klebsiella* spp. may be resistant. Additionally, it appears that mecillinam resistance is not associated with ESBL production in our isolates. These observations arguably warrant further prospective investigation.

**P1538** In vitro antimicrobial resistance of *Escherichia coli* strains isolated from patients with urinary vs. intra-abdominal infections

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**Objectives:** *Escherichia coli* is one of the most prevalent causative pathogens of a variety of diseases, including urinary tract infections (UTI) and intra-abdominal infections (IAI). Unfortunately, it continues to acquire and express resistance to many antimicrobial agents, including those commonly used for the treatment of these infections. This report aimed to evaluate the in vitro antimicrobial resistance of *E. coli* strains isolated from patients with UTI and compare them with isolates recovered from IAI.

**Methods:** We studied *E. coli* strains isolated from community-acquired (CA)-UTI and from patients with community onset IAI, diagnosed in our hospital, during the period October 2008–October 2011. Only one isolate per patient was accepted into the study. All clinical samples were cultured under standard conditions. Positive urine cultures were defined by bacterial grow  $> \text{or} = 10^5$  colony forming units/mL. Patients with polymicrobial urine cultures were excluded from the study. Identification of *E. coli* was performed by means of standard methods and susceptibilities to different antimicrobials were tested by agar disk diffusion method according to the CLSI criteria. Intermediate susceptibility to either of the antimicrobials studied was considered as resistant for data analysis.

**Results:** We obtained 442 *E. coli* isolates from an equal number of patients with CA-UTI and 263 strains from patients with IAI. The resistance rates for the UTI and the IAI isolates were as follows: ampicillin/sulbactam 38% and 49%, cefepime 6% and 11%, cefotaxime 4 and 11, ceftazidime 5% and 11%, ceftriaxone 6% and 12%, trimethoprim/sulfamethoxazole 22% and 37%, ciprofloxacin 15% and 32%, levofloxacin 15% and 36%, piperacillin/tazobactam 4% and 9%, respectively. Finally, amikacin, imipenem, meropenem, ertapenem and tigecycline had excellent in vitro activities against the isolates tested in this study.

**Conclusion:** Periodic monitoring of the in vitro resistance profiles of community-acquired infections is critical for an adequate empirical therapy. Overall, the isolates obtained from IAI exhibited reduced susceptibility levels compared to the urinary strains. Notably, an increased prevalence of fluoroquinolone non-susceptible IAI strains was observed. However, combining all *E. coli* isolates, the most active agents in vitro were amikacin, imipenem, meropenem, ertapenem, piperacillin/tazobactam and tigecycline. These drugs were the only agents with overall percentage susceptible values  $>90\%$ .

**P1539** Risk factors of fluoroquinolone resistance in community-acquired acute pyelonephritis caused by *Escherichia coli*

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**Objectives:** *Escherichia coli* is the most common pathogen in community-acquired acute pyelonephritis (CA-AP) and practice guidelines recommend oral fluoroquinolones (FQ) as initial therapy. The emergence of *E. coli* resistance to FQ increased in recent years and spread gradually worldwide leading to treatment failure. The goals of

this study were to identify risk factors for acquiring FQ resistance in *E. coli* isolated from CA-AP.

**Methods:** Retrospective analytic study included all *E. coli* isolated from urine samples of patients admitted for CA-AP at infectious diseases department in the university hospital of Monastir between 1999 and 2009. Clinical and epidemiological features were collected. Patients aged >14 years who presented temperature  $\geq 37.8^\circ\text{C}$ , flank pain and/or costovertebral tenderness, urinary tract symptoms, leukocyte count  $>10^4/\text{mL}$  and bacteriuria  $>10^5/\text{mL}$  were enrolled. Identification of *E. coli* was performed by API20E. The study of antibiotic susceptibility was performed by agar diffusion according to CA-SFM. Univariate analyses were run to describe the distribution, central tendency and variability. Covariates found to be associated with FQ resistance on univariate analysis at a level of significance  $p < 0.2$  were eligible for inclusion in a multivariate logistic regression model. SPSS version 17.0 was used for analysis.

**Results:** A total of 433 cases of CA-AP were included. The mean age was 44.4 years (15–89) and 128 (29.6%) were male. Dominant comorbid conditions were diabetes (90, 20.8%) and urinary abnormalities (65, 15%) particularly urinary stone (35, 53.8%). Thirty one strains (7.1%) were resistant to FQ. Of them, 12 (38.7%) were extended-spectrum beta-lactamase-producing. In univariate analysis FQ resistance was correlated to urinary catheterization ( $p = 0.002$ ), antibiotic use in the previous 12 months ( $p = 0.038$ ) and hospitalization in the previous 12 months ( $p = 0.024$ ). The independent predictor of FQ resistance in *E. coli* CA-AP was male sex (OR 3.5,  $p = 0.023$ , 95% CI 1.19–10.35). If we analyze only women, menopause was only significant risk factor for FQ resistance (OR 2.8,  $p = 0.01$ , 95% CI 1.23–6.53).

**Conclusion:** CA-AP due to FQ resistant *E. coli* strains are increasing in Tunisia. Efforts are needed to curtail the increase of resistance and empiric antimicrobial regimens should be evaluated.

#### **P1540** Susceptibility to oral antimicrobials among beta-lactam-resistant *Escherichia coli* strains causing community urinary tract infections

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**Objectives:** Urinary tract infections (UTIs) are among the most common infectious diseases occurring in either the community or healthcare setting. The increase in the multi-resistant Gram-negative rods prevalent in UTIs has resulted in difficulties in determining an appropriate empirical antimicrobial treatment and has also limited the number of oral therapeutic options in patients with community UTIs. The aim of our study was to compare percentages of susceptibility to oral antimicrobials among *Escherichia coli* isolates with extended-spectrum beta-lactamases (ESBLs), AMPc production and none of the two last resistance mechanisms causing UTI.

**Methods:** All significant *E. coli* strains isolated from urine over a 10 month period were included. The chromogenic agar (CPS ID 3, bioMérieux) was used for identification and the Vitek 2 system (bioMérieux) was employed for antibiotic susceptibility testing. Strains with ESBLs or AMPc production by the automatic method were checked by phenotypic disk-diffusion assays. A total of six antimicrobial agents were compared: fosfomicin, trimethoprim-sulfamethoxazole, nitrofurantoin, ciprofloxacin, amoxicillin-clavulanic acid and nalidixic acid. All statistical tests were performed with SPSS 15.0 software. Differences were considered significant if  $p < 0.05$ .

**Results:** A total of 3632 *E. coli* isolates were included. Among the strains studied, 75.6% were isolated from women and medium age ( $\pm\text{SD}$ ) was 48.5 (28.2). Of all the *E. coli* isolates studied, 91.3% were community acquired. AmpC hyperproduction and ESBLs were seen in 1.4% (51/3632) and 9.4% (341/3632) of the strains respectively. Oral antibiotic susceptibility percentages of all the isolates, AmpC production strains, isolates with ESBLs and none of the two last resistance mechanisms strains are shown in the table. Differences

among percentages of susceptibility were statistically significant (Chi-square test).

	% Susceptibility					
	fosfomicin	trimethoprim - sulfamethoxazole	nitrofurantoin	ciprofloxacin	amoxicillin-clavulanic acid	nalidixic acid
All isolates (n= 3632)	96.2	59.4	92.7	53.3	77.6	54
AMPc (n= 51)	95.5	51.1	84.3	25.5	0	24.4
ESBL (n= 341)	89.6	30	74.9	14.1	54.0	13.1
No AMPc no ESBL (n= 3240)	96.9	62.6	94.7	57.8	81.1	57.2

**Conclusion:** Overall, *E. coli* strains causing UTIs with mechanisms of resistance to beta-lactam antibiotics showed higher percentages of resistance to oral antibiotics, specially isolates with ESBLs compared with AMPc production, which makes difficult the choice of an adequate oral antimicrobial treatment in community UTIs.

#### **P1541** Revision of the guideline for empiric treatment of complicated urinary tract infections (c-UTI) using surveillance data on antimicrobial resistance, the Netherlands

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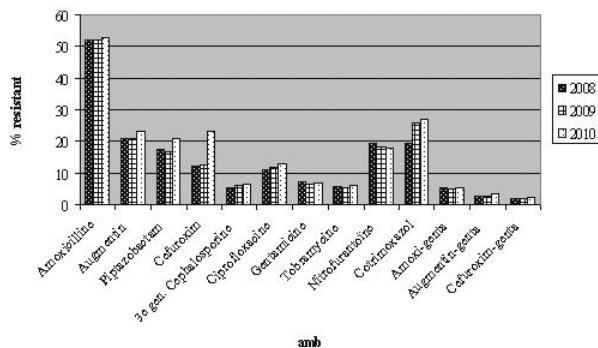
**Objectives:** Currently, the national guidelines for empiric treatment of c-UTI are being revised. Therefore we assessed the antimicrobial resistance rates in Enterobacteriaceae. The recommended empiric therapy for c-UTI was amoxicillin-gentamicin, a second or third generation cephalosporin, or ciprofloxacin.

**Methods:** The first urine isolate per patient per year (2008–2010) was selected from 23 hospitals reporting to the Dutch Infectious Diseases Surveillance Information System on Antimicrobial Resistance (ISIS-AR). c-UTI was defined as a urine-isolate from hospitalised patients. Based on the susceptibility pattern of all Enterobacteriaceae combined, it was determined to which degree the different recommended antibiotic regimens would cover the (combination of) bacteria isolated for 2010.

**Results:** A total of 32 785 isolates from 26 711 patients were reported to ISIS-AR in 2010. The most prevalent bacteria causing c-UTI were *E. coli* (45%), *Enterococcus* spp. (15%), *P. mirabilis* (8%), and *K. pneumoniae* (7%), and combinations between these groups. From 2008 to 2010, resistance to cefuroxim varied from 12% to 23% and to third generation cephalosporins from 6% to 7%, to ciprofloxacin from 11% to 13%, and to amoxicillin-gentamicin from 5% to 6% in Enterobacteriaceae. In the figure the resistance rates are depicted for the drug (combinations) tested. Resistance under 5% was found only for the combinations amoxicillin-clavulanic acid-gentamicin and cefuroxim-gentamicin. For the following combinations a significant increase in resistance rates was observed: amoxicillin-clavulanic acid, piptazobactam, cefuroxim, third generation cephalosporins, ciprofloxacin, and cotrimoxazol.

**Conclusion:** The recommendations of the SWAB guideline are greatly supported by our data, except for cefuroxim due resistance rates above 20%. Empirical use of 3 day generation cephalosporins (>5% R) and

resistance of Enterobacteriaceae, 2008-2010



ciprofloxacin (>10% R) should be dependent on the local resistance data and patient characteristics.

**P1542** Enteric carriage of antibiotic-resistant bacteria in care homes in Wales: preliminary results from the PAAD Study

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**Objectives:** Care home residents may play a significant role in the epidemiology of antibiotic resistance, particularly MDR Enterobacteriaceae. The PAAD Study (Probiotics for Antibiotic-Associated Diarrhoea) is examining the role of probiotics in Care Homes, and baseline stool samples are taken to assess the prevalence of faecal pathogens. We are analysing these samples to assess the prevalence of carriage of Gram negatives resistant to ciprofloxacin (CIP), cefotaxime (CTX), Ceftazidime (CAZ), gentamicin (GEN), meropenem (MER), and vancomycin-resistant enterococci (VRE) among Care Home residents.

**Methods:** Following consent/assent, baseline stool samples were obtained from 236 residents from 11 care homes in South East Wales. Stool was suspended in water and 0.05 mL was inoculated using a spiral plater. Agars including Blood agar, VRE agar, Chromogenic UTI agar (CHROM), and CHROM incorporating different selective agents (CIP (0.25 mg/L), CTX (2 mg/L), CAZ (2 mg/L), GEN (4 mg/L), MER (1 mg/L)) were incubated aerobically prior to counting of colonies.

**Results:** Summary results for Enterobacteriaceae carriage are shown in the table. 90.5% of CTX or CAZ resistant isolates were ESBL-producers, and no carbapenem-resistant Enterobacteriaceae were confirmed. VRE carriage was 1.3%, predominantly *E. faecalis*. Carriage of carbapenem resistant *Pseudomonas* spp. was 3.4%; no carbapenemase activity was detected.

Table: Enterobacteriaceae resistance in systematically obtained stool samples from care home residents

	Enterobacteriaceae		
	Number positive by screening agar	Confirmed resistance	% Resistance carriage
CIP	114	105	44.5
CTX	23	17	7.2
CAZ	23	17	7.2
GEN	26	18	7.6
MER	1	0	0

**Conclusions:** The carriage of resistant organisms is significant in this patient group, particularly for CIP-resistant Enterobacteriaceae. Carriage of 3rd generation cephalosporin-resistance is higher than

published resistance rates for urinary isolates in Wales (4.8%), and should be considered when selecting empiric therapy for these patients.

**P1543** A study of antimicrobial resistance of bacterial isolates from catheter-associated urinary tract infection in intensive care unit and medical wards of a tertiary care teaching hospital in Delhi, India

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**Objectives:** The study aimed to find the antimicrobial resistance of isolates from Catheter Associated Urinary Tract Infection (CAUTI), in Intensive Care Unit (ICU) and Medical Wards (MW) as per the CDC CAUTI guidelines.

**Methods:** One hundred and three unique bacterial isolates obtained from 200 adults with urethral catheters chosen by blocked randomization ensuring "Allocation Concealment" were included from MAMC and LN Hospital, Delhi, India, from August 2010 to September 2011. Standard disc-diffusion method and VITEK2 COMPACT® (BIOMERIEUX) were employed to determine susceptibility and Minimal Inhibitory Concentrations (MIC, expressed in micrograms per mL) for different antibacterials.

**Results:** Out of 103 bacterial isolates that fitted to the CDC CAUTI criteria, 13 were *Enterococcus* spp. and the rest were gram negative rods including *Escherichia coli* (42), *Klebsiella pneumoniae* (25), *Enterobacter* spp. (7), *Pseudomonas* spp. (5), *Acinetobacter baumannii* (3), *Proteus mirabilis* (2), *Providencia stuartii*, *Burkholderia cepacia*, *Serratia fonticola*, *Citrobacter freundii*, *Pantoea agglomerans* and *Strenotrophomonas maltophilia* (1 each). Upto 92.8% of all the Enterobacteriaceae isolates were resistant to ampicillin, ceftriaxone, cefepime, aztreonam, ampicillin + sulbactam and piperacillin + tazobactam. Prevalence of carbapenem resistance in *Klebsiella* spp. (13 out of 25) and *E. coli* (21 out of 42) were nearly identical (50%). Amikacin resistance for *Klebsiella* spp. and *E. coli* matched brilliantly with carbapenem resistance except for four strains of *E. coli*, where amikacin performed even better (in three strains MIC was 16, i.e. Sensitive and in one strain MIC was 32, i.e. Intermediate sensitive). Nearly 36% of the Enterobacteriaceae except *Proteus* and *Providencia* were sensitive to nitrofurantoin (MIC ≤ 32). Tigecycline sensitivity was seen in 98% of *E. coli*, 100% of Enterococci but only 56% of *Klebsiella*. Colistin and polymyxin-B were sensitive in 100% of gram negative isolates. Grimmet was the condition of ciprofloxacin with ≥95% of all the strains being resistant. Out of 13 *Enterococcus* isolates, seven were high-level gentamicin resistant, nine were vancomycin resistant and four were Linezolid resistant.

**Conclusion:** A comparatively low tigecycline prescription in the Medical wards (8.33%) as well as ICU (21%) was reflected in its overall low Resistance percentage. High prevalence of fluorquinolone, beta-lactam and carbapenem resistance among nosocomial isolates in the era of New Delhi Metallo-beta-lactamase is a serious cause of concern.

**P1544** *Staphylococcus aureus* susceptibility to vancomycin and alternative agents at a comprehensive cancer centre

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**Objectives:** To determine the current susceptibility of methicillin-susceptible and methicillin-resistant *S. aureus* isolates to vancomycin and alternative agents at a Comprehensive Cancer Center.

**Background:** *S. aureus* is a common pathogen in cancer patients, causing 20–30% of gram-positive infections. Vancomycin is recommended for empiric and target therapy of gram-positive infections in this setting by various guidelines (IDSA, NCCN, and ICHS). There have been multiple recent reports of vancomycin failures for *S. aureus* isolates with MICs of ≥1.0 µg/mL.

**Methods:** Retrospective review of microbiological records between Jan 1st – August 31st 2010 for identification of consecutive clinical *S. aureus* isolates and their susceptibility/resistance patterns. Susceptibility testing was performed in accordance with CLSI recommendations (M7-A7).

**Results:** Of the 689 *S. aureus* isolates, 383 (56%) were methicillin-resistant (MRSA). The anatomical sites of isolation are shown in Table 1. Of the 306 methicillin-susceptible isolates (MSSA) 193 (63%) had a vancomycin MIC of  $\geq 1.0$ . Similarly, of the 383 MRSA isolates 250 (65%) had a vancomycin MIC of  $\geq 1.0$   $\mu\text{g/mL}$ . All isolates were uniformly susceptible to daptomycin (MIC  $\leq 1.0$   $\mu\text{g/mL}$ ). The majority of MSSA isolates were susceptible to linezolid (99.5%), TMP-SMX (98.5%), tetracycline (92.3%), and rifampin (99%). For MRSA resistance rates ranged from 4% to 18% for these agents.

**Table 1. Vancomycin susceptibility of MSSA and MRSA**

Site	MSSA		MRSA	
	No.	MIC $\geq 1.0$ No. (%)	No.	MIC $\geq 1.0$ No. (%)
Blood	32	22 (69)	46	30 (65)
Urine	29	18 (62)	19	13 (68)
Sputum	88	50 (57)	121	80 (66)
Wound	152	100 (66)	168	108 (64)
Other	5	5 (100)	29	19 (65)
Total	306	193 (63)	383	250 (65)

**Conclusion:** Vancomycin may no longer be the preferred agent for *S. aureus* infections at our institution.

**P1545** **Antimicrobial resistance of *Staphylococcus aureus* isolates from primary care patients and nursing home residents in the Netherlands and Germany**

C. van der Donk\*, E. Stobberingh on behalf of the EurSafety Health-net EMR group

**Objectives:** We assessed the antibiotic resistance of *Staphylococcus aureus* isolated from primary care (GP) patients and nursing home (NH) residents in the Netherlands (NL) and Germany (G) in the Euregion Meuse-Rhine. High resistance rates could have implications on empiric treatment.

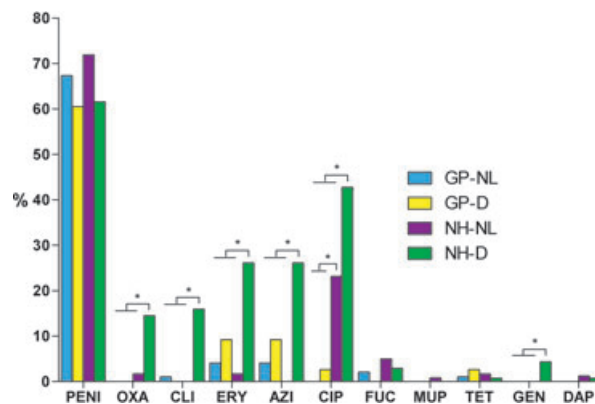
**Methods:** A total of 98 and 76 *S. aureus* strains were isolated from 315 and 200 nasal swabs from Dutch and German GP patients, respectively. From 356 and 343 nasal swabs from Dutch and German NH residents 121 and 138 isolates were collected. Quantitative susceptibility testing was performed with the micro broth dilution method according to EUCAST guidelines. Putative MRSA isolates were confirmed for the presence of *mecA* with PCR. Determination of the *spa* locus was performed on all MRSA isolates.

**Results:** Prevalence of *S. aureus* carriage was 32% among the Dutch groups and 26% and 39% among the German GP patients and NH residents, respectively.

Significant differences between the German NH isolates and the other groups of isolates were found for oxacillin ( $p < 0.001$ ), the macrolides ( $p < 0.001$ ), ciprofloxacin ( $p < 0.001$ ) and gentamicin ( $p < 0.005$ ) MRSA prevalence was 6% among the German NH residents ( $n = 19$ ), and is  $< 1\%$  in the other groups. Among the MRSA isolates from the German NHs three *spa* types were demonstrated i.e. t003, t032 and t151. The *spa* types for the Dutch isolates were t091 and t223.

Point of concern was the high resistance to ciprofloxacin among the Dutch (23%) ( $p < 0.001$ ) and German (43%;  $p < 0.001$ ) NH isolates compared with those from the GP (0% and 3%).

Overall resistance to fusidic acid, mupirocin, tetracycline, gentamicin and daptomycin was  $< 5\%$ , while resistance to linezolid, trimethoprim-sulfamethoxazole and vancomycin was not observed.



**Conclusions:** Resistance of *S. aureus* was relatively high among German NH isolates as was the prevalence of MRSA. This is very likely due to high antibiotic use and different infection control measures in Germany compared with the Netherlands. Also the resistance to ciprofloxacin was high among NH isolates both in the Netherlands and Germany. Reason for this might be the high use of fluoroquinolones in NHs compared with GP patients.

**P1546** ***Staphylococcus aureus* nasal colonisation in army recruits**

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**Objective:** To detect *Staphylococcus aureus* (and MRSA) nasal colonization in army recruits and investigate potential correlates for carriage of resistant strains.

**Methods:** Swabs obtained from both anterior nares of each individual were immediately streaked onto Chapman's agar and 5% sheep blood agar. Plates were incubated at 35°C for 48 hours. Species identification was performed by standard methods. Susceptibility profile to antimicrobials was determined by BD Phoenix™ Automated Microbiology System. Isolates resistant to oxacillin/cefoxitin were considered methicillin resistant (MRSA). Participants were less than a week in recruitment camp. Chi-square test was used to assess the correlation of staphylococcal carriage and MRSA isolation with variables such as personal and household demographic details, medical history, habits of living and recent use of medications and antibiotics (as collected by an anonymous questionnaire).  $p < 0.05$  was considered significant.

**Results:** Samples were taken from 151 recruits (mean age 23.7 years, SD 6.8) with mean BMI 26.7  $\text{kg/m}^2$ . *S. aureus* was cultured in 34/151 (22.5%). Carriage was only associated with use of exercise supplements ( $p = 0.045$ ). Oxacillin (OXA) resistance (cefoxitin also) was detected in 5/34 strains (14.7%). Resistance rates (%) to the rest of the antibiotics panel was: erythromycin 20.6-tetracycline (TET) 14.7-amoxicillin/clavulanate 14.7- clindamycin 11.8- chloramphenicol (CHL) 5.9-cotrimoxazole (COT) 3. No resistance was detected against glycopeptides, fluoroquinolones, rifampicin and mupirocin. The phenotypes for the five MRSA strains were: only OXA 1 strain- OXA/CHL 1 strain- OXA/FUS/TET/COT 3 strains. OXA-resistant strains came from recruits from different camps. Oxacillin resistance was associated with antibiotic consumption in the previous 3 months ( $p = 0.05$ ) and living with a person with a chronic disease ( $p = 0.05$ ).

**Conclusions:** *Staphylococcus aureus* carriage is prevalent among healthy young adults in the recruitment phase for military service. MRSA carriage rates were higher when compared to the recently published relevant rates from employees in Hellenic Air Force ( $\sim 1\%$ ), keeping in mind the different time of collection (2004–2005) and the age distribution (mean age 33 years) of the sampled population (Eurosurveillance 2008; 40(2)). Further prospective studies are



necessary in order to examine MRSA acquisition and transmission patterns in military settings (if present), with a view to elucidate preventive strategies.

**P1547** Influence of intensive cross-border traffic on antibiotic resistance and the population structure of *Staphylococcus aureus*

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**Objectives:** To study the influence of cross border traffic we evaluated both the antimicrobial resistance and population structure of *Staphylococcus aureus* isolated from general practice (GP) patients and nursing home (NH) residents in the province of Limburg (L) (near the borders with Germany and Belgium) in comparison with those obtained in the remaining part of the Netherlands (NL).

**Methods:** A total of 617 and 418 *S. aureus* isolates were isolated from 2691 and 1351 nasal swabs from GP patients and NH residents, respectively. Quantitative susceptibility testing was performed using a microbroth dilution. Putative MRSA isolates were tested for presence of *mecA* with PCR. Spa typing was performed on all isolates, multi locus sequence typing (MLST) on 2–4 isolates per spa-clonal complex (CC).

**Results:** The prevalence *S. aureus* among GP patients was 23% and 31% among NH residents ( $p < 0.001$ ). The isolates from the NH residents demonstrated a significantly lower resistance for larithromycin ( $p = 0.031$ ) and trimethoprim-sulfamethoxazole ( $p = 0.003$ ) in Limburg province compared with the Netherlands. No significant differences were demonstrated between the two groups of GP isolates. The 329 different spa types were clustered into 16 spa-CCs. Among isolates from NH residents in the Netherlands the prevalence of spa-CC 084 (6.92%) was significantly lower ( $p = 0.001$ ) and the prevalence of spa-CC 002 (17.30%) significantly higher ( $p = 0.002$ ) compared with isolates from NHs in Limburg province. There was also a difference in prevalence of spa-CC 024, which was higher among isolates from the NH compared with those from the GP.

The percentage of isolates with a spa type associated to a MRSA associated MLST CC was 71% of NH isolates in Limburg while this is 80% for NH isolates from the Netherlands ( $p = 0.065$ ). For the same groups of isolates there was also a difference in the percentages of isolates with a spa type associated with a MSSA MLST CC. This was 22% and 13% ( $p = 0.021$ ), respectively. The MLST CCs of the 40 isolates, on which MLST was performed, matched with the MLST CCs associated through the Ridom SpaServer.

**Conclusions:** From these data we can conclude that there were not many differences between isolates collected from Limburg and the remaining parts of the Netherlands regarding antibiotic resistance and population structure. We can also conclude that the location of the province of Limburg as a border region does not appear to be a risk factor.

**P1548** Characterisation of coagulase-negative *Staphylococcus* clinical isolates from a hospital in southern Ireland

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**Objectives:** Coagulase negative *Staphylococcus* (CoNS), particularly *Staphylococcus epidermidis* are the causative agents in the majority of nosocomial biofilm infections. Their presence and persistence can cause significant difficulties in terms of treatment owing to the high prevalence of such bacteria on human skin and mucous membranes. *S. epidermidis* has also the capacity to harbour the mobile genetic element SCCmec thereby facilitating extensive recombination and gene transfer between staphylococcal species including *S. aureus*. Until recently CoNS were deemed non-pathogenic and, when isolated in the lab, were thought to be present due to specimen contamination which has subsequently led to a noticeable paucity of related epidemiological

data and clinical studies. Our aim was to characterise a cohort of nosocomial CoNS isolates, particularly their biofilm forming capacity (the principal virulence factor), using standard phenotypic and genotypic methods.

**Methods/results:** Since January 2010, 157 CoNS isolates, originally isolated from blood were collected from a teaching hospital located in the south of Ireland. The study revealed 50% ( $n = 79$ ) of all CoNS isolates demonstrated a capacity to form a biofilm and of these 20% ( $n = 31$ ) were considered strong biofilm producers based on optical density measurements (OD at 570 nm of  $>0.240$ ). OD readings of 0.12 to 0.240 signified moderate biofilm formation and isolates with an OD of  $<0.12$  were recorded as biofilm negative. These findings were further substantiated by genotypic analysis of the isolates based on the detection of *icaA*, *icaD*, *aap* and *atlE* genes. Additionally, antibiotic resistance profiles for all isolates were ascertained and of the ten antibiotics examined particular resistance was observed for erythromycin (66%), cefoxitin (57%), fusidic acid (72%) and oxacillin (76%). The *mecA* gene responsible for methicillin/oxacillin resistance was present in 79% of the isolates.

**Conclusion:** Our study demonstrates the clinical significance of CoNS and their virulence potential as evidenced by the high incidence of biofilm formation and antibiotic resistance. It also supports the introduction of routine speciation and antibiotic resistance surveillance to provide up to date and comprehensive epidemiological data with a view to improving the management of CoNS nosocomial biofilm infections and the spread of antibiotic resistance.

**P1549** Morbidity and mortality of intensive care unit patients with vancomycin-resistant enterococcal colonisation at ICU admission

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**Objective:** The aim of this study was to determine the prevalence, the risk factors for vancomycin-resistant enterococcal (VRE) colonization at Intensive Care Unit (ICU) admission and the impact on the outcome of ICU patients.

**Methods:** A prospective study was carried out at the ICU of the University Hospital of Patras, Greece, from February 2009 to October 2011. All hospitalized patients had an initial rectal swab taken within 48 hours of admission. Epidemiologic data were collected from the ICU computerized database and patient chart reviews. Rectal swabs were inoculated into Brain-Heart Infusion Broth with colistin and caspofungin for 24 hours before subculture in chromogenic agar (ChromID VRE, bioMerieux). The presence of enterococci was established by Gram stain and Vitek 2 (bioMerieux) identification. Antibiotic susceptibility testing was performed by the disk diffusion method and Etest according to CLSI guidelines. The strains were tested for the presence of *vanA* and *vanB* genes by PCR. Clones were identified by PFGE of chromosomal SmaI DNA digests. Univariate statistical analysis was performed by SPSS ver. 17.0.

**Results:** From the 368 patients admitted, 58 (15.8%) were VRE colonized at admission. Fifty-five isolates were *E. faecium* (48 vanA-positive, seven vanB-positive) and three *E. faecalis* (one vanA-positive, two vanB-positives). One major clone was identified among *E. faecium* (19/39 strains) while the remaining isolates belonged to seven different clones. The variables studied and the results of the univariate analysis are shown in Table 1. Although no differences in the APACHE II Score at ICU admission between the two groups (VRE-positives and VRE-negatives) could be found, ICU mortality was significantly higher in the group of VRE colonized patients.

**Conclusion:** In the present study we could demonstrate that previous hospitalization, especially in ICUs, abdominal surgery, duration and the number of antibiotics administered are significantly correlated to VRE colonization. Furthermore, the number of chronic diseases and especially the insulin-dependent diabetes mellitus, chronic obstructive pulmonary disease, prior malignancies, cortisone administration and

Table 1: Univariate analysis comparing VRE-positive and negative patients admitted at a general ICU.

	VRE-NEGATIVES (n=309)	VRE-POSITIVES (n=59)	Significance (p)
FEMALE PATIENTS	88 (31.7%)	27 (45.8%)	*
MEAN AGE (years)	55.3 ± 19.5	60.9 ± 16.9	ns
BMI	27.4 ± 5.3	27.9 ± 7.2	ns
APACHE II SCORE	14.5 ± 7.2	16.7 ± 8.55	ns
PREVIOUS HOSPITALIZATION (days)	3.5 ± 9.5	30.8 ± 48.2	***
PREVIOUS ICU HOSPITALIZATION (days)	0.9 ± 6.4	12.3 ± 35.7	***
CO-MORBIDITIES			
-CHRONIC DISEASES (number) *	0.8 ± 0.9	1.44 ± 1.19	***
-INSULIN-DEPENDENT DIABETES MELLITUS	6 (1.9%)	10 (16.9%)	***
-CHRONIC OBSTRUCTIVE PULMONARY DISEASE	33 (10.7%)	18 (30.5%)	***
-HEMODYALYSIS	6 (1.9%)	6 (10.2%)	**
-MALIGNANCIES	36 (11.7%)	15 (25.4%)	**
-CORTISOL USE	18 (5.8%)	10 (16.9%)	**
DURATION OF ANTIBIOTICS ADMINISTERED (days)	3.1 ± 9.4	29.3 ± 43.4	***
NUMBER OF ANTIBIOTICS ADMINISTERED	1.1 ± 2.1	2.6 ± 3.1	***
CARBAPENEM ADMINISTRATION (days)	1.6 ± 7.3	17.32 ± 30	***
GLUCOPEPTIDE ADMINISTRATION (days)	1.8 ± 6.4	16.8 ± 25.6	***
QUINOLONE (days)	0.5 ± 2.3	7.4 ± 9.1	***
THIRD-GENERATION CEPHALOSPORIN (days)	0.8 ± 3.2	12.2 ± 9	ns
MORTALITY	76 (24.7%)	25 (42.3%)	***
ICU LENGTH OF STAY (days)	14.8 ± 14.9	15.6 ± 17.9	ns

Data are in numbers (N), ns = not significant, \* = p < 0.05, \*\* = p < 0.01, \*\*\* = p < 0.001

\*Chronic diseases: Congestive Heart Failure, Chronic Renal Failure, Hepatic Insufficiency, Obesity, Chronic Obstructive Pulmonary Disease, Diabetes Mellitus (insulin dependent or not dependent).

treatment with chronic haemodialysis were also found to be independent risk factors. However, VRE colonization at ICU admission seems to have an important influence on survival of ICU patients per se.

### P1550 Risk factors for colonisation with high-level aminoglycoside resistant *Enterococcus* species during hospitalisation in the intensive care unit

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**Objectives:** To determine the incidence and risk factors for colonization with high-level aminoglycoside resistant enterococci (HLARE) among patients hospitalized in the intensive care unit (ICU). **Methods:** This prospective study was carried out in the ICU of the Department of Infectious Diseases, University Medical Centre Ljubljana, Slovenia, from April 2004 through June 2005, in patients whose expected length of stay was at least 5 days. Colonization was investigated by performing surveillance samples every week during ICU stay. Patients with isolated high-level aminoglycoside *Enterococcus faecalis/faecium* from stool specimen or rectal swab during the hospital stay were considered as cases. All the other included patients were considered as controls. Demographics and known risk factors were retrieved and assessed by univariate and multivariate statistical methods.

**Results:** One hundred and nine patients were included: six were discharged or died before any repeat culture were obtained, 14 were colonized with HLARE at admission and of the rest 89 patients 46 (53%) acquired HLARE during their stay in the ICU. Eighty-one percent of HLARE isolates showed resistance to ampicillin and two were resistant to vancomycin. HLARE cases had longer mean duration of hospitalization than controls (39.4 day vs. 11.5 day;  $p < 0.0001$ ). In univariate analysis other risk factors were found to be urinary tract infection (UTI) ( $p = 0.03$ ), chronic kidney diseases ( $p = 0.03$ ), arterial line ( $p = 0.004$ ), days of arterial line ( $p = 0.005$ ), mechanical ventilation (MV) ( $p = 0.004$ ) and days of MV ( $p = 0.05$ ), total consumption of antibiotics in DDD ( $p = 0.03$ ), antibiotic days ( $p = 0.03$ ), ciprofloxacin use ( $p = 0.05$ ) and vasopressor treatment ( $p = 0.01$ ). Multivariate analysis showed that independent risk factors for acquisition of HLARE were UTI ( $p = 0.038$ ; odds ratio (OR) 27.6; 95% confidence interval (CI) 1.2–637.6) and days of antibiotic treatment ( $p = 0.017$ ; OR 1.3; CI 1–1.6). Arterial line (OR 3.5; CI 0.9–14.7) and days of mechanical ventilation (OR 1.12; CI 1–1.3) nearly approached the statistical significance ( $p = 0.082$  and  $p = 0.069$ , respectively) and were included as confounders.

**Conclusion:** The results suggest that UTI, MV, arterial line and antimicrobial use are important selective risk factors for HLARE colonization independent of other ICU and demographic characteristics. Prolongation of antibiotic treatment for 1 day increases the odds of colonization with HLARE for 30%. Further efforts to optimize antimicrobial use in high risk patients are proposed.

### P1551 Spectrum and potency of ceftaroline against leading pathogens causing skin and skin-structure infections in Europe and South Africa, 2010

R. Flamm\*, D. Farrell, H. Sader, R. Jones (North Liberty, US)

**Objective:** To determine the spectrum and potency of ceftaroline (CPT) against recent (2010) leading pathogens causing complicated skin and skin structure infections (cSSSI) isolated in Europe and South Africa (SAF). CPT, the active metabolite of the prodrug ceftaroline fosamil, is a novel cephalosporin exhibiting broad-spectrum in vitro bactericidal activity against Gram-positive organisms, including *Streptococcus pneumoniae* and methicillin-susceptible (MS) and -resistant (MR) *Staphylococcus aureus* (SA), as well as common Gram-negative organisms.

**Methods:** A total of 2943 isolates from the 2010 Assessing Worldwide Antimicrobial Resistance Evaluation (AWARE) Programme were identified as cSSSI pathogens by the infection type and/or specimen type recorded by the submitter. Isolates were collected from patients in 54 medical centres in 19 European countries (including Israel and Turkey) and in SAF (54 isolates, one medical centre) during 2010. Susceptibility testing for CPT and commonly used antimicrobials was performed by CLSI broth microdilution methodology. Susceptibility interpretations for the comparators were as published in CLSI and EUCAST guidelines. Extended spectrum beta-lactamase (ESBL) phenotype was determined as per CLSI guidelines.

**Results:** The potencies of CPT against the leading pathogens isolated are shown in the Table. CPT was very active overall against SA (MIC<sub>50/90</sub>, 0.25/1 mg/L) and inhibited 100.0% of all isolates at a MIC ≤ 2 mg/L (see Table). Activity against MRSA was good (MIC<sub>50/90</sub>, 1/2 mg/L) but lower than observed against MSSA (MIC<sub>50/90</sub>, 0.25/0.25 mg/L). CPT was also very active against 466 beta-haemolytic streptococci (BHS), including 198 *S. pyogenes* and 142 *S. agalactiae*, and 94 viridans group streptococci (VGS). CPT was very active against *Escherichia coli* (EC) and *Klebsiella pneumoniae* (KPN) not expressing ESBLs but, similar to other extended spectrum beta-lactam agents (ceftriaxone, cefepime and ceftazidime), was not active against the majority of EC and KPN demonstrating an ESBL phenotype.

Organism (n)	MIC (mg/L)										MIC <sub>50</sub>	MIC <sub>90</sub>	
	≤0.06	0.12	0.25	0.5	1	2	4	8	16	≥32			
<i>S. aureus</i> (1402)	4	164	987	168	141	41						0.25	1
MRSA (839)			13	144	141	41						1	2
MSSA (1116)	4	164	524	24								0.25	0.25
BHS (466)												≤0.008	0.015
VGS (94)	88	1	4	1								0.03	0.06
EC-ESBL (102)	2	1	2	1	0	1	3	3	89			>32	>32
EC-non-ESBL (232)	97	68	24	25	15	3	2	2	2			0.12	0.5
KPN-ESBL (65)	2	0	0	1	1	1	1	1	55			>32	>32
KPN-non-ESBL (85)	43	20	7	5	3	5	1	1	1			0.06	1

**Conclusions:** This study demonstrated the potent in vitro activity of CPT against the great majority of recent (2010) pathogens, including MRSA strains, isolated from patients with documented cSSSI from Europe and SAF. These data suggest that ceftaroline fosamil could emerge as an important therapeutic alternative for cSSSI occurring in Europe and SAF.

### P1552 Ceftaroline activity against clinical isolates from United States Hospitals: results from the 2011 Assessing Worldwide Antimicrobial Resistance Evaluation programme

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**Objective:** To evaluate the activity of ceftaroline (CPT) tested against prevalent Gram-positive and -negative species isolated in USA hospitals (2011). CPT, the active form of CPT fosamil, is a new, parenteral, broad-spectrum cephalosporin exhibiting in vitro bactericidal activity against Gram-positive organisms, including MRSA and multidrug-resistant (R) *Streptococcus pneumoniae* (SPN),

as well as common Gram-negative pathogens. CPT is approved in the USA for treatment of acute bacterial skin and skin structure infection (ABSSSI) and community-acquired bacterial pneumonia (CABP).

**Methods:** Five thousand six hundred and thirty-five consecutive, nonduplicate isolates from bloodstream, ABSSSI, and respiratory tract infections were collected from 52 medical centres and tested for susceptibility (S) to CPT and comparator agents at a central laboratory using the reference CLSI broth microdilution method. CLSI and EUCAST breakpoint criteria were used to determine S/R rates for comparator agents. USA-FDA interpretive criteria were used for CPT. **Results:** CPT inhibited all *S. aureus* strains (49.3% MRSA) at  $\leq 2$  mg/L and 98.8% of MRSA were S to CPT (Table). CPT was 8- to 16-fold more active than ceftriaxone (CRO; MIC<sub>50/90</sub>, 4/4 mg/L) against MSSA. CPT inhibited all tested SPN at  $\leq 0.5$  mg/L and remained active against penicillin-R and CRO-non-S SPN (MIC<sub>90</sub>, 0.25 mg/L for both subsets; see Table). The highest CPT MIC value among beta-haemolytic streptococci was only 0.03 mg/L. CPT activity against coagulase-negative staphylococci (CoNS; 61.6% methicillin-R) was similar to that against *S. aureus*. CPT showed only moderate activity against *E. faecalis* (MIC<sub>50/90</sub>, 2/8 mg/L). *Haemophilus influenzae* (MIC<sub>90</sub>, 0.03 mg/L; 27.2% beta-lactamase [BL] producers), *H. parainfluenzae* (MIC<sub>90</sub>, 0.12 mg/L) and *Moraxella catarrhalis* (MIC<sub>90</sub>, 0.12 mg/L) were highly CPT-S. CPT activity against the most frequently isolated Enterobacteriaceae species (MIC<sub>50</sub>, 0.12–0.25 mg/L) was similar to that of CRO (MIC<sub>50</sub>,  $\leq 0.06$ –0.25 mg/L) and ceftazidime (MIC<sub>50</sub>, 0.12–0.25 mg/L). Extended-spectrum BL (ESBL) phenotype was observed in 9.9% of *E. coli* and 12.4% of *Klebsiella* spp., and all cephalosporins tested showed limited activity against ESBL-producing strains.

Organism (no. tested)	No. of isolates (cumulative %) inhibited at ceftaroline MIC (mg/L) of:							
	$\leq 0.03$	0.06	0.12	0.25	0.5	1	2	9
<i>S. aureus</i> (1496)	0(0.0)	4(0.3)	124(8.6)	629(50.6)	500(84.0)	230(99.4)	9(100.0)	-
MSSA (738)	0(0.0)	4(0.5)	121(16.5)	611(97.1)	22(100.0)	-	-	-
MRSA (738)	0(0.0)	0(0.0)	3(0.4)	18(2.9)	478(67.6)	230(99.8)	9(100.0)	-
<i>S. pneumoniae</i> (956)	669(70.0)	69(7.2)	131(90.9)	841(99.4)	6(100.0)	-	-	-
Penicillin-R (222)	0(0.0)	12(5.4)	123(80.8)	81(21.6)	6(100.0)	-	-	-
Ceftriaxone-non-S (124)	2(1.6)	0(1.6)	39(32.3)	118(95.1)	6(100.0)	-	-	-
$\beta$ -haemolytic strep. (360)	360(100.0)	-	-	-	-	-	-	-
CoNS (172)	1(0.6)	49(29.1)	26(44.2)	56(76.7)	36(96.7)	3(99.4)	1(100.0)	-
<i>E. faecalis</i> (132)	0(0.0)	0(0.0)	0(0.0)	2(1.5)	5(5.3)	26(25.0)	65(74.2)	-
Viridans group strep. (63)	57(90.5)	5(9.4)	1(100.0)	-	-	-	-	-
<i>H. influenzae</i> (389)	367(94.3)	16(98.5)	4(99.5)	1(99.7)	1(100.0)	-	-	-
$\beta$ -lactamase-neg. (283)	281(99.3)	2(100.0)	-	-	-	-	-	-
$\beta$ -lactamase-pos. (106)	86(81.1)	14(94.3)	4(98.1)	1(99.1)	1(100.0)	-	-	-
<i>H. parainfluenzae</i> (56)	50(89.3)	0(99.3)	3(94.6)	1(96.4)	0(96.4)	2(100.0)	-	-
<i>M. catarrhalis</i> (63)	23(36.5)	18(65.1)	16(90.5)	5(98.4)	1(100.0)	-	-	-

**Conclusions:** CPT demonstrated enhanced activity against staphylococci, including MRSA, various streptococcal groups, and *H. influenzae* strains recently isolated from USA hospitals. CPT activity against Enterobacteriaceae was similar to that of currently marketed broad-spectrum cephalosporins.

#### P1553 The CDC definition of nosocomial infections revised: a prospective study with 1694 patients

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**Introduction:** The Centers for Disease Control and Prevention (CDC) defines a nosocomial infection (NI) as an infectious event that is diagnosed >48 hours after admission without evidence that the pathogen was already in the incubation phase. This definition will overestimate the true NI rate, since not all infections acquired outside the hospital are identified on admission. We prospectively studied the gap between NIs based on CDC definition compared to a predefined, published clinical definition that defines true NIs.

**Methods:** The study was performed at the University Hospital of Basel, a tertiary care center in Switzerland. All data were recorded in a standardized database, and yearly checked for consistency. Since 2002, all patients that were put on contact isolation were prospectively assessed if they acquired the pathogen in the hospital (=true NI) or detection has been delayed for other reasons to >48 hours

and were classified as CDC-NI. True NI was defined as a patient with an epidemiological link to an index patient, phenotypic identical species and identical molecular typing by pulsed-field electrophoresis. The CDC-NI was reclassified to non-NI, if the patient had no exposure to a patient colonized or infected with the same pathogen, and there was no match by molecular typing.

**Results:** Overall, 1694 patients were surveyed from January 1, 2002 until September 28, 2011: 742 had MRSA, 18 VRE, 865 ESBL, and 69 other multidrug resistant gram-negative bacteria other than ESBL. Out of the 1694 patients 306 (18.06%) were classified as CDC-NI and 163 (9.6%) as true NI. On average, only 53.3% of all CDC-NI fulfilled the definition of true NI (Table 1).

Table 1: Proportion of patients with CDC-NI and true NI

Microorganism	Number of patients	CDC-NI	True NI	Proportion true NI/CDC-NI
MRSA	742	109 (14.7%)	67 (9.0%)	61.5%
VRE van A/B	18	6 (33.3%)	5 (27.8%)	83.3%
ESBL	865	167 (19.3%)	72 (8.3%)	43.1%
other MDR	69	24 (34.8%)	19 (27.5%)	79.2%
<b>Total</b>	<b>1694</b>	<b>306 (18.06%)</b>	<b>163 (9.6%)</b>	<b>53.3%</b>

MRSA: Methicillin-resistant *Staphylococcus aureus*, VRE: Vancomycin-resistant enterococci, ESBL: Extended-spectrum betalactamase, other MDR: Multidrug-resistant gram-negative bacteria other than ESBL.

**Discussion:** The limitation to resistant pathogens in this study allowed a clear discrimination between true-NI and CDC-NI, a distinction difficult to assess in common NIs with patients not requiring contact isolation. Approximately half of the patients classified as NI by CDC really are true NIs. This is particularly important in countries who work with the reimbursement system DRG (Diagnosis Related Groups). Insurance companies are not necessarily obliged to pay for treatment of NI, bringing hospitals in precarious financial situations, however, only transmissions within the hospital can be prevented by an infection control program.

#### P1554 Resistance of *Porphyromonas gingivalis* and *Prevotella intermedia* isolated from periodontitis infections in the north of Portugal – myth or reality?

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**Objectives:** Our goal was to identify porphyromonas and prevotella species from periodontal pockets of Portuguese adults suffering from periodontitis infections and test their beta-lactamase production. Strains susceptibility to five antibiotics commonly prescribed in odontology in Portugal was studied.

**Methods:** Forty-three isolates of black-pigmented gram negative strict anaerobes rods were identified by the Rapid ID 32 A (bioMérieux, France) and confirmed by PCR analysis for *Porphyromonas gingivalis* and *Prevotella intermedia*. Beta-lactamase production was assayed by nitrocefin dryslide (BBL, EUA) and confirmed by disk diffusion synergy with amoxicillin/clavulanic acid. Strains antibiotic susceptibility was performed with amoxicillin, amoxicillin/clavulanic acid, metronidazole, clindamycin and tetracycline impregnated disks (Oxoid) by the disk diffusion method.

**Results:** *Prevotella intermedia* and *Porphyromonas gingivalis* represented respectively 44% and 20% of total isolates, with good correlation between employed methods. The remaining 36% strains belonged to other black-pigmented species.

Results from the disk diffusion method were confirmed by nitrocefin disks and no beta-lactamase production was detected in these strains. Isolated strains showed susceptibility to all tested antibiotics.

Only 2% of prevotella sp. (that were not *Prevotella intermedia*) showed beta-lactamase production with resistance to amoxicillin and susceptibility to amoxicillin/clavulanic acid.

**Conclusion:** The most frequently isolated anaerobic species from periodontal pockets was *Prevotella intermedia*. All *P. intermedia* and *P. gingivalis* isolates were susceptible to tested antibiotics. A low number of beta-lactamase producing strains was detected.

These results are not in accordance with most published studies that state high levels of resistance among anaerobes.

Further studies will be required where antibiotic resistance genes will be screened.

The success of antibiotic therapy in the oral cavity is dependent of the studies of antimicrobial susceptibilities, in order to optimize therapeutic decisions of most odontogenic local infections.

## Streptococcus pneumoniae – seroprevalence and antimicrobial resistance

**P1555** Distribution of serotypes and antimicrobial susceptibility of *Streptococcus pneumoniae* strains causing invasive disease in Madrid in patients 50 years and older

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**Objectives:** Recently the 13-valent pneumococcal conjugate vaccine (PCV-13) has been approved for adults >50 years old in Europe. The aim of this study was to analyze the distribution and the antimicrobial susceptibility of *Streptococcus pneumoniae* serotypes causing invasive pneumococcal disease in patients >50 years old over a 4-year period in Madrid.

**Methods:** From July 2007 to June 2011 1144 strains from patients >50 years old isolated in usually sterile clinical samples were studied. Serotyping was performed by Pneumotest-Latex and Quellung reaction (Statens Serum Institut, Copenhagen, Denmark). Susceptibilities to penicillin, erythromycin and levofloxacin were determined by E-test (AB biomérieux, Solna Sweden) according to the CLSI 2008 breakpoints.

**Results:** Twenty-nine serotypes accounted for 92.3% of the isolates: 3 (13.5%), 19A (12.3%), 7F (7.2%), 8 (6.7%), 1 (5.5%), 14 (4.5%), 22F (3.8%), 11A (3.6%), 6C (3.6%), 12F (3.3%), 31 (2.4%), 5 (2.3%), 9V (2.2%), 35B (2.1%), 4 (2%), 16F (2%), 19F (1.7%), 15A (1.6%), 9N (1.5%), 10A (1.3%), 23F (1.3%), 23B (1.1%), 25A (1.1%), 15B (1%), 24F (1%), 6B (1%), 6A (0.9%), 18C (0.8%) and 23A (0.8%). Overall, the PCV-13 serotype coverage was 55.2%.

One point two percent of the strains showed intermediate susceptibility to penicillin (no resistance to penicillin was detected). All penicillin non susceptible strains belonged to four serotypes: 14 (57.1%), 19A (28.6%), 9V (7.1%) and 6B (7.1%). The erythromycin resistance was 27.2%. The resistant serotypes were: 19A (32.2%), 8 (11.3%), 14 (7.4%), 11A (6.1%), 15A (5.1%), 19F (4.5%), 6C (3.9%), 6B (2.9%), 9V (2.6%), 15B (2.6%), 23F (2.3%), 24F (2.3%), 3 (1.9%), 35B (1.3%), 6A (1.3%), 7F (1.3%) and 4 (1%). Three point seven percent of all strains showed resistance to levofloxacin. Levofloxacin resistant strains belonged to 8 serotypes: 8 (68.4%), 14 (7.9%), 9V (7.9%), 7F (5.3%), 15A (2.6%), 19A (2.6%), 19F (2.6%) and 6B (2.6%).

**Conclusion:** The PCV-13 serotype coverage in this age group was higher than 50%. The proportion of strains with intermediate susceptibility to penicillin was limited to four serotypes covered by the PCV-13. More than half of erythromycin resistant strains were covered by the PCV-13. Levofloxacin resistance was mainly due to not PCV-13 covered serotype 8. The use of the recent approved PCV-13 could change the distribution of serotypes in this age group. As antimicrobial susceptibility is serotype dependent, the resistance pattern in adults could also be modified.

**P1556** Epidemiological and microbiological characteristics of invasive *S. pneumoniae* infection in the south of Galicia, Spain, before and after the introduction of seven-valent pneumococcal conjugate vaccine

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**Objectives:** Serotype 19A invasive pneumococcal disease (IPD) has increased after the introduction of 7-valent pneumococcal conjugate vaccine (7-PCV) in 2001. This has been related to a clonal expansion of multidrug-resistant strains. The aims of this study were to determine the prevalence of the IPD before (1992–2001, identified as pre-7-PCV) and after (2002–2008, identified as post-7-PCV) the introduction of 7-PCV and to identify the clonal expansion and antibiotic susceptibility of *S. pneumoniae* serotype 19A.

**Methods:** A total of 249 *S. pneumoniae* strains were isolated from IPD (blood, CSF, and pleural fluid) between 1992 and 2008. Serotype was determined in 124 randomized strains (41 strains from pre-7-PCV period and 83 from post-7-PCV period). Penicillin (PE), cefotaxime (CT), erythromycin (EM), clindamycin (CM) and levofloxacin (LE) susceptibility were established following CLSI 2009 break points. Presence of ermB or mefA and mutations in parC or gyrA were detected by PCR. Reserpine was employed to detect the presence of efflux pumps. BOX-A PCR was used to study the clonal relationship of the strains.

**Results:** The prevalence of vaccine serotype (4, 6B, 9V, 14, 18C, 19F, or 23F) before and after the introduction of 7-PCV was 44% and 40%, respectively ( $p = 0.7$ ). The more common serotypes in pre-7-PCV period were 19F (17%), 3 (12%), 6B (10%), 1 (7%), 8 (7%), 14 (7%), 19A (7%) and in the post-7-PCV period 19A (15%), 3 (12%), 9V (12%), 14 (11%), 7F (7%). Serotypes 14, 19F and 9V were more likely to be PE non-susceptible (92%, 88%, and 75%, respectively). All the 15 *S. pneumoniae* serotype 19A were isolated from blood. The MICs were, two strains PE = 4 µg/mL (13%), none PE ≥ 8µg/mL, 1 (7%) CT = 2 µg/mL, 9 (60%) EM ≥ 1 µg/mL (one isolate was mefA positive and eight isolates were ermB positive), eight CM ≥ 0.5 µg/mL (six strains CM ≥ 1 µg/mL). Two strains were PE and EM non-susceptible (13%). All serotype 19A pneumococci were susceptible to LE, however two strains showed a MIC > 1/≤2 µg/mL. Mutations in parC, gyrA or efflux pumps were absent. Only two strains belong to the same clonal type by BOX-A PCR.

**Conclusions:** Serotype 19A *S. pneumoniae* has become the most prevalent in the post-7-PCV period, but there was not a statistically significant increase in relation to the pre-7-PCV period. Most of the strains were EM resistant; the majority of them were susceptible to PE and CT and all of them fully susceptible to LE. Clonal expansion of serotype 19A was not observed.

**P1557** Serotypes and antimicrobial susceptibilities of *Streptococcus pneumoniae* associated with carriage and non-invasive infection amongst a paediatric population

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**Objective:** The pneumococcal conjugate vaccine 13 (PCV13) was introduced in Ireland in December 2010. The objective of the study was to analyze serotypes and antimicrobial susceptibilities of *Streptococcus pneumoniae* isolates associated with carriage and non-invasive infection amongst a paediatric population around the time the PCV13 was introduced.

**Methods:** Two hundred and fifty-two *S. pneumoniae* isolates which had been obtained from routine specimens from various sites (nasal discharge (n = 82), lower respiratory tract (n = 56), eye (n = 54), ear

(n = 26), throat (n = 19) and other sites (n = 15)), from 1 January 2009 to 31 August 2011, were retrieved from the clinical microbiology laboratory at the Children's University Hospital, Dublin. The isolates were serotyped using multiplex PCR and serological co-agglutination. Susceptibilities to penicillin, tetracycline, erythromycin, clindamycin and levofloxacin were determined using the Etest method and interpreted according to CLSI guidelines.

**Results:** Thirty-seven different serotypes were identified. The commonest serotypes were 6B (n = 29), 19A (n = 27), 6A (n = 20), 11A (n = 20) and 19F (n = 18). Overall potential PCV13 vaccine coverage was 52%. The most common serotype varied amongst anatomical sites. Serotypes 11A (n = 11), 6B (n = 8) and 23F (n = 7) were common amongst nasal isolates. Potential PCV13 coverage amongst nasal isolates was 42.6%. Serotypes 6B (n = 14), 19F (n = 6) and 3 (n = 4) were associated with lower respiratory tract infections. Potential PCV13 coverage amongst this group was 60.7%. Serotypes associated with conjunctivitis were mostly non-typeable (n = 9), 6B (n = 6) and 11A (n = 6). Potential PCV13 coverage amongst this group was 40.7%. Serotypes 19A (n = 8), 6A (n = 4) and 3 (n = 2) were associated with otitis media. Potential PCV13 coverage was 73%. Regarding throat isolates, serotypes 19F, 6A, 31, 3, 19A were equally distributed at n = 2. Twenty-five percent of isolates displayed resistance to at least one antimicrobial, 85% of which were serotypes contained in PCV13.

**Conclusions:** PCV13 serotypes accounted for the majority of paediatric pneumococcal carriage and non-invasive infection isolates. Antimicrobial resistance was mostly associated with PCV13 serotypes. It is likely that continued use of PCV13 will impact positively on antimicrobial resistance amongst circulating pneumococci but further on-going surveillance is required.

#### **P1558** Prevalence of *Streptococcus pneumoniae* in respiratory samples from patients with tracheostomy in a long-term care facility

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**Objectives:** The study objectives were to: (i) study the prevalence of *Streptococcus pneumoniae* in institutionalized patients with chronic tracheostomy; (ii) compare pneumococcal carriage in nasopharyngeal (NP) vs. endotracheal (ET) cultures; and (iii) assess of demographic and microbiologic effects on isolation site.

**Methods:** The study was conducted as part of an infection control intervention in a long-term care institution. Three point-prevalence surveys of *S. pneumoniae* carriage were conducted in January 2009, January 2010 and June 2011. Both NP swabs and ET aspirates were obtained from patients with chronic tracheostomy. Bacterial identification and antimicrobial susceptibility testing were done using Vitek-2 system. *S. pneumoniae* isolates were characterized by serotyping. The rates of NP vs. ET-derived positive cultures were compared using McNemar's test and correlations between categorical variables were analyzed using Chi-square test.

**Results:** Two hundred and sixty-four pairs of NP and ET cultures were taken from 188 patients. Thirty patients were <18 years old. Fourteen (5%) and 45 (17%) of NP and ET cultures, respectively were positive (p < 0.001). ET positivity rate was higher than NP in all three surveys. Overall proportion of positive cultures in children (≤years old) vs. adults did not differ significantly (21% vs. 18%, respectively, p > 0.05). However, ET positivity rate was higher than that of NP cultures in adults (18% vs. 3%, respectively, p < 0.001), but not in children (17% vs. 17%, respectively). Serotype 23F was present in a higher proportion in ET-derived isolates (42%) than in NP-derived isolates (21%), whereas serotype 15A was more common in NP-derived isolates (21%) than in ET-derived isolates (9%, p < 0.05). Serotypes 17F and 19F were similarly prevalent in both groups of isolates. Serotypes 19F and 23F were more common in adults vs. children (72% vs. 30%, respectively), whereas serotypes 15A and 17F were more common

among children (70% vs. 12%, respectively, p < 0.01). The majority of isolates were non-susceptible to penicillin (72%). Ofloxacin non-susceptibility was high and more common in serotypes 19F (92%) and 23F (100%), compared with other serotypes (14%, p < 0.001).

**Conclusion:** In the studied institution, among patients with a chronic tracheostomy, *S. pneumoniae* was present in a higher proportion in ET than NP in adults, whereas the yield from both sites was similar in children.

#### **P1559** A population snapshot of *Streptococcus pneumoniae* serotype 19A causing invasive disease in Ireland

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**Objectives:** Serotype 19A is a common cause of invasive pneumococcal disease (IPD) in Ireland. The purpose of the study was to characterise serotype 19A isolates causing invasive pneumococcal disease (IPD) in Ireland.

**Methods:** *S. pneumoniae* isolates from blood and CSF were serotyped using multiplex PCR and slide co-agglutination. Antimicrobial susceptibility was assessed using the Etest method. Reduced susceptibility to penicillin was defined as MIC ≥ 0.12 mg/L. Multi-locus sequence typing (MLST) was performed using standard procedures and clonal complexes were assigned using the eBURST algorithm.

**Results:** A total of 74 serotype 19A isolates caused IPD from April 2007 to December 2010. Twenty two different sequence types (STs) were identified, of which, two were novel. ST2081 and ST199 (Netherlands15B-37) were the most common clones and accounted for 21% and 17% of isolates, respectively. Following eBURST analysis the major clonal complex (CC) identified was CC199, which comprised of ST199, ST2081, ST667, ST645 and ST419; 47% of isolates clustered within this complex. All but one CC199 isolates were susceptible to penicillin. All other STs within the data set grouped as singletons. Reduced susceptibility to penicillin was observed in 30% of isolates, and was associated with nine STs. Amongst these, the commonly multi-drug resistant ST320 (DLV Taiwan19F-14) demonstrated the highest levels of penicillin MICs. Six ST63 (Sweden15A-25) isolates were identified, the first of which occurred in late 2009.

**Conclusions:** A diverse range of serotype 19A clones caused IPD in Ireland. However, nearly half of the 19A isolates clustered within one clonal complex, namely, CC199. In addition, reduced susceptibility to penicillin was commonly associated with particular clones.

#### **P1560** In vitro antimicrobial activity against *Streptococcus pneumoniae* isolates from middle ear fluid from children: pre- vs. post- introduction of the seven-valent conjugate pneumococcal vaccine in Spain

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**Objectives:** To determine in vitro activity against otical *S. pneumoniae* isolates from children collected in 2000–2001 vs. 2010–2011 in Spain (pre- vs. post- introduction of the 7-valent pneumococcal conjugate vaccine -PCV7-).

**Methods:** Isolates from middle ear fluid from children received in the Spanish Reference Laboratory for Pneumococci in the periods May 2010–May 2011 and May 2000–May 2001 were tested. Susceptibility was determined by agar dilution and isolates were serotyped by Quellung reaction and/or dot blot assay.

**Results:** A total of 330 isolates were tested. Table shows MIC<sub>50</sub>/MIC<sub>90</sub> values (mg/L) for total population and for serotypes with >30 isolates (10% of total).

PCV7 = serotypes included in PCV7 (4, 6B, 9V, 14, 18C, 19F and 23F); PEN = penicillin; AMX = amoxicillin; CFX = cefuroxime; CDN = cefditoren; CTX = cefotaxime; ERY = erythromycin.

According to CLSI breakpoints, percentages of non-susceptibility (pre-/ post- PCV7) were: 59.2/61.9 for PEN (oral), 10.5/23.7 for AMX, 46.1/ 51.1 for CFX, 3.7/21.6 for CTX, and 49.2/55.4 for ERY.

While similar susceptibility rates to penicillin (oral) were found in both periods in total population, rates highly increased among PCV7 serotypes (from 17.7% to 30.0%) but highly decreased among 19A isolates (from 66.7% to 6.1%). Serotype 3 was fully susceptible to beta-lactams and erythromycin.

There are not current CLSI breakpoints for CDN.

n	Pre-PCV7				Post-PCV7			
	Total	PCV7	19A	3	Total	PCV7	19A	3
PEN	0.25/2	1/2	0.03/0.5	0.015/0.01	0.5/2	0.5/2	2/4	0.015/0.01
AMX	0.5/4	1/4	0.06/0.5	0.06/0.06	0.5/4	0.25/2	2/4	0.06/0.06
CFX	1/8	4/8	0.06/4	0.03/0.06	2/8	0.5/8	8/16	0.03/0.03
CDN	0.12/0.5	0.25/0.5	0.015/0.5	0.015/0.03	0.25/1	0.12/1	0.5/1	0.015/0.01
CTX	0.25/1	0.5/1	0.03/0.5	0.015/0.03	0.5/2	0.5/1	1/2	0.015/0.01
ERY	0.25/12	128/12	128/128	0.12/0.12	128/12	0.12/12	128/12	0.12/0.12

**Conclusions:** Among isolates from middle ear fluid from children, PCV7 serotypes decreased from 64.9% (124/191) to 7.2% (10/139), with an increase in serotype 19A from 9.4% (18/191) to 47.5% (66/139) strongly linked to a reduced susceptibility in this serotype. CDN exhibited the highest intrinsic activity in terms of MIC50/MIC90 values, with the lowest increase, among all betalactams tested, in MIC90 for serotype 19A isolates from the post-PCV7 period.

**P1561** **In vitro antimicrobial activity against non-invasive *Streptococcus pneumoniae* isolates from adults pre- and post- introduction of the seven-valent conjugate pneumococcal vaccine in Spain**

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**Objectives:** To determine in vitro activity against non-invasive *S. pneumoniae* isolates from adults collected in 2000–2001 vs. 2010–2011 in Spain (pre- vs. post- introduction of the 7-valent pneumococcal conjugate vaccine -PCV7-).

**Methods:** Lower respiratory tract isolates (sputum or bronchoalveolar lavage) received in the Spanish Reference Laboratory for Pneumococci in the periods May 2010–May 2011 and May 2000–May 2001 were tested. Susceptibility was determined by agar dilution and isolates were serotyped by Quellung reaction and/or dot blot assay.

**Results:** A total of 1274 isolates were tested. Table shows MIC50/MIC90 values (mg/L) for total population and for serotypes with >50 isolates (4% of total population).

PCV7 = serotypes included in PCV7 (4, 6B, 9V, 14, 18C, 19F and 23F); NT = non-typeable; PEN = penicillin; AMX = amoxicillin; CFX = cefuroxime; CDN = cefditoren; CTX = cefotaxime; LVX = levofloxacin.

According to CLSI breakpoints, percentages of non-susceptibility (pre-/ post- PCV7) were: 54.1/ 36.7 for PEN (oral), 12.3/8.9 for AMX, 41.0/ 21.2 for CFX, 6.1/4.5 for CTX, 38.7/32.6 for erythromycin, and 4.8/5.6 for LVX.

While similar susceptibility rates to penicillin (oral) were found in both periods in total population, rates highly increased among PCV7

n	Pre-PCV7				Post-PCV7			
	Total	PCV7	NT	19A	Total	PCV7	NT	19A
PEN	0.12/2	1/2	0.5/2	0.03/0.12	0.015/2	0.5/2	0.25/2	1/4
AMX	0.12/4	2/8	1/4	0.06/0.12	0.06/2	0.5/4	0.25/2	0.5/4
CFX	0.5/8	4/8	1/8	0.06/0.25	0.03/4	2/8	1/4	4/16
CDN	0.06/0.5	0.25/0.5	0.12/0.5	0.015/0.03	0.015/0.5	0.25/1	0.12/0.25	0.5/1
CTX	0.12/1	1/2	0.5/1	0.03/0.06	0.015/1	0.5/2	0.25/0.5	1/2
LVX	1/1	1/1	1/1	1/1	1/1	1/16	1/1	1/1

serotypes (from 8.2% to 31.0%) and NT (from 16.9% to 29.4%), but highly decreased among 19A isolates (from 81.8% to 28.6%).

There are not current CLSI breakpoints for CDN.

**Conclusions:** Among non-invasive lower respiratory tract isolates from adults, PCV7 serotypes decreased from 43.2% (281/650) to 13.9% (87/624), with an increase in serotype 19A from 1.7% (11/650) to 6.7% (42/624) strongly linked to a reduced susceptibility in this serotype. CDN exhibited the highest intrinsic activity in terms of MIC50/MIC90 values against isolates collected in both study periods and for all serotypes.

**P1562** **In vitro activity of ceftaroline and comparative agents against multidrug-resistant clinical isolates of *S. pneumoniae* collected from an ongoing Canada-wide surveillance programme: 2008–2011**

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**Background:** Multidrug-resistance (MDR) is increasing in *S. pneumoniae* (SPN). In particular, increasing resistance to beta-lactam antibiotics, including penicillin (PEN), amoxicillin (AMOX) and ceftriaxone, pose challenges for the selection of optimal antimicrobial therapy for pneumonia.

**Objectives:** To compare the activity of ceftaroline and comparative agents against Canadian clinical isolates of SPN from 2008 to 2011.

**Methods:** The Canadian Bacterial Surveillance Network (CBSN) is a collaborative network of microbiology laboratories from across Canada that submit bacterial isolates to a central laboratory for broth microdilution antimicrobial susceptibility testing performed according to CLSI standards. CBSN has conducted surveillance for antimicrobial resistance in SPN since 1987. Since 2008, three new antimicrobials (ceftaroline, the active metabolite of the prodrug ceftaroline fosamil, ceftobiprole and cethromycin) have been included in susceptibility testing. MDR is defined as resistant to at least three classes of antibiotics; for this analysis, parenteral non-meningeal breakpoints are used to define susceptibility to penicillins and cephalosporins for the definition of MDR.

**Results:** From 2008 to date, 6328 SPN isolates have undergone susceptibility testing: 2650 (42%) from blood, 194 (3.1%) from CSF/ other sterile site, 2786 (44%) from sputum/BAL, 595 (9%) eye/ear swabs and 103 (2%) other sites. Nine hundred and forty-nine (15%) of isolates were from children, 3078 (49%) from adults aged 15–64 year and 2301 (36%) from older adults. Overall, 24/ 287 (0.38/4.5%) isolates PEN resistant (R)(nonmeningeal parenteral and oral breakpoints, respectively), 202 (3.2%) AMOX R, 26/278 (0.41/4.4%) ceftriaxone (nonmeningeal/meningeal breakpoints) R, 1565 (24.7%) erythromycin (ERY) R, 815 (12.9%) trimethoprim-sulfa (TS) R and 776 (12.3%) tetracycline (TET) R. Of the 394 (6.0%) MDR isolates, all were ERY and TS R, 386 (97%) were TET R, 200 (51%) AMOX R, 24 (6.1%) PEN R (nonmeningeal parenteral), and 26 (6.6%) ceftriaxone (non-

MIC (mg/L) (N)	Ceftriaxone		Ceftaroline		Ceftobiprole		Cethromycin	
	MIC <sub>50</sub>	MIC <sub>90</sub>	MIC <sub>50</sub>	MIC <sub>90</sub>	MIC <sub>50</sub>	MIC <sub>90</sub>	MIC <sub>50</sub>	MIC <sub>90</sub>
PEN								
<8 (6218)	0.25	0.25	0.06	0.06	0.015	0.25	0.015	0.06
≥8 (23)	4	8	0.25	0.5	1	1	0.12	0.25
AMOX								
<8 (6041)	0.25	0.25	0.06	0.06	0.015	0.06	0.015	0.06
≥8 (200)	2	4	0.25	0.25	0.5	1	0.12	0.12
ERY								
<1 (4702)	0.25	0.25	0.06	0.06	0.015	0.03	0.015	0.015
≥1 (1539)	0.25	2	0.06	0.12	0.03	0.5	0.03	0.12
MDR								
No (5838)	0.25	0.25	0.06	0.06	0.015	0.06	0.015	0.03
Yes (403)	2	2	0.12	0.25	0.5	1	0.06	0.12

meningeal) R. Activity of ceftaroline, ceftobiprole and cethromycin against susceptible and R SPN is shown in the Table.

**Conclusions:** Ceftaroline, ceftobiprole and cethromycin exhibit more potent activity against MDR and penicillin resistant SPN than ceftriaxone. These drugs may be valuable in the treatment of patients with pneumococcal infection caused by MDR strains. Ceftaroline has the most potent in vitro activity among beta-lactam antibiotics tested.

**P1563** Spectrum and potency of ceftaroline against leading pathogens causing community-acquired respiratory tract infections in Europe and South Africa, 2010

D. Farrell, R. Flamm, H. Sader\*, R. Jones (North Liberty, US)

**Objective:** Ceftaroline (CPT), the active metabolite of the prodrug ceftaroline fosamil, is a novel cephalosporin exhibiting broad-spectrum in vitro bactericidal activity against Gram-positive organisms, including *Streptococcus pneumoniae* and methicillin-susceptible (MS) and -resistant (MR) *Staphylococcus aureus* (SA), as well as common Gram-negative organisms. The objective of this study was to determine the spectrum and potency of CPT against recent (2010) leading pathogens causing community-acquired respiratory tract infections (CA-RTI) isolated in Europe and South Africa (SAF).

**Methods:** A total of 1608 isolates from the 2010 Assessing Worldwide Antimicrobial Resistance Evaluation (AWARE) Programme were identified as CA-RTI pathogens by the infection type and/or specimen type recorded by the submitter. Isolates were collected from patients in 53 medical centres in 19 European countries (including Israel and Turkey) and in South Africa (45 isolates, one medical centre) during 2010. Susceptibility testing for CPT and commonly used antimicrobials was performed by CLSI broth microdilution methodology. Susceptibility interpretations for comparators were published in CLSI and EUCAST guidelines.

**Results:** The potencies of CPT against the leading pathogens isolated are shown in the Table. CPT was very active overall against *Streptococcus pneumoniae* (SPN; MIC50/90,  $\leq 0.008/0.12$  mg/L) and inhibited 100.0% of all isolates at a MIC  $\leq 0.5$  mg/L. CPT was very potent against penicillin (PEN)-R and -intermediate (I) SPN (MIC50/90, 0.12/25 and 0.03/0.12 mg/L, respectively) but potency was lower than seen against PEN-S isolates (MIC50/90,  $\leq 0.008/\leq 0.008$  mg/L). CPT was also very active against 536 *Haemophilus influenzae* (HI) isolates with activity being slightly lower against beta-lactamase positive (BLP) isolates compared to BL negative (N) isolates. CPT also demonstrated good activity against 211 *Moraxella catarrhalis* isolates (MCAT; MIC90, 0.12 mg/L).

Subset (n)	No. (cum. %) of isolates inhibited at Ceftaroline MIC (mg/L)								
	$\leq 0.008$	0.015	0.03	0.06	0.12	0.25	0.5	MIC50	MIC90
All SPN (861)	553 (64.2)	66 (7.7)	42 (4.9)	41 (4.8)	133 (15.4)	25 (2.9)	1 (0.1)	$\leq 0.008$	0.12
PEN-S (800)	550 (68.8)	44 (5.5)	6 (0.8)	1 (0.1)	1 (0.1)	1 (0.1)	1 (0.1)	$\leq 0.008$	$\leq 0.008$
PEN-I (112)	3 (2.7)	22 (20.0)	36 (32.1)	32 (28.6)	19 (17.0)	1 (0.9)	1 (0.9)	0.03	0.12
PEN-R (148)	1 (0.7)	9 (6.1)	114 (77.0)	25 (16.9)	1 (0.7)	1 (0.7)	1 (0.7)	0.12	0.25
All HI (536)	398 (74.3)	88 (16.4)	38 (7.1)	7 (1.3)	4 (0.8)	0 (0)	1 (0.2)	$\leq 0.008$	0.015
BLN (469)	382 (81.5)	65 (13.9)	20 (4.3)	1 (0.2)	1 (0.2)	1 (0.2)	1 (0.2)	$\leq 0.008$	0.015
BLP (67)	16 (23.9)	23 (34.3)	19 (28.4)	6 (9.0)	3 (4.5)	0 (0)	1 (1.5)	0.015	0.06
MCAT (211)	12 (5.7)	13 (6.2)	67 (31.8)	63 (30.0)	43 (20.4)	11 (5.2)	2 (1.0)	0.06	0.12

**Conclusions:** This study demonstrated the potent in vitro activity of CPT against recent (2010) pathogens isolated from patients with documented CA-RTI from Europe and South Africa. These data suggest that ceftaroline fosamil could emerge as an important therapeutic option for CA-RTI in Europe and South Africa.

**P1564** Antimicrobial activity of ceftaroline and comparator agents against contemporary (2010) *Streptococcus pneumoniae* from Europe and South Africa

D. Farrell, H. Sader\*, R. Flamm, R. Jones (North Liberty, US)

**Objectives:** To determine the activity of ceftaroline against recent (2010) *S. pneumoniae* (SPN) isolated in Europe (EU) and South Africa

(SAF). Ceftaroline, active metabolite of the prodrug ceftaroline fosamil, is a novel cephalosporin exhibiting broad-spectrum in vitro bactericidal activity against Gram-positive organisms including multidrug-resistant (MDR)-SPN, methicillin-resistant *S. aureus* and common Gram-negative enteric bacilli.

**Methods:** Susceptibility testing for ceftaroline and commonly used antimicrobials was performed by CLSI broth microdilution methodology on a total of 1257 isolates from the 2010 Assessing Worldwide Antimicrobial Resistance Evaluation Programme (AWARE). Susceptibility interpretations for the comparators assessed in this study were performed using CLSI and EUCAST guidelines. Isolates were collected from patients in 55 medical centres in 19 EU countries, including Turkey and Israel, and in SAF (22 isolates, one medical centre). MDR-SPN status was determined by resistance (R) to two or more classes of antimicrobials from penicillin (PEN; CLSI oral breakpoints), erythromycin (ERY), levofloxacin, tetracycline (TET) and trimethoprim-sulfamethoxazole (SXT).

**Results:** Ceftaroline was very active against PEN-susceptible (S) and non-MDR isolates, and retained potent activity against PEN-intermediate (I), PEN-R, and MDR isolates (Table). The highest ceftaroline MIC found was in one isolate at 0.5 mg/L (a MDR strain from Romania with a ceftriaxone (CRO) MIC of 4 mg/L). The ceftaroline MIC50 was at least four-fold higher in SAF isolates (0.03 mg/L) than in EU isolates ( $\leq 0.008$  mg/L) due to the higher prevalence of MDR-SPN in the SAF region (54.5% vs. 26.8% in SAF vs. EU), however the MIC90 values were identical (0.12 mg/L for both; note the low number of SAF isolates [22]). Using CLSI oral PEN breakpoints, 26.0 and 72.7% of isolates were non-S for EU and SAF, respectively. By EUCAST breakpoints, 15.6% of all isolates were non-S to CRO (5.0% by CLSI non-meningitis breakpoints). Other antimicrobial resistances (CLSI) were: ERY, 23.6%; TET, 23.1%, SXT, 19.3%.

Subset (n)	No. (cum. %) of isolates inhibited at Ceftaroline MIC (mg/L)								
	$\leq 0.008$	0.015	0.03	0.06	0.12	0.25	0.5	MIC50	MIC90
All (1257)	836 (66.5)	104 (8.3)	56 (4.4)	67 (5.3)	160 (12.7)	33 (2.6)	1 (0.1)	$\leq 0.008$	0.12
PEN-S (920)	831 (90.3)	73 (7.9)	11 (1.2)	5 (0.5)	1 (0.1)	1 (0.1)	1 (0.1)	$\leq 0.008$	$\leq 0.008$
PEN-I (155)	5 (3.2)	31 (20.0)	45 (29.0)	50 (32.3)	24 (15.5)	1 (0.6)	1 (0.6)	0.03	0.12
PEN-R (182)	1 (0.5)	12 (6.6)	138 (74.7)	33 (18.1)	33 (18.1)	1 (0.5)	1 (0.5)	0.12	0.25
non-MDR (939)	794 (84.5)	86 (9.2)	33 (3.5)	25 (2.7)	20 (2.1)	1 (0.1)	1 (0.1)	$\leq 0.008$	0.015
MDR (318)	42 (13.2)	38 (12.0)	23 (7.3)	42 (13.2)	140 (44.0)	32 (10.1)	1 (0.3)	0.12	0.25

**Conclusions:** This study demonstrated the potent in vitro activity of ceftaroline against recent (2010) EU and SAF SPN isolates including MDR strains. These data suggest that ceftaroline fosamil may emerge as an important therapy for infections caused by SPN resistant to beta-lactams and other commonly used antimicrobials as well as MDR strains.

**P1565** Invasive pneumococcal disease (non-meningitis) in adult patients at a University Hospital, Asturias

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**Objectives:** The new antimicrobial susceptibility interpretations for *Streptococcus pneumoniae* and the introduction of conjugate vaccines are aspects to reconsider in pneumococcal disease. To assess its effect on the prevention and the treatment strategies in systemic pneumococcal infections, the serotypes and the antimicrobial resistance were analyzed in consecutive nonmeningeal episodes in adult patients.

**Methods:** All nonmeningeal strains isolated in blood and sterile fluids from adult patients (>14 years and clinically confirmed) were studied at the Central Hospital (Asturias); university center serving an adult population of 281 849–303 568 during 2001–2010. 98.3% of strains were serotyped at The National Reference Center (Madrid). The oxacillin disk and the commercial microdilution method were used. CLSI 2008 breakpoints were applied. The Chi-square test was used, a p value <0.05 was significant.

**Results:** Three hundred and forty-five cases were evaluated: 76.8% pneumonia, 16.2% sepsis, 4.1% empyema, 2.9% others. The average rates of incidence (cases/100 000/year) were 11.0 in 2001–2005 (158 cases) and 12.5 in 2006–2010 (187 cases). A total of 36 serotypes were identified. The most frequent (%) were: 3 (14.2), 14 (11.2), 7F (9.7), 19A (7.7), 8 (6.5), and 4 (5.3). Relevant trends between 2001–2005 and 2006–2010 were: 7-valent conjugate vaccine serotypes decreased from 38.1% to 25.5% ( $p = 0.013$ ); 13-valent conjugate vaccine serotypes showed a not significant decrease, 73.5% vs. 65.2% ( $p = 0.098$ ); the coverage of the 23-valent vaccine was 93.5% vs. 85.9% ( $p = 0.022$ ) corresponding with a significant increase (6.5% vs. 14.1%) in the prevalence of the nonvaccine serotypes. Penicillin susceptibility rates were 70.7% (19.4% intermediate, 9.8% resistant) for oral penicillin breakpoints and 98.3% for parenteral penicillin breakpoints. Susceptibility to cefotaxime and amoxicillin were 95.9% and 94.2% respectively. Resistance to erythromycin was 24.6% (temporal trend: 26.6% to 22.9%) and clindamycin resistance was 21.4%. Levofloxacin resistant isolates (3.8%) were uncommon.

**Conclusions:** Local data were actualized. There were a significant changes in the serotypes distribution. Continuous surveillance is desirable for immunization practice. Penicillin (iv), cefotaxime, amoxicillin and levofloxacin are good choices for the treatments in the hospitalized patients, by contrast the macrolide and clindamycin are not recommended for empirical therapy.

**P1566** **Clinical outcomes of patients with penicillin-nonsusceptible *Streptococcus pneumoniae* bacteraemia: impact of revised penicillin breakpoints in CLSI M100-S18**

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**Objectives:** On January 2008, the Clinical and Laboratory Standard Institute (CLSI) published revised penicillin breakpoints for non-meningeal *Streptococcus pneumoniae* infections in M100-S18. Impact of the revision on clinical outcomes of patients with penicillin-nonsusceptible invasive non-meningeal pneumococcal infections has not been reported. We compared clinical outcomes of patients with penicillin-nonsusceptible *S. pneumoniae* bacteremia (PNSPB) to those of patients with penicillin-susceptible *S. pneumoniae* bacteremia (PSSPB), according to the revised CLSI penicillin breakpoints.

**Methods:** An age- and sex-matched case-control study was conducted in 39 patients with PNSPB and in 78 patients with PSSPB, using the revised penicillin CLSI breakpoints.

**Results:** PNSPB group more frequently had hospital acquisition (38.5% vs. 20.5%,  $p = 0.038$ ), recent surgery (12.8% vs. 2.6%,  $p = 0.04$ ), congestive heart failure (12.8% vs. 2.6%,  $p = 0.04$ ), Pitt bacteremia score  $\geq 2$  (43.6% vs. 25.6%,  $p = 0.049$ ), previous antimicrobial therapy (46.2% vs. 16.0%,  $p = 0.001$ ), and inappropriate antimicrobial therapy (40.5% vs. 4.1%,  $p < 0.001$  within 3 days; 29.7% vs. 2.7%,  $p < 0.001$  within 7 days) than PSSPB group. The 30-day mortality rate was similar between them (30.8% in PNSPB vs. 23.1% in PSSPB,  $p = 0.37$ ). Whereas penicillins were used only in 16.1% of the study patients (18/112) within 3 days of SPB, extended-spectrum cephalosporin/carbapenem or vancomycin were used in 77.7% (87/112). Among the 14 patients who received penicillin monotherapy within 3 days of SPB, the 30-day mortality rate was 57.1% (4/7) in penicillin-nonsusceptible SPB, and 28.6% (2/7) in penicillin-susceptible SPB ( $p = 0.59$ ). Independent risk factors for 30-day mortality were ceftriaxone nonsusceptibility (adjusted OR = 9.54, 95% CI = 1.53–59.37,  $p = 0.016$ ), Charlson comorbidity index score  $\geq 3$  (adjusted OR = 34.86, 95% CI = 3.55–341.91,  $p = 0.002$ ), and Pitt bacteremia score  $\geq 2$  (adjusted OR = 61.70, 95% CI = 6.27–607.11,  $p < 0.001$ ).

**Conclusion:** Even with the implementation of the revised CLSI penicillin breakpoints, fatal clinical outcomes attributable to penicillin nonsusceptibility may be rarely encountered in the current clinical practice of using wide-spectrum empirical antimicrobial agents. The

occurrence of ceftriaxone-resistant pneumococcal isolates and their clinical impact should be continuously monitored.

## Urinary tract infections

**P1567** **Asymptomatic bacteriuria and urinary tract infections in pregnant women with and without diabetes mellitus**

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**Objectives:** The prevalence of asymptomatic bacteriuria (ASB) and the associated incidence of urinary tract infections (UTI), which may contribute to adverse pregnancy outcomes, are increased during pregnancy. Diabetes mellitus (DM) is an important risk factor for both ASB and UTI. The aim of this study was to investigate differences in prevalence of ASB and incidence of UTI including causative organisms in pregnant women with and without DM to provide a background for screening policies.

**Methods:** For this case-control study we used data from medical records of 213 pregnant women who received antenatal care in the Women's and Children's Hospital in Adelaide, Australia, during 2010. Cases were women with a clinical diagnosis of DM. Controls were matched on birthdate. ASB was defined as the growth of at least  $10^5$  colony forming units per milliliter of one organism or the presence of group B streptococcus in the first urine culture collected during pregnancy without complaints of a UTI; and UTI as symptoms in combination with a positive urine culture during the duration of pregnancy. ASB and UTI were compared by chi-square test.

**Results:** No association was found between ASB and UTI. Group B streptococcus was the most common causative organism of ASB in both women with and without DM (66.7% and 50%). There were no significant differences in prevalence of ASB (5.7% and 3.7%,  $p > 0.20$ ) and incidence of UTI (2.8% and 4.7%,  $p > 0.20$ ) between pregnant women with and without DM.

**Conclusion:** In contrast with the current literature no differences in prevalence of ASB and incidence of UTI were found between pregnant women with and without DM.

**P1568** **Cefixime vs. amoxicillin/clavulanate in pregnant women with asymptomatic bacteriuria: multicentre randomised study**

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**Objectives:** To compare the efficacy and safety of cefixime and amoxicillin/clavulanate in pregnant women with asymptomatic bacteriuria (AB).

**Methods:** One thousand and eighty pregnant women were evaluated for inclusion in the study during 2009–2011 years in three Obstetrics and Gynecology Outpatient Clinics in Russia (Smolensk, Kursk and Kirov). After giving informed consent in the study pregnant women with significant AB ( $\geq 10^5$  CFU/mL in two consecutive urine culture analysis separated  $>24$  hours) were included. Exclusion criteria were anatomical or functional abnormalities of urinary tract, hypersensitivity to the study medications, presence of urinary catheters, immune deficiency, diabetes mellitus, malignant tumors and severe renal and hepatic failure. The study was approved by Interregional Independent Ethic Committee. Pregnant women were randomized in 1:1 ratio to two study groups. Patients of Group 1 were treated by cefixime (Ceforal Solutab<sup>®</sup>, Astellas Pharma Europe) 400 mg OD for 7 days and pregnant women of Group 2 were treated by amoxicillin/clavulanate (Amoxiclav<sup>®</sup>, Sandoz/Lek) 625 mg t.i.d. for 7 days. Clinical and bacteriological evaluation was performed on days  $10 \pm 1$  (visit 2) and  $35 \pm 2$  (visit 3) after the start of treatment.

**Results:** AB occurrence rate was 11.9% (128/1080) and 112 of pregnant women with significant AB met the study criteria. Fifty-eight women were included in the study Group 1 and 54 in the study Group



2. The average age of the women in Group 1 and 2 were  $25.2 \pm 6.6$  years and  $26.6 \pm 5.8$  years, respectively. At visit 2 eradication in Groups 1 and 2 were 94.8% (55/58) and 98.2% (53/54), respectively ( $p = 0.35$ ) and at visit 3–92.7% (51/55) and 92.5% (49/53), respectively ( $p = 0.96$ ). During the study the rates of adverse events (AE) were 1.7% (one woman has diarrhea) and 13% (five women has nausea and two has diarrhea) in Groups 1 and 2, respectively ( $p = 0.02$ ) (Table 1).

Table 1. Efficacy and safety of 7-days course of cefixime 400 mg OD vs amoxicillin/clavulanate 625 mg t.i.d. in pregnant women with asymptomatic bacteriuria.

	Group 1 (cefixime 400 mg OD, 7 days)	Group 2 (amoxicillin/clavulanate 625 mg t.i.d., 7 days)	P-value <sup>†</sup>
Eradication on visit 2, % (n/N)	94.8 (55/58)	98.2 (53/54)	$p=0.35$
Eradication on visit 3, % (n/N)	92.7 (51/55)	92.5 (49/53)	$p=0.96$
Adverse events, % (n/N)	1.7 (1/58)	13 (7/54)	$p=0.02$

<sup>†</sup> The P-value was calculated with the use of chi-square test.

**Conclusion:** There were no significant differences in eradication rates of asymptomatic bacteriuria in pregnant women treated with cefixime and amoxicillin/clavulanate for 7 days. I was found that treatment with amoxicillin/clavulanate were significantly more developed adverse events (mainly gastrointestinal) than cefixime therapy.

#### P1569 Efficacy of pivmecillinam in empirical treatment of community-acquired ESBL-positive urinary tract infections – comparison with ESBL-negative infections

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**Objectives:** European guidelines have recently recommended pivmecillinam as one of four antibiotics in the empirical treatment of uncomplicated lower urinary tract infections (UTI). Our aim was to explore the efficacy of pivmecillinam as empirical treatment of ESBL positive UTI.

**Methods:** This is a prospective cohort study of 66 ESBL positive and 103 ESBL negative eligible Norwegian patients treated for UTI. The patients were enrolled during 2009–11. Inclusion criteria were patients treated for community acquired UTI (coUTI) with urine culture positive for *Escherichia coli* or *Klebsiella pneumoniae* without prior ESBL infection. The history of antibiotics use from the Norwegian Prescription Registry and microbiological results were collected. The major endpoint was relapse of infection defined as recurrence of antibiotic prescription at day 2–31. Differences between groups were examined by Mantel–Haenszel stratification analysis.

**Results:** A total of 92% females (median age 55 years, range 18–92) and 88% females (median age 64 years, range 19–88) were among the ESBL positive and the ESBL negative patients, respectively. Relapses were observed in 39 (59%) ESBL positive and 32 (31%) ESBL

negative patients. Crude odds ratio (OR) of relapse was 3.2 with a 95% confidence interval (CI) of 1.7–6.1,  $p < 0.001$ .

A Mantel–Haenszel stratification analysis showed that pivmecillinam therapy, age, gender and other antibiotic were not significant confounders for the association between positive ESBL and relapse of infection (Fig. 1). Relapses occurred significantly more often in patients receiving trimethoprim as compared to patients not receiving trimethoprim (OR = 12.4, 95% CI: 3.3–47.0 vs. OR = 1.87, 95% CI: 0.87–4.0).

**Conclusion:** The risk of relapse was significantly higher in ESBL positive patients as compared to ESBL negative patients receiving antibiotic treatment for coUTI. Pivmecillinam therapy did not influence the association between patients with ESBL positive coUTI and relapse. The relapse rate was higher in patients receiving trimethoprim as compared to not receiving trimethoprim.

To our knowledge this is the first systematic observational study on use of pivmecillinam in the treatment of ESBL positive UTIs. Our results confirm the previous case reports and in vitro observations that pivmecillinam has an activity against ESBL positive Enterobacteriaceae. The results will be discussed in relation to ESBL-characteristics and antibiotic susceptibility profiles.

#### P1570 Clinical characteristics of community-acquired urinary tract infections caused by extended-spectrum beta-lactamase-producing Enterobacteriaceae

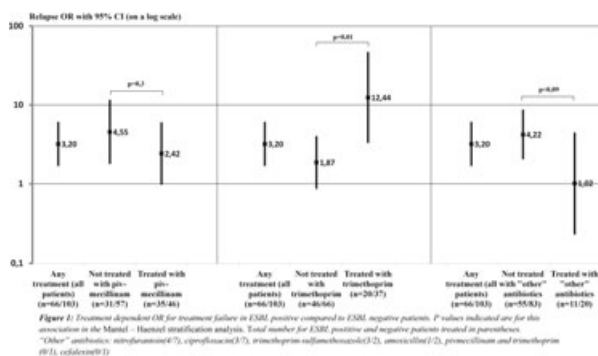
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**Background:** In order to identify the risk factors and clinical characteristics of community-acquired urinary tract infections caused by extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae, a prospective multi-center study was performed.

**Methods:** From March 2010 to February 2011, the patients of UTI were enrolled from 11 hospitals in Korea. Case-control study was performed between the patients with ESBL-producing Enterobacteriaceae in urine culture (ESBL group) and those with non-ESBL-producers (non-ESBL group). Demographic data, clinical findings and treatment results were recorded prospectively, and history of UTI, hospitalization, urinary catheterization and antibiotic usage within 1 year were investigated. Susceptibilities to several antibiotics were studied.

**Results:** Enterobacteriaceae was identified from urine of 526 patients. Forty-six of 526 isolates of Enterobacteriaceae (8.7%) and 38 of 497 *Escherichia coli* (7.6%) produced ESBL. Clinical characteristics between ESBL group and non-ESBL group did not differ. For the treatment results, ESBL group had a tendency to higher clinical and microbiological failure, however, the differences were not significant (9.5% vs. 5.5%,  $p = 0.516$ ; 20.8% vs. 9.8%,  $p = 0.164$ , respectively). Duration of hospitalization was longer in ESBL group with a marginal significance ( $11.11 \pm 7.0$  vs.  $9.0 \pm 7.0$ ,  $p = 0.052$ ). Risk factors for UTI by ESBL-producing organisms were high Charlson's score ( $2.13 \pm 1.80$  vs.  $1.30 \pm 2.00$ ,  $p = 0.007$ ), diabetes (43.5% vs. 28.8%,  $p = 0.044$ ), hemiplegia (8.7% vs. 2.3%,  $p = 0.034$ ), intermittent catheterization (4.3% vs. 0.4%,  $p = 0.040$ ), history of hospitalization within 1 year (50.0% vs. 23.1%,  $p < 0.001$ ), history of antibiotic use within 1 year (54.3% vs. 23.8%,  $p < 0.001$ ) and history of urinary catheterization within 1 year (4.3% vs. 0.8%,  $p = 0.009$ ) in univariate analysis. In multivariate analysis, history of antibiotic use within 1 year was a significant risk factor for UTI caused by ESBL-producing organisms (OR = 2.802,  $p = 0.024$ ). Antimicrobial susceptibility to FOX, CIP or LVX, GM and SXT showed significantly higher resistance rate in ESBL-producing organisms (58.1% vs. 3.9%, 69.6% vs. 17.5%, 45.5% vs. 19.3%, 47.5% vs. 25.8%, respectively).

**Conclusion:** Clinical characteristics between ESBL and non-ESBL groups did not differ. History of antibiotic use within 1 year was a significant risk factor for UTI by ESBL-producing organisms.



**P1571 Adults patients with bacteraemic urinary tract infection discharged from the emergency department**

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**Background:** Urinary Tract Infections (UTI) are one of the most common infectious diseases seen in every Emergency Department, and their investigation often includes performance of blood cultures. However, it often takes at least 48 hours before being detected by use of standard laboratory equipment, so some patients are discharged before receipt of blood culture results. The aim of this study was to know the outcome of these patients discharged early.

**Methods:** A prospective observational cohort study was conducted from September 2010 through March 2011 in an Emergency Department of a Spanish tertiary-care hospital.

**Results:** We identified 162 bacteremic infections, 63 of them (39%) were bacteremic UTI (B-UTI). The median age was 65 years and 44% were males. Twenty-two percent of patients with B-UTI had previous urologic surgery, and 12.7% had urologic tumors. Twenty-four out of 63 B-UTI were discharged early (30.9%). The independent risk factors for hospital admission were: previous urologic surgery (relative risk (RR), 5.5; 95% confidence interval (CI), 1.08–29.8,  $p = 0.047$ ) and severe sepsis (RR, 21.1; 95% CI, 2.5–176.4,  $p = 0.005$ ).

Fifty-five of 63 B-UTI were finally followed-up for at least 5 months (median:162.6 days). 8/55 patients (14.5%) developed a recurrence. The number of recurrences was  $1.77 \pm 1.09$ , and the median to the first recurrence was 38 days. Five out of 22 patients with early hospital discharge suffered a recurrence (22.7%) compared with patients admitted to hospital (9%,  $p = 0.24$ ). The recurrences were mild in 75% of patients and nobody died. In the Univariate analysis of recurrence, the need of a previous admission to hospital before the B-UTI, the presence of solid neoplasm, previous urologic surgery or a gram-negative Extended-spectrum beta-lactamases (ESBLs) bacteremia tender to produce more recurrences of UTI.

**Conclusions:** 14.5% of patients with B-UTI discharged at an Emergency Department suffered a recurrence during the follow up. We consider that it could be necessary to guarantee an adequate monitoring of them unless during the first 30 days after the hospital discharge.

**P1572 Epidemiology and outcome of complicated pyelonephritis caused by extended-spectrum beta-lactamase producing bacteria**

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**Objectives:** The incidence of UTI caused by ESBL-producing uropathogens has increased in recent years. However, information about the prognosis of complicated pyelonephritis (CP) produced by these pathogens is very low. In this study we analyze the main epidemiological characteristics and the prognosis of CP caused by ESBL-producing microorganisms.

**Methods:** Descriptive, retrospective, cross-sectional study which included 698 patients older than 14 years, diagnosed with CP between January 2001 and December 2010. The diagnosis of CP was based on IDSA criteria. All patients were treated for a period equal to or >14 days.

**Results:** The age was  $59.6 + 18.1$  years (range, 14–95 years), 461 cases (68%) were women, 393 (56.3%) had functional or structural urologic abnormalities, 230 (33%) were diabetic, 82 (11.7%) were immunosuppressed, 53 (7.6%) had undergone urinary instrumentation in the previous 15 days, 49 (7.6%) had indwelling catheter and 18 (2.5%) nephrostomy or double J catheter. The infection was nosocomial in 30 cases (4.3%). The duration of symptoms was  $5.5 + 6.5$  days,

55.5% of cases had bacteremia, 252 patients (36.1%), severe sepsis, 107 (15.3%) septic shock and 100 (14.3%) required ICU admission. The hospital stay was  $11.9 + 9.2$  days and the attributable mortality of 5.3%. Of the total sample, 92 cases (13.2%) were caused by an ESBL-producing strain; 59.8%, *E. coli* and 15.2% *Klebsiella-Enterobacter* spp. All these strains were susceptible to meropenem. The CP-ESBL were significantly more common in men, RR 2.2 (95% CI, 1.4–3.4), previous history of CP, RR 2.2 (95% CI, 1.5–3.2), immunocompromised, RR 1.9 (95% CI, 1.1–3.4), previous urinary tract instrumentation, RR 2.3 (95% CI 1.1–4.5), patients with urinary catheter, RR 4.1 (95% CI, 2.1–7.7) and without mictional syndrome, RR 2.2 (95% CI, 1.5–3.3). The CP-ESBL patients had higher risk of septic shock RR 1.6 (95% CI, 1.05–2.8) and had a higher attributable mortality, 8.7% vs. 4.8%.

**Conclusion:** The incidence of CP-ESBL is considerable. Its frequency is significantly higher in men, patients with previous CP, without mictional syndrome, immunocompromised, urinary tract instrumentation or indwelling catheter. The etiological spectrum of CP-ESBL does not differ from other complicated UTI. By having a worse prognosis, while the causative agent is identified and its antimicrobial susceptibility known, the empirical treatment of patients at highest risk should be based on carbapenem.

**P1573 Health-care-associated, community and hospital-acquired bacteraemic urinary tract infections: a prospective cohort study**

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**Background:** Recent changes in healthcare delivery and the increase of multidrug resistance bacteria may be causing epidemiological changes in bacteraemic urinary tract infections (BUTI). This study aims to assess the clinical features and outcome of BUTI taking into account the new classification of infections.

**Methods:** Prospective observational cohort study conducted at eight tertiary care hospitals in Spain, from October 2010 to June 2011. Clinical and microbiological data of community onset healthcare-associated (HCA) BUTI in patients needing hospital admission were compared with those with community acquired (CA) and hospital acquired (HA). A logistic regression model was performed to identify risk factors for BUTI related-mortality.

**Results:** Six hundred and sixty-seven episodes were included (241 HCA, 284 CA and 142 HA). Differences between CA and HCA were sex (32% vs. 60%,  $p < 0.001$ ), Charlson index (median 1 vs. 3,  $p < 0.001$ ), ultimately or rapidly fatal disease (McCabe II-III) (14% vs. 49%,  $p < 0.001$ ), Pitt score  $\leq 1$  (69% vs. 60%,  $p = 0.03$ ). ESBL-producing enterobacteria isolates (5% vs. 13%,  $p < 0.001$ ), median hospital stay (7 day vs. 9 day,  $p = 0.03$ ), inappropriate empirical antibiotic therapy administration (13% vs. 19%,  $p = 0.04$ ) and in-hospital BUTI related-mortality (3% vs. 10%,  $p = 0.001$ ). Differences between HCA and HA were isolation of *Pseudomonas aeruginosa* (3% vs. 16%,  $p < 0.001$ ), median hospital stay (9 day vs. 13 day,  $p < 0.001$ ), and in-hospital BUTI related-mortality (10% vs. 19%,  $p = 0.01$ ). Independent factors associated to mortality were age (OR 1.06; CI 1.02–1.11), McCabe score (OR 3.10; CI 1.27–7.57), Pitt score (OR 7.32; CI 2.88–18.5) and HA BUTI (4.14; CI 1.32–12.9).

**Conclusions:** HCA BUTI showed clinical differences with CA BUTI. Isolation of ESBL-producing enterobacteria was more frequent in HCA and HA, while pathogens as *Pseudomonas aeruginosa* were isolated predominantly in HA. HCA and HA had a longer hospital stay and more frequent inappropriate empirical therapy administration compared with CA. In-hospital BUTI related-mortality was associated with patient condition, severity of infection and hospital acquisition.

**P1574** Qualitative and quantitative digestive carriage of ESBL-producing *Escherichia coli* in patients with community-onset urinary-tract infections: a multinational study

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**Context:** It remains unclear if intestinal colonisation with E-ESBL is associated with increased risk of UTI with these bacteria.

**Objectives:** To assess the relationship between faecal carriage of extended-spectrum beta-lactamase-producing *Escherichia coli* (ESBL-E) and the development of community urinary tract infections (UTI).

**Methods:** Between 2008 and 2011, 100 outpatients from Bucharest (Romania), 192 from Chisinau (Moldova), 100 from Izmir (Turkey) and 12 from Athens (Greece) with *E. coli* UTI brought the first stool emitted after the urine sample was taken. Stools with ESBL and non ESBL *E. coli* UTI were frozen, centralised and screened for ESBL-E on 1 mg/L cefotaxime Drigalski agar. The relative concentration (RC) of ESBL-E/total Enterobacteriaceae (ESBL-E RC) was determined. ESBL (PCR and sequencing) and *E. coli* (REP-PCR) were typed

**Results:** Prevalence (%) of ESBL-E in urine and stools were respectively 7.3 and 9.9, 11.0 and 19.0, 31.0 and 53.0 and 0.0 and 16.7 in Moldova, Romania, Turkey and Greece. The positive predictive value (PPV) of ESBL-E faecal carriage for ESBL UTI was 42%, whereas the negative PV was 94%. Of 29 patients who had ESBL-E in both stool and urine, clones with the same REP-PCR patterns was detected in only 12 (42%) of them. The ESBL-E RC was 8.2% vs. 0.18% patients with and without antibiotics, respectively ( $p < 0.001$ ). It was not significantly different between patients with ESBL-E and non ESBL-E UTI. In contrast, it was significantly higher in patients with different ESBL-E in urine and stool than in those with the same ones (10.0% vs. 0.1%,  $p < 0.02$ ).

**Conclusion:** We showed that ESBL-E faecal carriage was not a good predictor of ESBL-E UTI, yet the negative predictive value was high. Surprisingly, in patients with ESBL-E in both stool and urine the faecal strain was often not the infecting one. Together with the finding that ESBL-E RC was higher in patients with discordant clones than in those with concordant ones it suggests intestinal densities does not play a major role in ESBL-E UTI pathogenesis. Other factors such as virulence characteristics of the various strains are currently under investigation.

**P1575** Optimised patient transfer using an innovative multidisciplinary assessment in Canton Aargau (OPTIMA II) – an observational survey in urinary tract infections, Switzerland

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**Background:** Current guidelines provide limited evidence to allocate the most appropriate treatment site in patients with urinary tract infection (UTI). We assessed current triage practices and tested if different sets of criteria including biomarkers have the potential to improve triage decisions in UTI.

**Methods:** Consecutive adults with UTI presenting to our emergency department were recruited in an observational study. We compared the actual triage decisions based on the physician judgement with virtual treatment site allocation and analysed the added impact of cut-offs of proadrenomedullin (proADM) and urea with the best performance regarding efficacy and safety. We defined that hospitalisation was required if any of clinical criteria (i) severe disease with malaise, fever ( $T > 38^\circ\text{C}$ ) or flank pain, (ii) dehydration or need for IV therapy, (iii) doubtful compliance, (iv) pregnancy, (v) complications of pyelonephritis, (vi) comorbidities requiring hospitalisation) were fulfilled (clinical group) or if any of clinical criteria 2–6 were fulfilled or on admission either proADM  $\geq 1.5$  nM (proADM group)

or urea  $\geq 14$  mM (urea group). Phone interviews were performed 30 days after enrolment. Adverse outcome was defined as transfer to ICU, death, recurrence of UTI or rehospitalisation for any reason.

**Results:** We recruited 127 patients (age:  $61.8 \pm 20.8$ ; 73.2% females) and analysed data of those 123 with final diagnosis of UTI: 10 with uncomplicated and 32 with complicated cystitis, 21 with uncomplicated and 60 with complicated pyelonephritis. Twenty-seven (22.0%) were treated as out- and 96 (78.0%) as inpatients. Mean admission levels of proADM and urea were  $1.4 (\pm 1.2)$  nM and  $7.9 (\pm 6.3)$  mM, respectively. Virtual triage based only on clinical signs would have treated only 22 (17.9%) as outpatient with higher proportions of outpatients in the proADM group (28.5%;  $p = 0.05$ ) and the urea group (29.3%;  $p = 0.04$ ). There were no significant differences in observed adverse events between outpatients according to the clinical (4.5%), proADM (2.9%) or urea group (2.7%). Mean length of hospitalisation was 5.1 days including 1.7 days after reaching medical stability.

**Conclusions:** Current rates of hospitalisation are high and the length of stay could be shortened in patients with UTI. Adding biomarkers to clinical criteria might improve the risk-based triage without compromising safety. An intervention study to test this hypothesis is currently underway.

**P1576** Diagnostic approach to urinary tract infections in male general practice patients

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**Objectives:** To create a diagnostic algorithm for male general practice (GP) patients suspected of a urinary tract infection (UTI) based on clinical information and dipstick results. To assess the potential impact of the new diagnostic algorithm by comparing its predicted care with care as usual.

**Methods:** General practitioners (GPs) from the Dutch Sentinel General Practice Network ( $n = 42$ ) collected clinical information, i.e. age, history of UTI in the past year and clinical symptoms, and dipstick results (nitrite and leucocyte-esterase (LE)) from male patients ( $\geq 18$  years) with symptoms indicative of UTI. Also, prescribed antimicrobial treatment was recorded and collected urinary samples were sent to the laboratory for microbiological analysis. Clinical information and dipstick results predictive of UTI, determined by multivariable logistic regression analysis, were incorporated in a diagnostic algorithm for which positive and negative predictive values were determined (PPV and NPV respectively).

The new diagnostic algorithm and care as usual were compared in terms of sensitivity (antibiotic recommended when UTI was confirmed) and specificity (no antibiotic recommended when no UTI was observed). A  $p$ -value  $\leq 0.05$  was considered statistically significant.

**Results:** From January 2009 to June 2011, 603 patients were included, of whom 490 (81%) had all clinical information and dipstick results available. A microbiologically confirmed UTI ( $\geq 10^3$  CFU/mL) was observed in 321/490 (66%). A diagnostic algorithm recommending antimicrobial prescription in case of a positive nitrite test or a positive LE test in men above 60 years of age showed the best performance (area under the ROC curve: 0.78, 95% CI: 0.74–0.82). This algorithm had a PPV of 83% (95% confidence interval (CI): 78–87) and a NPV of 60% (95% CI: 52–66), respectively. When both dipstick results were positive in men over 60 years of age, the PPV increased to 90% (95% CI: 83–94), whereas the highest NPV (71%, 95% CI: 59–81) was observed in men  $\leq 60$  years of age with negative dipstick results. Sensitivity and specificity of the predicted UTI care and care as usual did not differ (75% vs. 79% and 70% vs. 63% respectively, both  $p > 0.05$ ).

**Conclusions:** UTI care provided to Dutch male general practice patients is as accurate as predicted care from a diagnostic algorithm. Clinical information and dipstick tests are useful for ruling in UTI in men, but have limited value in ruling out this diagnosis.

## Tropical medicine

### P1577 Infectious diseases in internationally adopted children

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**Objectives:** The number of international adoptions in Italy has substantially increased during the last decade, exceeding 4000 children in 2010. Most adopted children are at increased risk of infections acquired in their country of origin. Aim of our study was to determine the prevalence of and factors associated with various infectious diseases in a cohort of internationally adopted children.

**Methods:** We performed a retrospective study of 254 international adoptees that came to Piedmont, Italy, between 2010 and 2011. Clinical assessment, biochemical and radiological tests were performed in an international adoption centre following the national screening protocol for health assessment of international adoptees (Italian Society of Paediatrics). Statistical analysis was performed using Paws statistics software.

**Results:** The study population included 140 males and 114 females, with a mean age of 4.6 years (range 0.5–14.6). The four leading countries of origin were Ethiopia (19.3%), Vietnam (12.2%), China (9%) and Russian Federation (5.5%). One hundred and thirty-five children (53.1%) had at least one infection (Fig. 1). One hundred and two adoptees had a parasitic infection, such as amebiasis (38), giardiasis (38), toxocariasis (29), other intestinal protozoa (13) or roundworms infestation (7), schistosomiasis (1), and malaria (1). Cutaneous infections were observed in 28 children. Latent or active tuberculosis were diagnosed in eight children each. One child had active hepatitis C and three had hepatitis B. Being born in Eastern Europe was a risk factor for Toxocara infection (OR 8.64; 95% CI 3.55–21.01), while African origin was a risk factor for amebiasis and giardiasis (respectively, OR 2.64; 95% CI 1.35–5.17 and OR 3.1; 95% CI 1.53–6.27). Children with congenital CMV infection and neurological defects came more likely from the Russian Federation.

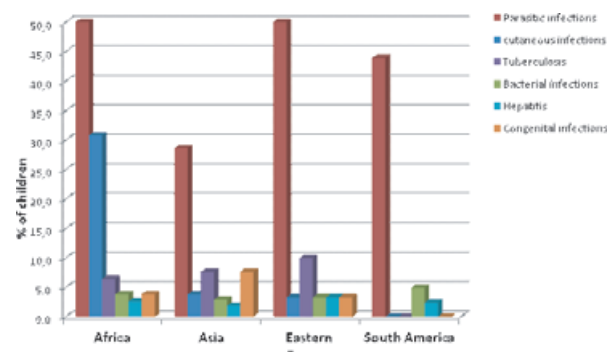


Figure 1. Prevalence of infectious diseases according to country of origin

**Conclusions:** We found a high prevalence of infectious diseases in our cohort of adoptees, particularly of parasitic infections. We recommend early health assessment and appropriate screening of internationally adopted children to recognise latent or active infections, to provide appropriate treatment, and to prevent further transmission of communicable diseases to family members or other children.

### P1578 First case of *Salmonella salamae* infection associated with consumption of reptile meat in humans

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**Objectives:** *Salmonella enterica* subsp *salamae* (subspecies II) is usually isolated from cold-blooded animals and the environment.

Human infections are rarely reported and the few cases have been associated to direct contact with reptiles. However, there are no previous reports of *S. salamae* infection linked with consumption of flesh of reptiles. We report a case of salmonellosis caused by *S. salamae* in an African man who frequently eats reptile products.

**Methods:** Stool cultures for enteropathogen bacteria were made by standard procedures. Biochemical identification was performed by Api® 20E (bioMérieux) and MicroScan® 53 (Siemens). Virulence factor genes were examined by PCR. Investigation of intestinal and blood parasite were also performed.

**Results:** We report the case of a 42-year-old man who had lived in Equatorial Guinea for the last two years and came to Spain 1 month ago. He presented to the Hospital with symptoms of malaise and mild diarrhea in the last month. In the background, he referred he was a regular consumer of reptile meat, mainly flesh from sea turtles. He did not report any other contact with reptiles. He had been diagnosis of malaria by *Plasmodium falciparum* and treated 2 weeks ago. At the time of admission, hematological examination showed the presence of anemia. In the microbiological study, a pure culture of *Salmonella salamae* in McConkey and *Salmonella-Shigella* agar plates was observed. Intestinal parasites were not detected. The most remarkable biochemical findings for identification were ONPG negative, hydrolysis of gelatin positive after 48 hours, and fermentation of sorbitol and mannitol positive. ipaH, stx1, stx2 and eae virulence factors genes were not detected. The strain was susceptible to amoxicillin/clavulanic acid, ampicillin, aztreonam, cefepime, cefotaxime, ciprofloxacin, imipenem, piperacillin/tazobactam, and trimethoprim/sulfamethoxazole. *P. falciparum* was detected with a low parasitemia.

The patient was treated with ciprofloxacin with resolution of his diarrhea. At 1-month follow-up, *Salmonella salamae* was again yielded from stool samples but only a few colonies and not in pure culture.

**Conclusion:** Salmonellosis by *S. salamae* after consumption of reptile meat is possible. Microbiological diagnosis can be performed easily by traditional methods. Epidemiological data as eating habits or country of origin are factors that can guide the diagnosis.

### P1579 Haemorrhagic colitis complicated by haemolytic uraemic syndrome in Georgia

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**Background:** The incidence of diarrhea has significantly increased in Georgia recently. The prevalence of hemorrhagic colitis increased 3–4 times (2005–2010). Furthermore, patients with HUS have been dramatically increased in 2009–2011.

**Methods and objectives:** Manifestation of hemorrhagic colitis clinico-epidemiological features, evaluation its etiological structure, and the analyze of the cases complicated by HUS was the aim of our research. The research is conducted in the Centers of Infectious Diseases and National Center for Disease Control., Tbilisi, Georgia. In addition to the bacteriological analyze of the stool specimens shiga-toxin is identified by PCR and ImmunoCard STAT methods.

**Results:** We have studied 200 patients with hemorrhagic colitis, most patients (68%) were rural residents, women comparatively to men were (62% vs. 38%). Forty patients developed HUS and it was characterized by renal failure, hemolytic anemia and thrombocytopenia. Nineteen patients (47%) with HUS required dialysis, died five patients, lethality – 12.5%. Bacteriological studies of the patients with bloody-diarrhea revealed enteropathogenic bacteria in 63.5% of cases. (STEC strains-0157, 0111, 026, 0103, 045 in – 67%, shigella-8%, *Klebsiella*-10%, *Salmonella*-5%, and other pathogens in 8.5%.) STEC culture was isolated four times more often in patients with bloody-diarrhea than in patients with non-bloody diarrhea (67% vs. 16%).

**Conclusion:** Shiga toxin-producing *E. coli* circulate in Georgia and they represent the leading causative agents of *Haemorrhagic colitis* and HUS. There are not only 0157 but non-0157 strains as well (0111, 026, 045, and etc.). Recently, by the adoption of the bacteriological and serological investigations of the patients important success in diagnosis

was achieved. Bacteriological confirmation of the HUS cases has been increased – 44–63.6%, resulted in 2.2 time decrease of HUS cases and diminution of lethality from 20% to 0%.

#### P1580 Dynamics of clinical symptoms in patients with scrub typhus

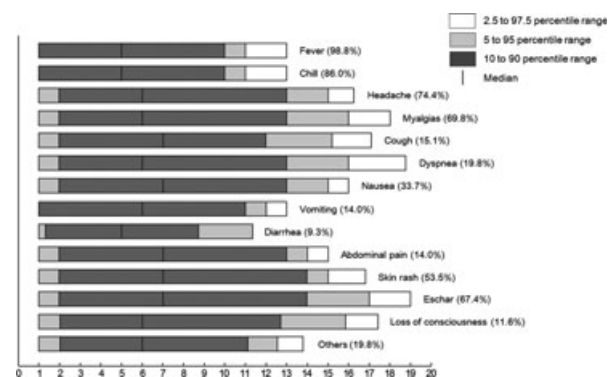
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**Introduction:** Scrub typhus is a potentially fatal infectious disease caused by the organism *Orientia tsutsugamushi*. Clinically, the manifestations and complications of scrub typhus are protean. Severe complications were reported in scrub typhus cases including acute respiratory distress syndrome (ARDS), encephalitis, interstitial pneumonia, myocarditis and pericarditis, acute renal failure, and acute hepatic failure.

**Methods:** The diagnosis of scrub typhus was made on clinical manifestations and indirect immunofluorescence assay (IFA). A definite case of scrub typhus was defined by an increased titer of IFA against *O. tsutsugamushi* ( $\geq 1:80$ ) in a single serum sample or by a 4-fold or greater increase of titer in the follow-up [11]. The sera of patients were assayed by previously described methods for detecting the IgM and IgG antibodies against *O. tsutsugamushi*.

This study was conducted from October to December 2011 at five university-affiliated hospital in South Korea. Respiratory symptoms were defined as cough, and dyspnea. The symptom score was average, and the range was from 0 to 3. To quantify the symptoms score, we asked all subjects to complete a daily log at the same time every morning to document the degree of their scrub typhus-related symptoms in terms of the four-point scale. The study protocol and questionnaire were approved by Institutional Review Board of the Chonbuk National University Hospital.

**Results:** During the evaluation period, 89 patients were diagnosed with scrub typhus. Of the 85 patients studied, 57 (67.1%) were male. The mean age was  $23.4 \pm 14.3$  years. Among the clinical manifestations, fever (98.8%) and cough (86.0%) were the most frequent, followed by headache (74.4%), myalgia (69.8%), skin rash (53.5%), nausea (33.7%). The longest median duration of clinical symptoms were cough, nausea, abdominal pain, skin rash and eschar, which was persisted for 7 days. Regarding the severity of symptoms, systemic symptoms peaked the earliest and most severe, these systemic symptoms were resolved slowly for 2 weeks. Gastroenterology, respiratory symptoms, neurologic symptoms were mild, and the duration was within 2 weeks (Fig. 1). The skin rash was severe first 7 days, and then resolved slowly for 2 weeks.



**Conclusion:** If we know the dynamics of clinical symptoms, we can use these findings to different diagnosis in scrub typhus endemic area.

#### P1581 A review of human anthrax infection in Georgia

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**Objectives:** Anthrax infection is endemic zoonose for Georgia. Annually the incidence of human anthrax remains a considerable health problem in our region, periodically with peaks of cases. The aim of the present study was to review human anthrax outbreak in Georgia during the last 5 years. To evaluate features and emphasize the importance of the disease in Georgia.

**Methods:** The research retrospectively reviewed all the cases of human anthrax during 2006–2011 year admitted at the scientific research centre of the Infectious disease, aids and clinical immunology of Georgia, Tbilisi. From the patient's files, epidemiology, data on age, gender, clinical findings and severity of infection, treatment and outcome of patients were recorded. Diagnose was based on samples investigations with bacteriological and /or PCR methods.

**Results:** Totally 73 cases were diagnosed as human anthrax in the last 5 years. Seventy male, three female, mean age 28.3, range 16–71. In 2006 and 2007 year – 8–8 patients; 2008 – 14; 2009 – 7; 2010 – 6; 2011 – 30 (till October). All the cases with cutaneous anthrax were reviewed, in which in two cases developed anthrax sepsis and infectious toxic shock. Source of infection were: contact with infected animal or animal products – 88%, contact with ground, grass – 12%. The predominantly affected sites were fingers (48%), followed by forearms, hands (40%), foot (4%), eyelids (4%) and necks (4%). One case with anthrax sepsis recovered in spite of there was beforehand surgical intervention, but no concomitant diseases. Mortality was induced: In one case- with severe infectious toxic shock and such important comorbidities as lung cancer with chemotherapy, renal failure and late hospitalisation. In second one- chronic hepatitis C, cirrhosis, esophageal varices, chronic renal failure. The mortality rate was 2.7%. All patients treated with penicillin and/or ciprofloxacin.

**Conclusion:** Anthrax infection remains as an important problem for Georgia. That indicates irrelevant control of anthrax in animals. Human anthrax morbidity dramatically rise during 5 years, especially last year. All cases are cutaneous anthrax. In all outcome of human anthrax was crucial premorbid background, preliminarily surgical interventions and late hospitalisation.

#### P1582 Randomised double-blind clinical trial to assess the efficacy of dexamethasone in reducing local inflammatory reaction in Bothrops snake envenoming

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**Objectives:** We performed the first clinical prospective, randomized, double-blind study to evaluate the development of local edema after the administration of antivenom in Bothrops accidents associated or not with glucocorticoids.

**Methods:** The study was carried out in Vital Brazil Hospital (Butantan Institute) São Paulo, Brazil, from February 2002 to May 2004. Patients were randomly allocated in two groups by randomization in different interchangeable blocks. The first group received anti-histaminic and serumtherapy and another one received anti-histaminic, serumtherapy and corticosteroids. Edema was assessed daily according to its intensity by measuring 5 limb circumferences segments. These measures were used to calculate the volume variation in percentage, in different days, the hemostatic parameters. The venom kinetics were assessed in both groups.

**Results:** One hundred and five patients were included: 53 in the placebo group and 52 in the corticosteroids group. Eighty-three patients (79.0%) were classified as mild cases and twenty two (21.0%) as moderate. The placebo and dexamethasone groups were homogeneous

in the epidemiological variables. The comparison between both groups in different times showed that the volume of the member was less intense and declined more rapidly compared with the group receiving corticosteroids ( $p = 0.0002$ ). The hematological results in both groups were similar; however, in the dexamethasone group there was a significant slower recovery of the fibrinogen, but without delay in the coagulability capacity. Serum venom level was detected in 63 patients (60%) at T0 (admission time), the mean in mild cases ( $n = 83$ ) was  $6.6 \pm 0.8$  ng/mL and in moderate,  $23.4 \pm 5.8$  ng/mL ( $p = 0.0001$ ).

**Conclusions:** Bothrops accident is characterized by local swelling, systemic bleeding and coagulation disturbances. The local edema is progressive and can evolve to local complications, such as necrosis and amputation. The participation of inflammatory endogenous mediators in this local process has been demonstrated and the corticosteroids have a potent anti-inflammatory action. Up to the present moment, the unique therapy for treating Bothrops accidents is the antivenom. It was concluded that the glucocorticoids in high dosis at the admission time diminished the edema when compared to the other group. This result open new perspectives in the complementary therapy in accidents caused by snakes that presents venom with local inflammatory activities.

#### **P1583** Brucellar spinal epidural abscesses. Analysis of 19 cases

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**Introduction and objectives:** Brucellosis, a zoonosis with worldwide distribution, is a systemic infection caused by facultative intracellular bacteria of the genus *Brucella*, which can involve multiple organs and tissues. The spine is the most common site of musculoskeletal involvement. Brucellar spinal epidural abscesses are rare and very few series of them have been reported. We aimed to evaluate the clinical characteristics, laboratory, radiological findings and outcome of this entity.

**Patients and methods:** Of 146 patients with brucellosis, 19 (13%) patients were diagnosed to have spinal epidural abscesses during a period of 21 years (1990–2010). Diagnosis made on clinical presentation, laboratory findings, radiographic evidence and the Brucellar aetiology was considered when seroagglutination tests were positive at a titre of 1/160 or higher, and/or *Brucella* spp. were isolated in the blood.

**Results:** The mean age of patients was  $51 \pm 16$  years (14 males, 5 females; age range, 22–74 years). The median diagnostic delay was 3 months. Back or neck pain (100% of patients), fever (100%), and sweats (68.6%) were the most common symptoms. Cultures of blood specimens from four patients (21%) were positive for *Brucella melitensis*. Three patients (15.8%) had motor weakness or paralysis. Magnetic resonance imaging was performed in all cases. The lumbar vertebra was most frequently involved region with the rate of 84.2%, followed by thoracic (15.8%), cervical (5.3%), lumbosacral (5.3%), and thoraco-lumbar (5.3%) segments. A combination of rifampin, doxycycline and Trimethoprim-sulfamethoxazole was the most widely used therapy regimen (eight cases, 42%). The duration of antimicrobial therapy of brucellosis (median, 6 months; range, 3–13 months) varied according to clinical response. There were no deaths or severe sequelae in this study.

**Conclusion:** Brucellar spinal epidural abscesses should be considered in patients who have back pain and neurologic disorders as well as systemic symptoms and findings in or from endemic areas.

#### **P1584** Implementing a laboratory in a tropical remote area or what can be done with little funds and no electricity access

J.-F. Carod\* (Saint-Claude, FR)

Medical biology is often ignored or misconducted in developing countries especially in rural remote areas. However, basic clinical

laboratories may be implemented with little means and without regular electricity access. This presentation will show how an isolate laboratory can work without regular access to electricity and share algorithm that have been developed in Madagascar to valorize simple biological data. The solutions proposed are:

**1** having reliable and educated human resources with a reliable laboratory management

**2** implementing useful tools that can be performed without electricity: (a) gram and MGG staining and all what can be concluded from their examination: algorithms have been built to help physicians Interpreting Laboratory Test Results and orientating their antibiotic choice according to gram staining and available bioresistance data; MGG staining is useful for the diagnosis of leukemia, anemia but also leishmaniosis, filariasis, donovan bodies...

(b) direct examination will help for the diagnosis of *Tinea capitis*, *Tinea corporis*, vaginosis, vaginitis, fecal parasites, ectoparasitosis.

(c) rapid test is a revolution for developing countries: they are stable, easy to practice, cheap and may be used for screening: (i) biochemical disorders: urine test, HCG, cardiac markers (ii) infectious diseases: HIV, B hepatitis serologies, malaria, cholera, *Helicobacter pylori*.

(d) RPR test is still a first step in the diagnosis of Syphilis though Vidal test should not be performed anymore for the diagnosis of Typhoid Fever.

**3** having autonomous and proper accounting practices.

#### **P1585** Imported paracoccidiomycosis in Spain

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**Objective:** The growing presence of immigrants from Latin America and the higher frequency of travels to South America has increased the incidence of endemic mycosis as Histoplasmosis and Paracoccidiomycosis (PCM) in Europe. In spite of PCM is the most prevalent mycosis in Central and South America, there are a few imported cases reported in Spain. The aim of this study is to report one case of PCM to alert of this unusual illness in our continent.

**Methods:** Review of the clinic history of a patient diagnosed of PCM.

**Case report:** A 47-year-old man who was born in Ecuador and has been living in Spain since 11 years ago. He presented a 3-month history of cough and haemoptysis with weight loss. He had also skin lesions in thorax and head. CT Scan showed lung's nodular lesions, some of them cavitates and mediastinal adenopathies.

Biopsies of bronchial-tree and thorax skin showed chronic granulomatous infiltrates with yeast-like structures. *P. coccidioides* PCR of skin biopsy was positive. Sputum and bronchoaspirates were incubated at 25 and 37°C for 2 months but they were negatives as blood cultures did. As a result of pathologic study and posterior PCR result, treatment with Anfotericin B was initiated and then switched to itraconazol. At the moment the lesions have disappeared and the patient is asymptomatic.

**Conclusion:** In our country, PCM is an imported infection and it is necessary to have a high index of suspicion, to perform a detailed history and do pathologic and microbiologic studies to get a diagnosis. PCR is the most rapid and sensitive technique to confirm the diagnosis.

#### **P1586** Accident by stingray: soft tissue infection caused by *Aeromonas caviae*

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**Objectives:** We report a serious accident caused by freshwater stingrays, with secondary infection caused by *Aeromonas caviae*.

**Methods:** Review of clinical records from Hospital Vital Brazil, Butantan Institute, Brazil and Department of Infectious and Parasitic Diseases, Clinical Hospital of School of Medicine, University of São Paulo, SP, Brazil.

**Results:** On 6 January 2011, 25 years old, female, biologist, diving in an aquarium in São Paulo city, when, accidentally, kicked one of the stingrays that was in the tanks and immediately developed severe pain in the lower third of leg left with a wound of 3 cm. She received medical care and the lesion was sutured. Seven days later she returned to medical care with fever, diarrhea, local cellulitis, with purulent hemorrhagic secretion, until the left knee and blistering. The sutures were removed, the lesion drained and intravenous ceftriaxone and clindamycin were introduced and the patient initially improved. After the third day the fever returned, with worsening edema and erythema. Abnormal results of laboratory tests showed mild leukocytosis with neutrophilia and normal renal and hepatic functions. Oxacillin, levofloxacin and a new surgical debridement was performed and four days later she presented exposure of tendons and necrotic tissue. Culture of secretions from the second approach revealed growth of *Aeromonas caviae* sensitive to third generation cephalosporin, imipenem and piperacillin/tazobactam. In January, 27 were removed microsurgical flaps of the abdominal region and grafted into the ankle successfully. The patient presented good evolution and regression of the oedema and the infectious process, and began physical therapy at the time of discharge.

**Conclusions:** Although rarely reported, accidents caused by aquatic venomous animals are common in both: Atlantic coast and Brazilian rivers. The injuries caused by freshwater stingrays often cause severe pain and local inflammatory lesions that frequently evolve to necrosis and secondary infection. The genus *Aeromonas* is responsible for significant number of diarrhea in people who drank contaminated water. Moreover, accidents caused by fins of freshwater fish can develop secondary infection.

\*The authors wish to thank to the Department/Division of Plastic Surgery at Clinical Hospital, Faculty of Medicine, University of São Paulo, Brazil.

**P1587 Spectrum of neglected tropical disease in rural clinic of Mihango (Kenya) within 5 years**

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**Objectives:** Kenya belongs to countries of Sub-Saharan Africa with state supported health care system and health infrastructure are based on community clinics by basic health care group – clinical officer or nurse, midwife or nurse, laboratory technician and pharmacies or pharmanurse. The aim of this study was to asses spectrum of neglected tropical disease in rural clinic of Mihango in Kenya within 5 years.

**Methods:** St. Raphael and St. Bakhita Clinic of St. Elisabeth University Clinic Tropical Programme serve health care for rural population of Mihango since 2006. Number of patients in last 5 years was 10 375–12 646. All together 51 364 patients has been seen on outpatient department after January 2006.

**Results:** Common diagnoses were respiratory tract infections (including pneumonia) increasing from 34% in 2006 to 46% in 2010, forwarded by diarrhoea without fever (18% in 2006 to 14.2% in 2010) and diarrhoea with fever (10% in 2006 to 11.5% in 2010), sexually transmitted diseases (STD – 9% in 2006 to 8.2 in 2010), malaria (18.2% in 2006 to 7.9% in 2010) and skin and soft tissue infections (9.7% in 2006 to 8.8% in 2010). The significant trend has been observed only in the decrease of microscopically confirmed malaria (from 12.2% in 2006 to 7.4% in 2010;  $p < 0.05$ ).

**Conclusion:** Commonest diagnoses were respiratory tract infections, diarrhoea, sexually transmitted infections, malaria and skin and soft tissue infections. Mortality was minimal (only four children died). Comparing trend in occurrence of infectious disease, any statistically significant trend has been observed apart of the decrease of malaria between 2006 and 2010 possibly associated with increasing use of bed nets and intermittent preventive treatment in school children after 2007.

**P1588 A case of imported progressive disseminated histoplasmosis in Madrid**

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**Introduction:** *Histoplasma capsulatum*, a thermally dimorphic fungus, is the etiologic agent responsible of histoplasmosis. The fungus is primarily found in soil, where it exists in a mycelia form. The most important endemic areas of this mycosis surround the American continent however its incidence in Spain has increased in recent years.

**Objective:** The aim of this document is to describe a clinical case of a HIV positive patient with progressive disseminated histoplasmosis (PDH).

**Methods:** A 38-year-old bisexual male, from Venezuela, was attended at the emergency department due to a respiratory infection with several weeks of evolution. The patient lived in Spain since 2000. He presented flu-like symptoms, weight loss, weakness and was diagnosed of HIV. Physical examination were unremarkable except an epigastric mass (10 × 10 cm). No adenopathies were found and chest X-ray was normal. The analysis highlighted leucopenia with marked neutropenia. The CD4 count was 14/mL. An abdominal ultrasound showed a homogeneous splenomegaly (14 cm) and mesenteric, abdominal and retroperitoneal lymphadenopathies. Serum, blood and urine were obtained for different studies. A fine needle aspiration biopsy (FNAB) of lymph node, bone marrow biopsy and a laparoscopic lymph node biopsy were performed and samples were sent to the Microbiology department for conventional culture and molecular biology procedures.

**Results:** VHC, syphilis, *Toxoplasma* and *Histoplasma* serologies, Mantoux and criptococcal antigen were all negative. HBV serology indicated past infection. All tuberculosis findings were negative. Blood PCR for *Histoplasma*, *Paracoccidioides*, *Plasmodium*, *Leishmania*, and *Trypanosoma* were as well negative. However, biopsy PCR was positive for *Histoplasma capsulatum*. Progressive disseminated histoplasmosis was diagnosed. The microorganism also grew in the biopsy culture.

A treatment based on liposomal amphotericin B induction was started. The patient presented a great improvement. He was discharged with the following treatment: trimethoprim/sulfamethoxazole, folinic acid, itraconazole, and the anti-retroviral therapy: tenofovir, emtricitabine and lopinavir/ritonavir.

**Conclusion:** This report highlights the need to consider the diagnosis of PDH among patients with acquired immunodeficiency syndrome, originated in endemic areas and presented with an indolent, prolonged febrile illness and very low CD4 count.

**P1589 An outbreak of histoplasmosis following a biology field trip in the Ugandan rainforest**

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**Objectives:** To describe an outbreak of pulmonary histoplasmosis in a multinational group of students following a biology field trip in a Ugandan rainforest.

**Methods:** A 22 year-old biology graduate was referred to Liverpool with suspected pulmonary histoplasmosis. Her symptoms began in Quebec, Canada, 12 days after finishing a field trip studying insects, primates and bats in the rainforest near Fort Portal, Western Uganda, close to the Congolese border. She had fever and flu-like symptoms progressing to mild breathlessness on exertion, dry cough and chest pain. A chest X-ray showed diffuse miliary shadowing and induced sputum was negative for acid-fast bacilli. On return to the UK she was reviewed in Blackpool Victoria Hospital for ongoing chest pain and exertional dyspnoea, 3 weeks after symptom onset. Oxygen saturation was 93% on air and her chest X-ray remained unchanged.

Histoplasmosis was confirmed serologically and she made a gradual recovery without antifungal medication.

She knew that some of her colleagues had developed similar symptoms since leaving the rainforest. With her assistance and that of physicians who responded to our alert in ProMED-mail, we were able to accumulate data on most of the other cases. This included a severely ill 21 year-old man who required intensive care admission and antifungal therapy in Cambridge (UK) and a 23 year-old female with milder symptoms in Dublin, Ireland.

**Results:** Out of 24 taking part in the project in Uganda, 13 students from 10 different countries (including the cases above) developed respiratory symptoms, eight of whom have received a final diagnosis of pulmonary histoplasmosis. Five were not in their country of residence when they first needed health care (two in Kenya, one in each of Uganda, Indonesia and Canada). At least six patients were initially thought to have miliary tuberculosis and two commenced antituberculous medication.

The principal risk exposure was probably entering a hollow tree infested with bats. This was the second largest tree in the forest and many of the scientists ventured inside it for a popular photographic opportunity.



**Conclusion:** Outbreaks of histoplasmosis in travellers are unusual, particularly in Africa, but a common source is often obvious (photograph). Histoplasmosis should be considered in the differential diagnosis of returning travellers with miliary shadowing on chest X-ray and risk factors for exposure. On-line e-alerts (e.g. ProMED-mail) help to identify such outbreaks.

#### **P1590** Impact of gastric acidity on acquisition of cholera infection post gastric bypass

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**Introduction:** *Vibrio cholerae*, a gram-negative bacterium, is the causative organism of the diarrheal disease cholera. Although *V. cholerae* is categorized as being highly acid sensitive, it thrives in the acidic environment of the stomach through expression of "acid tolerant" genes. Despite the presence of intrinsic survival mechanisms by some adapted strains, previous studies have illustrated that decreased acid secretion may predispose to infection with *V. cholerae*, and furthermore, may contribute to a more severe form of disease. Roux-en-Y gastric bypass results in decreased parietal cells and gastrin levels due to removal of the gastric fundus and pyloric antrum; this, subsequently produces hypochlorhydria. Gastric pH levels obtained in patients post gastric bypass reveal reduced acid secretion both pre and post-prandially. This report reveals a unique case of acquisition of cholera after a Roux-en-Y gastric bypass.

**Case description:** A 34-year-old African American female with a medical history significant for morbid obesity status post Roux-en-Y gastric bypass, hypertension and sleep apnea, presented to the hospital with a chief complaint of abdominal pain and multiple episodes of diarrhea and emesis. She reported recent travel to Haiti for 4 days. Physical examination was remarkable for epigastric tenderness, and dry mucous membranes. Laboratory findings demonstrated acute renal failure, metabolic acidosis, and electrolyte abnormalities. Patient's admitting diagnosis was infectious diarrhea of amoebic or parasitic etiology. Medical management consisted of aggressive rehydration and antibiotic therapy. A total of 4.5 L of watery rice appearing stools were produced per day. On hospital day 6, stool WBC was positive and stool cultures revealed *V. cholerae* serotype O1 and *Aeromonas hydrophila*. Doxycycline was initiated resulting in significant reduction in bowel movements and improvement in overall hydration status. The patient was discharged after a hospital course of 11 days and was scheduled for follow-up in the bariatric clinic.

**Discussion:** This is a unique case of *V. cholerae* infection, illustrating the potential additive risk of its development in a patient in a hypochlorohydric state. Gastric acidity of the stomach provides a natural barrier to the establishment of cholera, which may be altered in patients after gastric bypass procedures. Further research involving gastric pH testing is warranted in order to evaluate this association.

#### **P1591** Clinical and epidemiological peculiarities of the tetanus cases in Georgia

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**Objectives:** Tetanus is a preventable disease. The morbidity from tetanus is directly related to the faults of vaccination. Despite the detailed knowledge of the molecular mechanism of the disease the lethality from tetanus remains high in Georgia as well as worldwide. This research aims to identify clinical and epidemiological aspects of tetanus in Georgia.

**Methods:** We retrospectively studied the case histories of patients hospitalized in the Infectious Diseases, AIDS and Clinical Immunology Scientific Practical Center of Georgia with the diagnosis of tetanus during 2006–2010.

**Results:** During 2006–2010 totally 22 cases of tetanus were registered in Georgia; the lethal outcome was identified in two cases (27, 3%). Twelve patients (eight males, four females) had undergone treatment in the Infectious Diseases, AIDS and Clinical Immunology Scientific Practical Centre of Georgia, with four lethal cases (33%). From 12 patients admitted at our hospital four were Azerbaijanian and four were Georgians. The age of the patients was as follows: one patient – 3 years old, one patient – 6 years old, 1 – patient 15 years old and nine patients >51 years old. The oldest patient was 82 years old. Two patients were not vaccinated, one patient was vaccinated once and the vaccination status of nine patients was unknown. Six patients had injuries on the lower extremities, two patients – on the upper extremities and four patients were injured on the head.

The average incubation period was 6 days. The patients were admitted at our hospital at the median 4th day of the illness. The onset of the cardinal manifestations was with the facial and palat pain and trismus. The pain irradiated towards the back and extended to the extremities on the following days. Opisthotonos was developed in five cases. All lethal cases were accompanied with hectic fever.

The following complications were detected: pneumonia – in half of the patients (50%), myocarditis – one patient (8%), necrotizing cellulitis on the injury site – one patient (8%). The treatment consisted of antitoxin and antibiotics.

**Conclusion:** The morbidity from tetanus was registered in our hospital mainly in patients older than 51 years, mainly in patients with unclear immunization status and patients with injuries on lower extremities. The mean onset of the disease from the injury was 6 days. They were hospitalized on the 4th day of the illness. Despite the adequate treatment the overall lethality was 33%.



**P1592** Fluoroquinolone resistance of *Orientia tsutsugamushi* and clinical use in severe scrub typhus

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**Objectives :** Although doxycycline remains the standard therapy for the treatment of scrub typhus. Some reports recommend levofloxacin may be effective in instances where treatment with doxycycline fails. On the other hand, there are clinical evidences that fluoroquinolones are ineffective. These discrepant results may be associated with fluoroquinolone resistance according to genotypes. Therefore, we analyzed genotypes and quinolone resistances and clinical characteristics

**Methods:** This prospective observational study included 49 patients admitted to a tertiary hospital with scrub typhus in 2010. We sequenced the *Orientia tsutsugamushi* – specific 56-kDa protein gene and quinolone resistance determining region (QRDR) of the *gyrA* gene, the target of fluoroquinolones with samples of blood.

**Results:** Of the 48 PCR- positive samples, 47 clustered with the Boryong previously isolated in Korea. Among them, seven had Ser83Leu mutation in their QRDR domain that is known to be associated with quinolone resistance. Five cases of quinolone resistant strains had complications including septic shock (two cases), pneumonia (1), meningitis (1), acute kidney injury (1).

**Conclusions:** We identified fluoroquinolone resistance in most common genotype, Boryong in the southern part of Korea and suggest that fluoroquinolones should not be used in the treatment of severe scrub typhus.

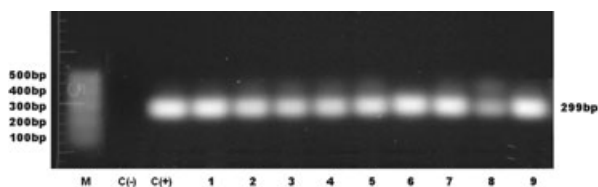
**P1593** Contribution of *Bacillus oleronius* to Demodex chronic blepharitis

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**Objective:** *Bacillus oleronius* was isolated for the first time from the hindgut of the termite *Reticulitermes santonenensis* in 1995. In 2007 presence of *B. oleronius* was demonstrated inside *Demodex mites*. In view of the above this study aimed at analysis of *Bacillus oleronius* involvement in pathogenesis of Demodex-induced chronic blepharitis.

**Materials and methods:** The studies were conducted on 68 adult patients, among whom ophthalmological and parasitological tests permitted to distinguish a group of 38 patients with diagnosis of Demodex-related chronic blepharitis (group 1, including a subgroup 1a with moderate blepharitis and a subgroup 1b with severe blepharitis) and a group of 30 healthy individuals (group 2). In every studied person six eyelashes were epilated from each eye and a number of Demodex per eyelash was scored. Demodex was detected under a light microscope using 10% solution of KOH. In parallel, cultures of eyelashes was performed in nutrient broth (Difco), tryptic soy agar and sheep blood agar, at the temperature of 37°C for 24 hours. *Bacillus oleronius* was identified conducting Flagella Stain Droppers test and using PCR to detect the gene sequence for 16S rRNA.

**Results:** In the studies intensity of Demodex infestation manifested no significant differences between subgroups 1a and 1b. From the epilated eyelashes 23 bacterial isolates were obtained, identified as belonging to *B. oleronius* species (Fig. 1). Using the epilated eyelashes, culture permitted to isolate 23 strains of bacteria, originating from 18 patients



of group 1 (two strains were isolated from patients of subgroup 1a and 16 strains were isolated from patients of subgroup 1b) and from five patients of group 2. Difference in the frequency of *B. oleronius* detection in patients of subgroup 1b and healthy persons (group 2) was significant ( $p = 0.0190$ ).

**Conclusion:** The result indicate that *B. oleronius* bacteria, most probably act as a co-pathogen in development of Demodex blepharitis severe forms.

Fig. 1. PCR detection of *Bacillus oleronius*. Ethidium bromide-stained agarose gel. M – 100 bp molecular weight standard. C(-) – negative control. C(+)- positive control. 1-9 – positive patients.

**P1594** Evaluation of the painful sensation in accidents caused by spiders, scorpions and lepidopteran larvae (caterpillars): Hospital Vital Brazil, Butantan Institute for the Health Secretariat of São Paulo

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**Objective:** The objective of this study is to compare the epidemiological, clinical and therapeutic accidents caused by spiders, scorpions and caterpillar seen at the Hospital Vital Brazil (HVB).

**Methods:** This is a prospective observational study, which collected information from the animal that caused the accident, the variables related to patients and also the circumstances of the accident, the clinical picture (mainly the pain), the treatment applied and the final evolution.

**Results:** The period of data collection began in July 2009 and intends to include patients treated until October 2011.

By June 2011, 162 protocols were completed, of which 75 met the inclusion criteria and will be reviewed in this summary. Among the epidemiological variables of patients, we have: 39 (52%) were men, 62 (83%) accidents occurred in urban areas. The accidents were caused by 35 spiders (46%), 17 scorpions (23%) and 23 caterpillars (31%). Regarding the assessment of pain intensity was used a numerical scale ranging from 0 to 10, resulting in a median of five to spiders, seven to scorpions and seven to caterpillars. Our study aims to include all patients admitted to the HVB who have been injured by spiders, larvae of Lepidoptera or scorpion regardless of whether or not brought the animal.

**Conclusions:** In Brazil, in 2009, 45.721 accidents were reported by scorpions, 18 687 by spiders and 3387 were caused by larvae of Lepidoptera. The key characteristic of these three injuries is the local pain, usually acute and intense. Sometimes there are other flogistic abnormalities.

This project will propose to the health professionals of HBV and other health facilities that meet envenomations some routines: (i) Establish an assessment of pain as the 5° vital sign; (ii) Therapeutic approach of the painful phenomenon based on the guidelines already established in literature and evaluation of its effectiveness; (iii)- Add items 1 and 2 in the routine care of all accidents caused by venomous animals; (iv) Allow the implementation of future projects of comparative evaluation of different therapeutic approaches, from the standpoint of efficiency and economic.

**P1595** Tropical pyomyositis in children: 10-year experience of a tertiary care hospital in northern India

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**Objective:** Tropical pyomyositis (TP) is characterised by supuration within skeletal muscles, manifesting as single or multiple abscesses. It has been reported frequently from Africa and Latin America. However there are only a few reports in children from India.

**Material and methods:** Between July 2001 to June 2010, 40 children with TP were admitted to our hospital, their retrospective review formed the study material.

**Results:** The disease was common in children above 5 years (mean age 7.75 years) with male to female ratio being 2:1. Majority (55%) were reported between July and September, which coincides with the rainy season. Most of the patients were admitted with short history of localised swelling (100%), pain (100%) and fever (95%). Muscles most commonly involved were thigh (58%), leg (35%), psoas (30%), gluteal (20%), arm (15%), chest wall (12%), back (10%), forearm (8%) and abdominal wall (3%). Number of muscles involved in each varied from 1 to 5 (mean 1.97). Majority (68%) presented with muscle abscess (stage II) and only few (25%) with septic shock (stage III). History of preceding trauma/intramuscular injection was present in 30% cases.

Diagnosis was confirmed by aspirating pus from the involved muscles. The pus grew organism in 42.5% cases and all of them (100%) showed methicillin sensitive *Staphylococcus aureus* (MSSA). Initial blood cultures in all of them were sterile; on later stay three of them grew *Acinetobacter* spp., probably hospital acquired. USG was investigation of choice in most of them, which showed bulky muscles, loss of intermuscular planes and pus collection in different muscle groups suggestive of pyomyositis. CT scan and MRI were done only in few, which confirmed the findings of USG only. All children were treated with antibiotics for a period of 7–45 days depending on severity (mean 21 days). Surgical drainage of pus was done in 87.5% cases, amount of pus aspirated varied from 5 to 2000 mL. The common complications were arthritis (25%), pneumonia (17.5%) and osteomyelitis (5%). Arthritis in most of them was reactive (sterile), which recovered spontaneously, while only two had purulent arthritis which needed drainage. Duration of stay in hospital varied from 2 to 42 days (mean 15.7 days). All of them recovered and discharged; no mortality.

**Conclusions:** TP is not an uncommon disease in children in Northern India. Physicians should become more familiar with this infective disease entity, as early recognition and aggressive treatment could be life saving.

#### **P1596 Clinical features of mortality cases with dengue during 2010–2011 epidemic in Caquetá, Colombia**

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**Objectives:** Dengue is the most important arthropod-borne viral disease of public health significance. We aimed to determine the clinical features and causes of mortality of patients with dengue during 2010–2011 epidemic (January 2010–November 2011) in Florencia – Caquetá, Colombia.

**Methods:** Retrospective analysis of hospitalized patients with dengue in two hospitals from Florencia – Caquetá, Colombia. Dengue mortality cases were confirmed with polymerase chain reaction and/or histopathology.

**Results:** During 2010–2011 epidemic, there were an incidence rate of dengue 176.4/100 000 inhabitants in Caquetá – Colombia. There were six mortality cases. The median age of mortality cases was 27.5 years (range 1–82) and five patients were male sex. Only one patient had comorbidities (mild chronic kidney disease). The median time from symptoms onset to hospital admission was 4.5 days (range 2–8). All patients had fever and nearly half had headache, jaundice, and abdominal pain at hospital admission. Thrombocytopenia was present in 50% of cases. All patients required ICU admission and mechanical ventilation. The main causes of mortality were respiratory failure and multiple organ dysfunctions. The median time from hospital admission to dead was 5.5 days (range 3–78). Only one patient had positive immunochromatographic test for the detection of IgM antibodies to dengue virus.

**Conclusion:** During the 2010–2011 dengue epidemic in Caquetá – Colombia, the mortality was low. Most patients who died were younger without comorbidities. In this study, immunochromatographic test for the detection of IgM antibodies to dengue virus was no useful for

identify patients with this disease. More specific diagnostic tools should be use for the diagnosis of dengue in this region.

#### **P1597 Dengue infection-associated brachial plexopathy: the first case and review of the literature**

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**Objectives:** Many viral infections have been reported to be associated with brachial plexopathy. To our knowledge, dengue infection-associated brachial plexopathy has never been reported in the literature. We report a case of dengue infection complicated by bilateral brachial plexopathies, and also review all cases with viral infection-associated brachial plexopathy in the English literature.

**Methods and results:** A 62-year-old Thai woman presented with fever, orthopnea, and pain at neck and interscapular area aggravated by movement for 3 days. Neurological examination revealed bilateral winged scapulae, bilateral diaphragmatic paralysis, and hyporeflexic weakness of right biceps brachii, right serratus anterior muscle, and infrapinatus muscles. Complete blood count was normal. Electrodiagnostic tests indicated bilateral brachial plexopathies. Polymerase chain reaction (PCR) showed negative results for Varicella-zoster virus (VZV), Herpes simplex virus both types 1 and 2, Cytomegalovirus (CMV), Epstein-Barr virus (EBV), pan-Enteroviruses, and West Nile virus (WNV). Reverse-transcription nested PCR test for 3' untranslated region of dengue virus showed positive results in serum and peripheral blood mononuclear cells, and negative result in the cerebrospinal fluid. And PCR test for Japanese encephalitis virus showed negative results in all samples. Three days after hospitalization, the patient developed ventilatory failure. She was doing well without BiPAP ventilatory support and nearly complete recovery of weakness of all muscles when last seen 6 months after being discharged. To date, there are 25 patients with viral infection-associated brachial plexopathy. Of DNA viruses, VZV is the most common causative agent, followed by Parvovirus B19, CMV, EBV. Of RNA viruses, there are hepatitis E, HIV, and WNV. There are 14 males, 10 females, and 1 patient with unknown gender. The age ranges from 9 to 86 years. The outcomes of brachial plexopathy were excellent.

**Conclusion:** Dengue infection has been reported to be associated with neurological complications including encephalopathy, transverse myelitis, Guillain-Barré syndrome, mononeuropathies, polyneuropathies, and aseptic meningitis. To our knowledge, dengue infection-associated brachial plexopathy has never been reported in the literature. Dengue infection should be included in the differential list of viral infection-associated brachial plexopathy.

#### **Pharmacodynamic analyses and antibiotic combinations: *S. aureus*, MDR-*Klebsiella* and others**

##### **P1598 Differential toxin gene expression and regulation in community-associated MRSA USA300 with pharmacodynamic antibiotic exposures in an in vitro hollow fibre model**

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**Objectives:** CA-MRSA virulence is a complex process due to its multiple toxins and their expression modulation at the transcript level with pharmacokinetic/pharmacodynamic (PK/PD) antibiotic exposures. The objective of this study was to model CA-MRSA strain USA300 kill and potential virulence modification with clinical exposures of five antibiotics in an in vitro hollow fiber PK/PD model.

**Methods:** USA300-0114, the predominant CA-MRSA strain, was cultured in the hollow fiber PK/PD model at  $10^6$  CFU/mL. PK simulations of clindamycin 600 mg q8h (CL), linezolid 600 mg q12h

(LI), minocycline 100 mg q12h (MI), trimethoprim 160 mg/sulfamethoxazole 800 mg q12h (TS), and vancomycin 1 g q12h (VA) were targeted individually at clinical free drug exposures. Relative quantification of three toxin genes, *sek*, *seq*, and *lukSF-PV* transcripts were determined against an endogenous control gene, *gyr*, by qRT-PCR first without antibiotics and then at 0, 8, and 24 hours of antibiotic exposure. Fold changes in gene expression with antibiotics were based on the differences of changes from the control (no antibiotic) and compared statistically by an analysis of variance model.

**Results:** All five antibiotics were bacteriostatic in the model. LI had the greatest maximum kill activity (2.7 log CFU/mL), followed by CL (2.2), VA (2.1), MI (2.0), and TS (0.7). LI was the only agent with sustained antibacterial activity, while >4 log CFU/mL bacterial regrowth occurred after 24 hours of exposure with CL, T/S, and MI. Toxin expression from the USA300-0114 strain increased during the exponential growth phase in PK/PD model and peaked at 8 hours. The highest relative gene expression occurred with *lukSF-PV* followed by *seq* and *sek*. CL was the most potent inhibitor of toxin gene expression with 7.9–23-fold reduction at 24 hours of therapy ( $p < 0.05$ ). LI inhibited the toxin genes expression 1.3–7.4 fold ( $p < 0.05$  with >5-fold change), while exposures of MI and TS had only minor effects on these transcripts ( $\pm 2$  fold). VA upregulated gene expression 5.3–8.8 fold at 24 hours ( $p < 0.05$ ).

**Conclusion:** In our hollow fiber PK/PD model, LI had the greatest sustained USA300-0114 kill along with downregulation of the *sek*, *seq*, and *lukSF-PV* transcription. VA increased virulence potential during therapeutic exposures and suggests the need for adjunct therapy with CL or LI for optimum virulence inhibition. Optimal antibiotic therapy for infections due to pandemic CA-MRSA clone USA300 may integrate bacterial kill and virulence inhibition.

**P1599** Population probability of target attainment of daptomycin at different levels of renal function against methicillin-resistant *Staphylococcus aureus* in European medical centres

A. Farkas\* (Nyack, US)

**Objectives:** Empiric dosing of Daptomycin (DAP) to achieve a Cumulative Fraction of Response (CFR) of >90% based on the MIC distribution of European MRSA isolates at various levels of renal function has not been examined. Consequently, the optimal empiric dosing regimen for DAP in serious infections remains unclear. The objective of this study was to describe the population Probability of Target Attainment (PTA) of DAP against recently documented MRSA isolates at the doses recommended by the manufacturer for less severe infections. Additionally, alternative dosing recommendations for DAP when treating serious infections will be established.

**Methods:** Published pharmacokinetic ( $n = 282$ ) and MIC data of MRSA isolates for DAP were used in this analysis. Doses up to 12 mg/kg were evaluated for population PTA with Monte Carlo Simulation (MCS,  $n = 10\,000$ ) for different levels of renal function at the MIC ranges of 0.125–2 mg/L. CFR was calculated for each regimen targeting AUC/MIC ratios necessary to achieve stasis, a killing of 1 and 2 log<sub>10</sub> CFU derived from seven *Staphylococcus aureus* isolates at different pre-treatment titer conditions. The minimum amount of drug and the respective dosing interval necessary to produce a CFR > 90% was then evaluated for the chance of clinically significant CPK elevation using MCS and a two compartment model with first order output.

**Results:** Manufacturer recommended 4 and 6 mg/kg DAP regimens are expected to achieve the CFR > 90% for stasis and a killing of 1 log<sub>10</sub> CFU at all levels of renal function from 20 to 120 mL/minute. The MCS also showed that the 6 mg/kg dose will result in sub-optimal population PTAs empirically for patients with serious infections where achieving the pharmacodynamic index that result in a killing of 2 log<sub>10</sub> CFU would be desired. The probability for clinically significant CPK elevations showed to be similar across all renal function categories with the newly proposed dosing recommendations.

**Conclusion:** We conclude that for the treatment of serious MRSA infections to achieve the killing of 2 log<sub>10</sub> CFU, the revised dosing

method would provide better empiric population PTA compared to manufacturer recommendations. Doses of 4 and 6 mg/kg provide adequate empiric coverage for patients with less severe infections.

**P1600** Influence of demographic and pathophysiological factors on the probability of target attainment for intravenous antibiotics used in the treatment of methicillin-resistant *Staphylococcus aureus* complicated skin and soft-tissue infections

P. McCormack\*, J. Borrill (Macclesfield, UK)

**Objectives:** Pharmacokinetic-pharmacodynamic (PK-PD) Monte Carlo simulation has been widely used as a tool to predict the probability of target attainment (PTA) for different antibiotics against specific bacteria. This study aimed to investigate the influence of patient demography and pathophysiology on the PTA for standard doses of intravenous antibiotics used in the treatment of hospitalised adults with complicated skin and soft-tissue infections (cSSTI) caused by methicillin-resistant *Staphylococcus aureus* (MRSA).

**Methods:** PK-PD models, sourced from the literature, were developed for daptomycin (4 mg/kg every 24 hours [q24hr]), linezolid (600 mg q12hr), teicoplanin (400 mg q24hr), tigecycline (50 mg q12hr), and vancomycin (1 g q12hr). Simulations were performed using MRSA MIC<sub>90</sub> values reported for European isolates. Patient demographics and pathophysiology were systematically varied in the model: age 30–50, 51–70, 71–90 years, body weight (BW): 51–80, 81–110, 111–140 kg and creatinine clearance (CLCR): mild (60–89 mL/minute), normal (90–160 mL/minute) and augmented (160–190 mL/minute). PTAs were assessed. An adult with suspected MRSA cSSTI aged 40 years, BW 70 kg and CLCR 110 mL/minute was chosen as a point of reference.

**Results:** This analysis suggests that the PTA for antibiotics used in the treatment of cSSTI caused by MRSA is dependent not only on the MIC<sub>90</sub> for the pathogen, but also on the demographic and pathophysiological characteristics of the patient. For illustration, of the antibiotics studied, the PTA for linezolid appeared most sensitive to variations in BW within these simulations (Table). The PTA for linezolid decreased within increasing BW. For patients with BW (51–80 kg), the PTA was 65% whereas the PTA for patients with higher BW (111–140 kg) was 39%. Further analysis on the other demographic characteristics will be presented.

Target attainment based on MIC<sub>90</sub> against MRSA for five antibiotics used in the empiric treatment of MRSA-suspected cSSTI: examination of the effect of body weight in a patient with mean age of 40 years and CLCR of 110 ml/min

Antibiotic	Probability of target attainment			MIC <sub>90</sub> (mg/l)
	51–80 kg	81–110 kg	111–140 kg	
Daptomycin (4 mg/kg q24hr)	82%	98%	100%	0.5
Linezolid (600 mg q12hr)	65%	47%	39%	2.0
Teicoplanin (400 mg q24hr)	5%	7%	7%	2.0
Tigecycline (50 mg q12hr)	98%	90%	79%	0.25
Vancomycin (1 g q12hr)	71%	77%	87%	1.0

**Conclusion:** In this study, the age, BW and CLCR of the patient population were shown to have a marked impact on the PTA for antibiotics used in the treatment of adults with cSSTI caused by MRSA. Although renal function is well recognised as an influential factor in PTA, clinicians may also want to consider other patient characteristics when deciding on the most appropriate choice of antibiotic.

**P1601** **In vitro evaluation of daptomycin in combination with other drugs against difficult to treat staphylococci and enterococci**

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**Objectives:** Daptomycin is a bactericidal antibiotic active against difficult to treat staphylococci and enterococci. As a substantial proportion of patients do not achieve adequate outcome, for the MDR phenotype of an increased number of strains, antimicrobial combinations are frequently used to provide broad-spectrum empiric coverage in treatment who are seriously ill and who may be septicemic. This study was designed to determine the in vitro interaction of daptomycin with different drugs against MDR *S. aureus* and MDR enterococci strains, to measure the cidal activity of antibiotic combinations with daptomycin and to obtain a dynamic picture of their possible interactions.

**Methods:** Thirteen *S. aureus* and 16 *Enterococcus* spp. strains were included in the study: seven HA and CA MRSA clinical isolates (including 2 hVISA); two MRSA controls (VISA and VRSA – NARSA collection) and four MSSA; 16 *E. faecalis* (including one VanA and five HLRG), five *E. faecium* (all AmpR and 2 HLRG), one *E. gallinarum* VanC and one *E. avium*. MICs for all drugs were performed by CLSI standard methods. Daptomycin activity in combination with different antibiotic was evaluated by time-kill curves, following standard methods; for staphylococci were used rifampin, gentamicin, linezolid, fosfomicin, fusidic acid, oxacillin, piperacillin/tazobactam, levofloxacin, co-trimoxazole and ampicillin; for enterococci were used rifampin, gentamicin and ampicillin.

**Results:** The combination of daptomycin and gentamicin or oxacillin was found to be synergistic for 7/9 MRSA including NARSA strains. Even the combination of daptomycin and piperacillin/tazobactam, and levofloxacin has been shown to exert killing activities for 6/9 MRSA. For all MSSA strains tested, time-kill studies confirmed synergy between daptomycin and levofloxacin or fosfomicin, while indifference between daptomycin and cotrimoxazole was observed.

Among *E. faecalis* strains, daptomycin was found to be synergistic in 9/16 strains with ampicillin, 10/16 with gentamicin or rifampin (including the VRE). Among *E. faecium* strains, a higher synergistic effect was observed with daptomycin plus ampicillin (5/5), gentamicin (4/5) or rifampin (2/5), including one HLRG strain. *E. gallinarum* and *E. avium* strains showed variable behaviors.

**Conclusions:** Our in vitro data demonstrate the good synergistic interaction of daptomycin in combination with diverse antibiotics, either for MDR *S. aureus* and MDR enterococci, suggesting to be microbiologically beneficial and not antagonistic.

**P1602** **Pharmacodynamic analysis of the susceptibility of intracellular methicillin-susceptible, methicillin-resistant and linezolid-resistant *Staphylococcus aureus* to ceftaroline**

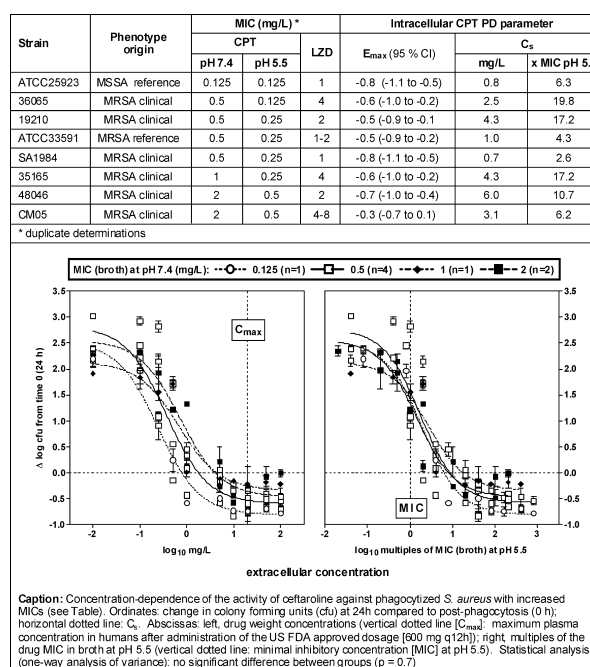
A. Melard, S. Lemaire\*, P. Tulkens, F. Van Bambeke (Brussels, BE)

**Objectives:** *S. aureus* is a facultative intracellular pathogen, explaining the persistent and recurrent character of staphylococcal infections. Ceftaroline (CPT; active metabolite of the prodrug ceftaroline fosamil), is a new broad spectrum cephalosporin with activity against *S. aureus* resistant to methicillin (MRSA) and linezolid (LZD). Our aim was to quantitatively assess the activity of CPT against intracellular forms of *S. aureus* using strains with different susceptibilities to CPT and LZD.

**Methods:** Strains: Nine strains with MICs (mg/L; pH 7.4) from 0.125 to 2 for CPT and 1 to 8 for LZD (see Table for resistance phenotype); MICs determined at pH 7.4 and 5.5 (to mimic plasma and intracellular environments, respectively) in MH broth (microdilution). Cells: human THP-1 macrophages (unstimulated). Phagocytosis: opsonized bacteria (45 minutes; fresh human serum) at a bacteria:cell ratio of 4 (1 hours; 37°C), and elimination of non-internalized bacteria by washing and 45-

minutes incubation with 50 mg/L gentamicin. Assessment of activity: Incubation of cells (24 hours) with CPT (0.01–100 mg/L), followed by cell collection (centrifugation and washing in PBS) and enumeration of cell-associated bacteria (CFU/mg cell protein). Results are expressed as the change in intracellular inoculum at 24 hours compared to time 0, with data used to fit a Hill equation of the dose-response for determination of static concentration ( $C_s$ ) and maximal relative efficacy ( $E_{max}$ ).

**Results:** Table: The MICs of CPT towards MRSA were systematically 1–2 dilutions lower at pH 5.5 vs. pH 7.4. Table and Figure: (i)  $E_{max}$  was between  $-0.3$  and  $-0.8$  log<sub>10</sub> CFU decrease compared to post-phagocytosis inoculum; (ii) left panel: strains with a higher MIC (broth pH 7.4) showed a trend (not significant) toward correspondingly higher  $C_s$ , with the highest value, however, still remaining  $< C_{max}$  (see Table); (iii) right panel: all strains behave alike when data are plotted against multiples of MIC at pH 5.5, with a  $C_s$  about eight-fold larger than MIC at that pH; (iv) a LZD resistance phenotype was without effect on CPT activity.



**Conclusions:** CPT is active against intraphagocytic *S. aureus* (disregarding their resistance phenotype to beta-lactams or LZD), with MICs (broth pH 7.4) up to 2 mg/L. The ~8-fold higher difference between the  $C_s$  and MIC at acid pH may be a function of the kinetics of CPT uptake or reflect effects of the intracellular environment on CPT activity.

**P1603** **High-dose daptomycin vs. vancomycin, alone or combined with clarithromycin or rifampin against methicillin-resistant *Staphylococcus epidermidis* in an in vitro pharmacokinetic/pharmacodynamic model of bacterial biofilm**

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**Objectives:** Medical device infections represent a treatment challenge for clinicians. We evaluated the activity of high dose D and V alone and in combination with R or C against a *S. epidermidis* isolate in an in vitro PK/PD model of bacterial biofilm.

**Methods:** One MRSE (R461) isolate was evaluated in a CDC biofilm reactor with titanium (TT), teflon (TE), and steel (ST) coupons for 112 hours (40 hours of biofilm maturation + 72 hours of drug therapy). Simulated regimens included D 10 mg/kg/day ( $fC_{max}$  11.3 mg/L,  $fAUC_{0-24}$  83.1 mg hour/L,  $t_{1/2}$  8 hours) and V 2g q12h ( $fC_{min}$  10 mg/L,  $t_{1/2}$  6 hours), alone or in combination with R 600 mg q24h ( $fC_{max}$  3.5 mg/L,  $t_{1/2}$  3 hours) or C 250mg q12h ( $fC_{max}$  1 mg/L,  $t_{1/2}$  3.5 hours). Media and coupons were assessed for the presence of viable planktonic (P) and biofilm embedded bacteria (EB) at 0, 4, 8, 24, 48, and 72 hours. Differences in CFU/mL were evaluated by ANOVA with a Turkey's post-hoc test. Bactericidal activity was defined as  $\geq 3$ -log<sub>10</sub> CFU/mL decrease in colony count.

**Results:** MIC values were D 0.25, V 1, R < 0.0625 and C > 32 mg/L. D + R produced rapid and sustained bactericidal activity against EB cells (4.69, 3.80 and 4.47 log<sub>10</sub> CFU/mL for TT, TE and ST, respectively). D + R was significantly more efficacious than D, D + C, V (24–72 hours,  $p < 0.05$ ), and V + R (24 hours,  $p < 0.05$ ) for decreasing EB CFU/mL. D + R demonstrated significantly more change from baseline (delta T0 to T72 hours log<sub>10</sub> CFU/mL) in EB compared to D, D + C, V and V + R ( $p < 0.05$ ). Resistance to R developed at 72 hours in the presence D + R and V + R in P and EB cells on all materials. Reduced susceptibility to D (>1.5 mg/L) was found in P and EB cells from TT, TE and ST at 72 hours with D monotherapy and D + C regimens, but not with D + R. Intermediate susceptibility to V developed with V monotherapy in P and ST EB cells.

**Conclusion:** High dose D in combination with R was the most effective regimen, displaying sustained cidal activity to 72 hours against mature biofilms of MRSE grown on ST, TT and TE coupons. This antimicrobial combination represents a promising option to treat persistent MRSE biofilm infections on these materials.

**P1604 Evaluation of standard and high-dose daptomycin vs. linezolid against vancomycin-resistant *Enterococcus faecalis* in an in vitro model of simulated endocardial vegetations**

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**Objectives:** Daptomycin (DAP) displays concentration-dependent pharmacodynamics. DAP minimum inhibitory concentrations (MIC) for enterococci are typically 1–2-fold higher than for *Staphylococcus aureus*. Based on Emax models, higher dosages of DAP may be needed to adequately treat enterococcal infections. We investigated the bactericidal activity of DAP at varying dose exposures vs. linezolid (LZD) against vancomycin-resistant *Enterococcus faecalis* (VREF) SF11496 in an in vitro Pharmacokinetic/Pharmacodynamic (PK/PD) simulated endocardial vegetations (SEV) model.

**Methods:** The killing effects of DAP 6 (D6,  $C_{max}$  93.9 mg/L,  $t_{1/2}$  8 hours), 8 (D8,  $C_{max}$  123.3 mg/L), 10 (D10,  $C_{max}$  141.1 mg/L), 12 (D12,  $C_{max}$  183.7 mg/L) mg/kg/day, AUC 0-24 632–1277 mg hour/L, and LZD 600 mg ( $C_{max}$  15.1 mg/L,  $t_{1/2}$  5 hours) q12h were evaluated against 1 VRE isolate (SF11496) in a 96 hours in vitro PK/PD SEV model and media supplemented with albumin (3.5 g/dL). Bactericidal and bacteriostatic activity were defined as a  $\geq 3$ -log<sub>10</sub> CFU/g (99.9% decrease) or a <3-log<sub>10</sub> CFU/g reduction in colony count from the initial inoculum, respectively. Early bactericidal activity was defined as  $\leq 24$  hours. Model samples were plated on DAP and LZD-containing agar for detection of resistance.

**Results:** Against VREF SF11496, (DAP MIC = 0.5 mg/L, LZD MIC = 1 mg/L), D10 and D12 displayed early (4 hours) bactericidal activity sustained to 96 hours. D10 and D12 had a significantly greater reduction in colony counts than D6, D8 and LZD (48–96 hours,  $p \leq 0.011$ ). LZD demonstrated no appreciable activity. Decreased susceptibility to DAP developed in SF11496 at 24 hours when exposed to DAP6 and DAP8, producing a 32-fold increase in MIC (0.5–16 mg/L). Reduced susceptibility (DAP MIC = 8 mg/L) was seen in one

sample in one model against DAP10. No resistance was seen with DAP12 or LZD.

**Conclusions:** DAP displayed a dose-dependent response against VREF. High-dose DAP (D10 and D12) had a more optimized pharmacodynamic profile producing bactericidal activity against VREF with minimal to no resistance. Further research is warranted.

**P1605 In vitro activities of fosfomicin and gentamicin combinations against clinical isolates of *Staphylococcus aureus***

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**Objectives:** Daptomycin has proven rapid bactericidal effect against methicillin-resistant and susceptible *S. aureus* (MRSA, MSSA), however persistent bacteraemia under daptomycin therapy has been reported. Different combinations have been described to improve outcome although controversies still exists.

**Methods:** Susceptibility testing was performed in duplicate by the E-test method using vancomycin (VAN), levofloxacin (LEV), daptomycin (DAP), Linezolid (LNZ), oxacillin (OX) and imipenem (IMP), gentamycin (GM) and Fosfomicin (FOS). Quality control was performed using *S. aureus* ATCC 29213. Four isolates from our collection recovered from patients with persistent bacteraemia (two MRSA and two MSSA), were evaluated by time kill experiments (TK) against VAN, DAP, LNZ and IMP at 1, 2, 4 8× MIC alone and at 1 and 4× MIC in combination with 0.5 and 2× MIC of FOS and GM (MRSA) and against OX, LEV, DAP and VAN at 1, 2, 4 8× MIC alone and at 1 and 4× MIC in combination with 0.5 and 2× MIC of FOS and GM (MSSA), using a starting inoculum of 5–6 log CFU/mL. Bactericidal activity was defined as  $\geq 3$  log<sub>10</sub> kill compared to the starting inoculum.

**Results:** DAP was the most effective drug against all tested isolates, exhibiting cidal activity within 4 hours at either 4× or 8× the MIC. VAN was cidal at 4 and 8× MIC at 8 hours against MRSA and at 24 hours against MSSA. LEV and OX were cidal against MSSA strains at 8 hours. In contrast, LNZ and IMP were bacteriostatic at either 4× and 8× MIC against the MRSA strains. DAP-FOS combination was the most effective against all strains tested being cidal at 4 hours three combinations (1 and 4× MIC [DAP] with 2× MIC [FOS] and 4× MIC [DAP] and 0.5× MIC [FOS]). IMP-FOS combinations at high doses were also cidal at 8 hours. Finally VAN-FOS combinations were cidal at high doses at 24 hours. GM combinations showed similar pattern being slightly more active than FOS combinations with VAN and LEV. Finally, LNZ combinations performed better than LNZ alone, being LNZ at 4× MIC with either FOS and GM at 2× MIC bactericidal at 24 hours.

**Conclusions:** At 4 and 8× MIC neither FOS nor GM combinations appeared to be better than DAP alone, however at 1 and 2 MIC, combination therapy with 2× MIC resulted in a significantly shorter time to achieve cidal activity. OX and VAN combinations with FOS did not result in shorter time to cidal activity suggesting a limited role of this combination. Further investigations are needed to better define the therapeutic value of DAP-FOS combinations.

**P1606 Assessment of antimicrobial combinations for KPC-producing *Klebsiella pneumoniae* infections**

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**Objectives:** The prevalence of Gram-negative bacteria harboring blaKPC continues to increase worldwide. Limited treatment options exist for infections caused by this multidrug-resistant (MDR) phenotype, which often necessitates combination therapy. We investigated the in vitro and in vivo efficacy of multiple two-agent combinations.

**Methods:** One KPC-2 (KPVM9) and one KPC-3 (KP6153) producing isolate were studied. The killing activities of six 2-agent combinations

of amikacin (AMK), doripenem (DOR), levofloxacin (LVX), and rifampin (RIF) were quantitatively assessed utilising a validated mathematical model. Time-kill studies (TKS) using  $\sim 3 \times 10^5$  CFU/mL were conducted with each antimicrobial alone and were repeated using 25 clinically relevant concentration combinations in a  $5 \times 5$  array. Bacterial burden observed at 24 hours in TKS were modeled using a three-dimensional response surface. The in vivo efficacy of selected combinations was further validated in a neutropenic murine pneumonia model using human-like dosing exposures. Bacterial burden in lung tissues at 24 hours and survival rate over 96 hours were compared for each combination investigated.

**Results:** AMK, DOR, LVX, and RIF MICs for KPVM9 ranged from 16 to 128 mg/L, and 8 to >256 mg/L for KP6153. The most enhanced killing effect was seen with AMK + DOR for both isolates in TKS. Compared with placebo controls, reduction in tissue bacterial burden at 24 hours was more pronounced with AMK + DOR for KP6153 (9.50 vs. 7.66 log CFU/mL;  $p = 0.003$ ) than for KPVM9 (9.75 vs. 9.28 log CFU/mL;  $p = 0.009$ ). This combination also significantly prolonged animal survival ( $p < 0.01$ ) at 96 hours against both isolates. In contrast, AMK + LVX was found to be antagonistic against KPVM9; an inferior trend in animal survival was observed as predicted, compared to placebo controls.

**Conclusions:** Our modeling approach appeared to be robust in assessing the effectiveness of various antimicrobial combinations. Utilising both in vitro and in vivo infection models, AMK + DOR was the most effective combination against two clinical KPC-producing isolates. Empiric selection of combination therapy for KPC infections may result in antagonism and should be avoided.

**P1607** Colistin and doripenem are synergistic in vitro against NDM-1 *Klebsiella pneumoniae*

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**Objectives:** The rapid spread of NDM-1 in Kp and other critical gram-negatives presents a global health concern. This study aimed to investigate Colistin (COL) as a last-line therapy for killing and preventing resistance of NDM-1 Kp in mono- and combination (combo) therapy with doripenem (DOR).

**Methods:** Time-kill studies (34 arms, in total) assessed the effect of COL (0.125–16 mg/L), DOR (initial conc.: 4–50 mg/L), and combos against a clinical NDM-1 *Klebsiella pneumoniae* (Kp) isolate in Australia. MICs were 0.25 mg/L for COL and 64 mg/L for DOR. Initial inoculum was  $10^{6.3}$  CFU/mL. Serial viable counts over 24 hours and real-time population analysis profiles (PAPs; with 0.5–10 mg/L COL) were conducted at 0 and 24 hours. DOR in broth was determined by HPLC-UV. The time-courses of all viable counts and of DOR conc. were described by population pharmacodynamic modelling in S-ADAPT.

**Results:** DOR monotherapy achieved 2.0–3.9 log<sub>10</sub> killing at 2–4 hours and COL alone achieved 2 to >5 log (below detection limit) killing in the first 2 hours. However, all viable counts for the monotherapy arms were within 0.6 log of growth control at 24 hours. Compared to 0 hour, COL 0.125–1 mg/L plus 25 mg/L DOR yielded stasis at 24 hours and COL 4 mg/L plus DOR 25 mg/L led to >5 log kill at 24 hours. DOR 4 to 25 mg/L in monotherapy was degraded rapidly with concentrations below the quantification limit (0.5 mg/L) even at 6 hours. COL PAPs indicated substantial emergence of resistance at 24 hours for COL monotherapy, whereas no colonies were on PAPs plates for the combos of 0.125–4 mg/L COL with 25 mg/L DOR. The model contained three populations and proposed that DOR was effective against the populations less susceptible to COL ( $r = 0.93$ , slope = 1.00, population fitted vs. observed log CFU/mL).

**Conclusion:** The combo of COL and DOR showed very promising activity against NDM-1 Kp. DOR 25 mg/L prevented emergence of

COL resistance over 24 hours. Further evaluation in dynamic in vitro and animal infection models is underway.

**P1608** Enhanced bactericidal activity of colistin and doripenem in combination (combo) against multidrug-resistant *Klebsiella pneumoniae* at a high inoculum in an in vitro PK/PD model

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**Objectives:** Emergence of Kp strains that are resistant to all antibiotics except polymyxins is a global concern. This study evaluated the activity of COL/DOR combos against a high inoculum of MDR Kp in the inoculum in an in vitro PK/PD model (IVPM).

**Methods:** A one-compartment IVPM was used to simulate clinically relevant COL/doripenem (DOR) regimens against a  $10^8$  CFU/mL initial inoculum of Kp. Four isolates were examined: ATCC 13 883 (MICs: colistin (COL) 1 mg/L and heteroresistant (HR), DOR <0.125 mg/L), M567470 (COL 1 mg/L and HR, DOR <0.125 mg/L), M320445 (COL 1 mg/L and non-HR, DOR 8 mg/L) and FADDI-KP035 (COL > 128 mg/L, DOR <0.125 mg/L). COL was administered as 0.5, 2 or 5 mg/L continuous infusion (5 mg/L for monotherapy only), while DOR was dosed every 8 hours ( $C_{max}$  2.5 or 25 mg/L,  $t_{1/2}$  1.5 hours), and in combos. Viable counts over 72 hours and real-time COL population analysis profiles (PAPs) were conducted.

**Results:** Against COL-susceptible strains, COL monotherapy achieved killing only at 5 mg/L (<6.4 log<sub>10</sub>), followed by extensive regrowth to control levels by 24 hours. Against the same strains, DOR 25 mg/L monotherapy achieved 4–5.3 log killing followed by differing extents of regrowth. All combos showed substantial killing except COL/DOR 0.5/2.5 mg/L against M320445. The combo of COL/DOR 2/25 mg/L was superior to monotherapy for all three isolates with counts at 72 hours below the limit of counting (1.3 log). PAPs generally confirmed that substantial emergence of resistance caused the extensive regrowth for COL monotherapy, whereas combos suppressed resistance. Against the COL-resistant strain, DOR alone at 25 mg/L achieved  $\sim 3.4$  log kill within 2–4 hours, whereas combos containing the same DOR regimen achieved marginally greater kill (4.3 log).

**Conclusion:** The COL/DOR combo showed very promising activity against multidrug-resistant (MDR) *Klebsiella pneumoniae* (Kp) with the ability to suppress COL resistance, even at a high inoculum. Further evaluation in animal infection models is underway.

**P1609** In vivo efficacy of tigecycline alone and in combination with colistin, meropenem and gentamicin against KPC-carbapenemase-producing Enterobacteriaceae in a mouse thigh infection model

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**Objectives:** KPC-carbapenemase-producing Enterobacteriaceae are endemic in many regions and cause severe and difficult-to-treat infections. The in vivo activity of tigecycline combinations has not been tested against KPC infections. We report results on the activity of tigecycline alone and in combinations with colistin, meropenem and gentamicin, which may be active against KPC producers, in experimental infections caused by KPC-producing Enterobacteriaceae.

**Methods:** A neutropenic mouse thigh infection model was used. Activities of tigecycline (50 mg/kg/24 hours), colistin (40 mg/kg/8 hours), gentamicin (5 mg/kg/12 hours) and meropenem (200 mg/kg/8 hours) alone and tigecycline combined with colistin, gentamicin or meropenem were tested against three KPC-producing *Klebsiella pneumoniae* and one *Escherichia coli* clinical isolates exhibiting meropenem MICs 1–4 mg/L and susceptible phenotypes to the remaining antibiotics. Thigh infections were performed in triplicate

by inoculating  $\sim 7 \log_{10}$  CFU of each isolate; colonies grown from excised thighs of treated and untreated euthanized mice were enumerated after 24-hours treatment. The efficacy of each antibiotic alone and of combinations was reflected by the reduction of mean CFUs grown after treatment vs. untreated control animals and vs. other regimens and estimated by a Student's t-test

**Results:** Results of the mice infections are presented in Table 1. Colistin and meropenem alone were ineffective ( $p > 0.05$ ) against all isolates, gentamicin alone was effective (significant reduction of CFU in treated mice vs. untreated controls,  $p < 0.05$ ) against one and tigecycline alone effective against two isolates. Tigecycline plus colistin was effective against three isolates, tigecycline plus meropenem was effective against three and tigecycline plus gentamicin effective against all isolates. When compared with tigecycline alone, the tigecycline plus colistin combination was significantly more effective ( $p < 0.05$ ) against one isolate, tigecycline plus meropenem significantly more effective against one and tigecycline plus gentamicin significantly more effective against two isolates.

Table 1. Results of the experimental thigh infections caused by KPC-producing isolates

ISOLATE	P value T	P value C	P value G	P value M	P value T+C	P value T+G	P value T+M	P value T vs. C	P value T vs. G	P value T vs. M	P value T vs. T+C	P value T vs. T+G	P value T vs. T+M
K 1	0.644	0.738	0.844	0.116	0.031	0.003	0.004	0.723	0.700	0.746	0.534	0.048	0.038
K 2	0.137	0.071	0.327	0.137	0.023	0.007	0.077	0.262	0.627	0.423	0.026	0.010	0.094
K 3	0.032	0.940	0.0001	0.184	0.109	0.004	0.004	0.025	0.048	0.049	0.081	0.095	0.148
E 1	0.001	0.176	0.170	0.232	0.015	0.005	0.029	0.005	0.001	0.052	0.231	0.541	0.870

K, *K. pneumoniae*; E, *E. coli*; T, tigecycline; C, colistin; G, gentamicin; M, meropenem

\*P value < 0.05 indicates efficacy

**Conclusions:** The in vivo results of the present study indicate that tigecycline as single antibiotic regimen exhibited superior activity from colistin, meropenem and gentamicin, while tigecycline plus gentamicin was the most effective tigecycline combination. Such results could have significant implications for the treatment of KPC infections.

#### P1610 Anti-mutant potentials of doripenem, imipenem and ciprofloxacin against *Pseudomonas aeruginosa* examined using an in vitro dynamic model

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**Objectives:** To compare the abilities of doripenem (DOR), imipenem (IMP) and ciprofloxacin (CIP) to restrict the amplification of resistant *P. aeruginosa*, their pharmacokinetics were simulated in vitro mimicking multiple antibiotic dosing.

**Methods:** A clinical isolate of CIP-resistant *P. aeruginosa* (MIC of DOR 1 mg/L, MIC of IMP 2 mg/L, MIC of CIP 4 mg/L) was exposed to thrice-daily DOR or IMP and twice-daily CIP for three days at comparable ratios of the 24-hours area under the concentration-time curve (AUC) to the MIC (50–170 hours with DOR, 30–140 hours with IMP and 55–180 hours with CIP). Given the mutant prevention concentrations (MPCs) of DOR (8 mg/L), IMP (1024 mg/L) and CIP (32 mg/L), the respective times inside the mutant selection window varied from 39% to 46% (DOR), from 41% to 66% (IMP) and from 77% to 90% (CIP) of the dosing interval. Based on daily population analyses (bacterial growth in the presence of 2x, 4x, 8x and 16x MIC of DOR, IMP or CIP), 72-hours areas under the bacterial mutant concentration – time curves (AUBCm) were calculated.

**Results:** DOR-, IMP- and CIP-resistant mutants were enriched in all simulations, except those at the highest AUC/MIC ratio with DOR. With each antibiotic, an increase in the simulated AUC/MIC ratio led to a systematic decrease in the AUBCm. A specific AUC/MIC relationship with AUBCm was established for each antibiotic. With DOR and IMP, the AUC/MIC plots of the AUBCm were lower than the CIP plot. At a given AUC/MIC ratio the effects of DOR and IMP on mutants resistant to 2x and 4x MIC were more pronounced than CIP. Less clear AUC/

MIC relationships with resistance were seen with mutants resistant to 8x and 16x MIC of the antibiotics. The different AUBCm-AUC/MIC relationships observed with DOR, IMP and CIP were in concordance with the differences in mutant growth after the end of treatment: numbers of mutants resistant to 2x, 4x, 8x and 16x MIC of DOR were smaller than those resistant to IMP and, especially, to CIP.

**Conclusions:** These data demonstrate greater abilities of DOR and IMP to restrict the enrichment of resistant *P. aeruginosa* compared to CIP.

#### P1611 In vivo efficacy of human simulated exposures of tigecycline against Enterobacteriaceae over 3 days of treatment in the murine thigh model

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**Objectives:** Historically in vivo pharmacodynamic (PD) assessments have been conducted over 24 hours. While these studies have been noted to correlate with clinical outcomes for rapidly bactericidal agents (i.e., fluoroquinolones) these PD endpoints appear more poorly correlated for agents with slower killing profiles. Herein we sought to determine if the extent of bacterial kill resulting from human simulated exposures tigecycline (TGC) was enhanced over a multiday treatment regimen.

**Methods:** An isolate of *K. pneumoniae* (KP404) and *E. coli* (EC363) both with TGC MICs = 0.125  $\mu\text{g}/\text{mL}$  were inoculated into the thighs of immunocompetent ICR mice. Two hours after inoculation, TGC was administered using a regimen that simulated the human steady state 24 hours area under the free concentration-time curve of 50 mg Q12. Thighs were harvested and processed after 24, 48 and 72 hours of treatment. Efficacy was determined by the change in bacteria density at each of the timepoints relative to the 0 hour controls.

**Results:** Human simulated exposures of TGC resulted in enhanced antibacterial activity with each subsequent day of therapy. After a 24 hours treatment period, 0.9 and 1.4 log CFU reductions in bacterial density were detected for KP404 and EC363. After 48 hours, CFU reductions of 1.7 and 2.2 log were observed, while the 3rd day of dosing resulting in overall reductions in CFU of 2.9 and 3.2 log, respectively for KP404 and EC363.

**Conclusions:** Exposures of TGC equivalent to 50 mg Q12 in humans displayed bacteriostatic activity at 24 hours as might have been anticipated; however, this activity was enhanced on subsequent dosing days. After the 3rd day of treatment these cumulative TGC daily exposures resulted in profound bactericidal effects as defined by the achievement of three logs kill. These data provide important insights into the in vivo killing profile of TGC and suggest that assessments of the PD profile beyond 24 hours may provide more predictive value when considering the potential clinical efficacy of TGC and structurally related investigational compounds in the management of infections in man.

#### P1612 Comparative pharmacodynamics of moxifloxacin, levofloxacin, erythromycin, roxithromycin, clarithromycin and azithromycin at simulated serum- and lung concentrations in the presence or absence of macrophages

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**Objectives:** Characterization of the anti-pneumococcal activities of two fluoroquinolones and four macrolides in a new in vitro infection model simulating pharmacokinetics in the presence or absence of macrophages.

**Methods:** Monolayers of J774 macrophages were grown in cell culture flasks in RPMI 1640 medium + 10% calf serum, +10% CO<sub>2</sub>, +95% humidity. A ten-fold excess of *S. pneumoniae* (Spn) was added. Fluctuating serum and lung concentrations following doses of 400 mg moxifloxacin (MXF), 500 mg + 750 mg LVX, 1 g erythromycin (ERY), 150 mg RXM, 500 mg each clarithromycin (CLR) and azithromycin (AZM) were simulated in vitro in an open one

compartment model. Extracellular drug concentrations and viable counts were measured at  $t = 0, 1, 2, 3, 4, 5, 6, 8, 10$  and 24 hours; intracellular drug concentrations and viable counts were measured at 24 hours.

**Results:** In the absence of macrophages, simulated serum concentrations of each of the macrolides reduced viable counts by 1.5–2 log titers for up to 8 hours; thereafter, Spn regrew. In the presence of macrophages, regrowth was less marked. MXF and both LVX doses eliminated Spn within 7–8 hours in the absence, and within 5–6 hours in the presence of macrophages. Simulated azithromycin (AZM) serum concentrations exerted no antipneumococcal activities, neither in the presence nor the absence of macrophages. Simulated lung concentrations augmented the activities of macrolides except azithromycin (AZM) by 1 log titer. But still bacteria regrew, which was prevented in the presence of macrophages. Spn was eliminated by moxifloxacin (MXF), levofloxacin (LVX) and azithromycin (AZM) lung concentrations within 5–10 hours; neither a more pronounced nor a more rapid reduction of viable counts was achieved by higher levofloxacin (LVX) concentrations. Antibacterial activities were accelerated by macrophages. Although intracellular drug concentrations increased in parallel to the concentrations simulated they were not paralleled by augmented antipneumococcal activities of the macrolides or levofloxacin (LVX). High intracellular moxifloxacin (MXF) concentrations translated into an increased bactericidal activity, which, however, was not dose related.

**Conclusions:** This new model adds cellular immunocompetence to the in vitro PK/PD model. Although high extracellular target site concentrations translate into increased antipneumococcal activities, high intracellular concentrations are not paralleled by an augmented antibacterial activity. Intracellular concentrations are only partially and non-consistently predictive for antibacterial activity.

#### **P1613** Effect of fidaxomicin on *C. difficile* spore germination and outgrowth

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**Objective:** Fidaxomicin (FDX) is a novel antibiotic that was recently approved in the US for the treatment of *C. difficile* associated diarrhea. Previously FDX was shown to inhibit sporulation in *C. difficile*. Such an inhibitory effect on sporulation may partly explain its superior clinical performance to vancomycin in reducing recurrences. This study further examines the effect of FDX, its major metabolite (OP-1118) and vancomycin (Vanc) on germination and outgrowth of spores.

**Methods:** *C. difficile* spores (epidemic UK-1 and UK-14 and historical CD196) were harvested from cells grown for ~1 week on BHIS agar medium. Spores were washed and purified through sucrose or HistoDenz gradient centrifugation. Spore germination with or without drugs was monitored spectrophotometrically at 600 nm in media containing taurocholate (a germinant). The ratio of A600 at time X (Tx) to the A600 at time zero (T0) was plotted against time and germination rates were determined using the slopes of the linear portions of the germination plots. Outgrowth experiments were performed at 37°C and samples were assayed for A600 at 5 minutes intervals. Spores were also monitored microscopically for loss of birefringence and appearance of vegetative cells.

**Results:** In the absence of drugs, refractile spores from both the epidemic (UK-1) and historical (CD196) strains germinated in the presence of taurocholate. After ~100 minutes, the A600 began to rise as the dark spores began to swell and vegetative cells grew from the germinated spores. However, in the presence of drugs (FDX, OP-1118, or Vanc), the spores appeared as unswollen, dark spores without any increase in A600, indicating inhibition of outgrowth but no effect on germination. Addition of high concentrations of FDX (200× MIC) or OP-1118 (25× MIC) did not affect spore germination in an epidemic strain (UK-14). The apparent Km for taurocholate (an estimate of apparent affinity of the spore for the germinant) was the same whether drugs (FDX, OP-1118, or Vanc) were present or not.

**Conclusions:** FDX inhibits sporulation and outgrowth of spores but not initial stages of germination. Such inhibitory effects on *C. difficile* sporulation and outgrowth may contribute to its performance in sustaining clinical response and reducing recurrences. Lower spore counts may also be beneficial in decreasing shedding and transmission of this pathogen.

## New pharmacokinetic data

#### **P1614** Prescription of high dose of ceftriaxone for treatment of meningitis: is therapeutic drug monitoring useful? A cohort study

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**Objectives:** The French guideline for meningitis treatment recommends the prescription of a high dose of ceftriaxone (100 mg/kg/day od or bid) without limitation of the dose, whereas american and european guidelines recommend not to exceed 4 or 6 g/day. In addition, summary of ceftriaxone characteristics does not advocate for an adjustment of the dosage, except for severe renal impairment (creatinine clearance below 5 mL/minute). The aim of this study was to describe the pharmacokinetic of ceftriaxone at this dosage for the treatment of meningitis (and/or others neurological infections).

**Methods:** It was a descriptive prospective cohort study, conducted in an French university hospital, designed to provide preliminary pharmacokinetic data in serum, and if possible in CSF, for ceftriaxone prescribed at high dosage ( $\geq 75$  mg/kg/day or  $\geq 4$  g/day). The trough serum levels of ceftriaxone were measured at steady-state for adult patients treated for neuro-meningeal infections.

**Results:** Forty-eight patients (25 men, 23 women) were included (mean age: 55.7 years old  $\pm$  16.3, mean weight: 75.4 kg  $\pm$  21.5, mean serum creatinine levels : 97.5  $\mu$ M  $\pm$  93.2). Mean ceftriaxone dose per day was 92.9 mg/kg  $\pm$  17.3. Sixty-seven serum dosages were obtained for 43 patients (no serum ceftriaxone concentrations were available for five patients, who had only CSF dosages). Mean trough serum levels at steady-state were 80.9 mg/L  $\pm$  66 (10.1–333 mg/L). A non parametric correlation was found between serum levels and renal function estimated by creatinine serum concentration.

Results in CSF were obtained for 16 patients (23 dosages). Mean concentrations were 14.9 mg/L  $\pm$  11.9 mg/L (0.8–39.6 mg/L).

**Conclusion:** These results showed a large inter-individual variability in ceftriaxone serum concentrations, which seems to be correlated with renal function. These results suggest the need of a monitoring to adjust doses of ceftriaxone, particularly in cases of renal impairment, even if creatinine clearance is above 5 mL/minute. A larger clinical study would be conduct to confirm these preliminary results and to find out the link between high trough serum levels and adverse events (neurotoxicity and lithiasis).

#### **P1615** Overton's rule helps to estimate the penetration of anti-infectives into the cerebrospinal fluid of patients without and with meningeal inflammation

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**Objectives:** The brain is surrounded by barriers consisting of lipid layers, which possess, however, several inward and outward active transport systems. In 1900, Ernst Overton found that the entry of anilin dyes through the cell membrane of living cells depended on the lipophilicity of the dyes. We assessed whether Overton's rule is useful to predict the entry of an anti-infective into the cerebrospinal fluid in humans (CSF).

**Methods:** We analyzed previously published data from patients without and with meningeal inflammation. Relevant publications were identified by a PUBMED search using the following algorithm:



Name of the antibiotic and (cerebrospinal or brain) and (concentration or concentrations) and human. We also contacted the manufacturers for information on the penetration of antiinfectives into the CSF.

**Results:** In the absence of meningeal inflammation, the CSF penetration of antiinfectives in humans estimated by the ratios of the areas under the concentration-time curves (AUC) in CSF and serum (AUCCSF/AUCS) correlated positively with the lipid-water partition coefficient at pH 7.0 (log D) (Spearman's rank correlation coefficient  $r_s = 0.40$ ,  $p = 0.01$ ) and negatively with the molecular mass (MM) ( $r_s = -0.33$ ,  $p = 0.04$ ). The ratio AUCCSF divided by the fraction unbound of AUCS (AUCCSF/AUCS free) strongly correlated with log D ( $r_s = 0.67$ ,  $p < 0.0001$ ).

In the presence of meningeal inflammation, AUCCSF/AUCS also correlated positively with log D ( $r_s = 0.46$ ,  $p = 0.002$ ) and negatively with the molecular mass (MM) ( $r_s = -0.37$ ,  $p = 0.01$ ). The correlation of AUCCSF/AUCS free with log D ( $r_s = 0.66$ ,  $p < 0.0001$ ) was as strong as in the absence of meningeal inflammation.

**Conclusions:** Despite these clear correlations, Overton's rule was able to explain only part of the differences in CSF penetration of the individual compounds. The site of CSF withdrawal (lumbar vs. ventricular CSF), the age of the patients, the underlying diseases, active transport and alterations in the pharmacokinetics by co-medications also appeared to strongly influence the CSF penetration of the drugs studied.

#### P1616 Cerebrospinal fluid distribution of cefotaxime in patients with acute brain injury

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**Objectives:** Cefotaxime is part of standard therapy of bacterial meningitis and ventriculitis. Its pharmacokinetics in human is well described except for central nervous system distribution. The purpose of this study was to explore cerebrospinal fluid (CSF) distribution of cefotaxime in patients with acute brain injury.

**Methods:** After local ethic approval and written informed consent, five patients (four men, one woman, age =  $55 \pm 12$  years, weight =  $68 \pm 19$  kg) with an external ventricular drainage (EVD) system inserted into the lateral ventricles of the brain to relieve elevated intracranial pressure (ICP) were enrolled. Cefotaxime was administered intravenously at a dose of 2 g over 30 minutes (2 g every 8 hours). At steady-state, CSF samples ( $n = 10$ ) were collected over 8 hours post-dosing with time intervals between 15 minutes and 1 hour. Blood samples were collected over the same period and plasma ultrafiltrates were obtained using Centrifree® (Millipore, Billerica, MA) for unbound plasma determination. Cefotaxime was assayed by HPLC. Pharmacokinetic parameters were estimated by non-compartmental analyses using WinNonlin® (Pharsight corporation, Mountain View, CA). Results are presented as mean  $\pm$  SD.

**Results:** Plasma profiles exhibited a sharp peak whereas CSF profiles were flat with much lower maximum concentrations ( $C_{max} = 1.5 \pm 1.0$   $\mu\text{g/mL}$  in CSF compared with  $C_{max} = 52.1 \pm 13.6$   $\mu\text{g/mL}$  in plasma ultrafiltrates). Plasma protein binding was estimated to  $58 \pm 8.4\%$ . Mean cefotaxime CSF to unbound plasma AUC ratio was evaluated to  $0.12 \pm 0.08$ .

**Conclusion:** This study has demonstrated that cefotaxime distribution in CFS is restricted possibly due to the presence of efflux transport systems at the blood brain barrier.

#### P1617 Drug levels in septic patients treated with continuous renal replacement therapy receiving new beta-lactam dosage regimen

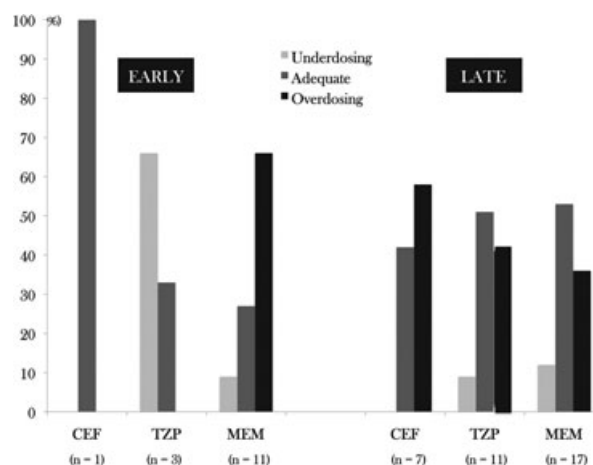
M. Beumier\*, M. Hites, F.S. Taccone, R. Surin, L. Seyler, J.L. Vincent, F. Jacobs (Brussels, BE)

**Objectives:** A recent prospective study on beta-lactams levels in septic patients receiving continuous renal replacement therapy (CRRT)

recommended the following dosage regimens: 2gq8h for ceftazidime or ceftipime (CEF), 4gq6h for piperacillin-tazobactam (TZP), and 1gq8h for meropenem (MEM).<sup>1</sup> We applied these regimens in our hospital since December 2009. The aim of our study was to evaluate the adequacy of this dosage strategy in a cohort of septic patients undergoing CRRT.

**Methods:** Retrospective observational study from 01/01/2010 to 31/05/2011. Inclusion criteria were: (i) sepsis, (ii) CRRT; (iii) treatment with CEF, TZP or MEM, and (iv) at least one measure of beta-lactam levels. Serum concentrations were measured by high-performance liquid chromatography (HPLC-UV). Blood samples were taken twice during the elimination phase after a 30-minutes intravenous administration of the drug. The two samples were used to calculate the time spent above the minimal inhibitory concentration (MIC), which was empirically considered as the clinical breakpoint for "difficult-to-treat" bacteria, such as *Pseudomonas aeruginosa*. Adequate therapy was defined as serum concentrations between 4 and eight times the MIC during optimal periods of time for each drug: >70% for CEF, >50% for TZP, and >40% for MEM. Underdosing and overdosing were defined as drug concentrations that were respectively <4 times or >8 times the MIC during optimal periods of time. Early and late phases of therapy were considered if drug levels measurement was performed within 48 hours or later on, respectively.

**Results:** Fifty drugs levels were obtained from 36 patients; 15 during the early and 35 during the late septic phase. Drug levels obtained for each antibiotic are shown in Fig. 1.



**Conclusions:** Administering a higher beta-lactam dosage regimen for septic patients receiving CRRT avoided insufficient drug concentrations in most patients. Nevertheless, therapeutic drug monitoring should be performed routinely in order to adapt beta-lactam doses and to avoid drug accumulation.

**Reference:** 1. Seyler L, Cotton F, Taccone FS, De Backer D, Macours P, Vincent JL, Jacobs F. Recommended beta-lactam regimens are inadequate in septic patients treated with continuous renal replacement therapy. *Critical Care* 2011, 15: R137.

#### P1618 Differences in clinical efficacy and colistin plasma concentrations in patients receiving three different dosage regimens of CMS for treating infections caused by multiresistant Gram-negative bacteria

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**Objectives:** Colistin use has been reemerged in the last years for the treatment of infections caused by multiresistant gram negative (MDR-GN) bacteria. Despite colistin was introduced in the market in the late

1950s, the available information regarding its pharmacokinetics (PK) and pharmacodynamics (PD) is scarce and data regarding the most efficacious and less toxic schedule of colistin are limited.

The aim of this study was to evaluate the clinical efficacy and the differences in colistin plasma concentrations in patients with MDR-GN infections receiving three different dosage regimens of parenterally sodium colistimethate (CMS): 1 million international units (MU)/8 hours, 2 MU/8 h and 3 MU/8 hours.

**Methods:** Prospective observational cohort study of patients who received intravenous CMS for >72 hours from January 2010 to November 2011. All included patients were treated with one of the three different CMS dosage regimens: 1 MU/8 hours, 2 MU/8 hours and 3 MU/8 hours. The selection of the CMS dose was based on the individual criteria of the responsible clinicians, who were not aware that this study was being performed. Collected data: demographic, clinical and pharmacokinetic data. Blood samples were collected on the third day of treatment (after steady-state was achieved), immediately before ( $C_{\text{min}}$ ) and 30 minutes after CMS infusion ( $C_{\text{max}}$ ). Plasma colistin concentrations were analysed by high-performance liquid chromatography (HPLC).

**Statistic tests:** ANOVA test for quantitative variables with normal distribution, RUSAL WALLIS for non-parametric analysis and Fisher exact test for proportions.

**Results:** Table 1 shows comparative data on patients according to the received CMS schedule.

Variable	Patients (75)			
	CMS dosage regimen			
	1 MU/8h	2 MU/8h	3 MU/8h	p
N° patients	26	39	10	
Men, n (%)	20 (76.9)	31 (75.9)	10 (100)	0.29
Age (years)*	67.92 ± 16.9	62.18 ± 16.26	53.20 ± 16.08	0.021
Body mass index (BMI) (m/kg <sup>2</sup> )	24.44 ± 5.13	25.67 ± 7.32	27.28 ± 3.6	0.214
Charlson score	4.92 ± 2.86	4.13 ± 2.68	3.9 ± 3.07	0.434
Sepsis, n (%)	10 (38.5)	24 (61.5)	6 (60)	0.17
Shock, n(%)	1 (3.8)	3 (7.7)	1 (10)	0.68
Plasmatic levels				
$C_{\text{min}}$ * (mg/L)	0.894 (0.51)	1.92 (1.49)	2.44 (1.97)	0.003
Renal function at baseline and at the end of treatment				
Serum creatinine (mg/dL) at baseline	0.85 ± 0.47	0.61 ± 0.28	0.54 ± 0.27	0.063
Glomerular filtration rate (GFR) at baseline (mL/min/m <sup>2</sup> )	109.11 ± 77.37	161.28 ± 119.37	219.82 ± 180.49	0.013
Serum creatinine (mg/dL) at the end of treatment	1.44 ± 1.42	1.17 ± 0.91	1.29 ± 0.87	0.85
Glomerular filtration rate (GFR) at the end of treatment (mL/min/m <sup>2</sup> )	106.9 ± 96.54	129.9 ± 121.09	136.06 ± 114.30	0.67
Acute Kidney Injury, n (%)	12 (46.2)	20 (51.3)	7 (70)	0.477
Clinical outcome				
Cure/improvement, n (%)	20 (76.9)	28 (71.8)	4 (40)	0.11
Mortality, n (%)	6 (23.1)	10 (25.6)	5 (50)	0.26

**Conclusions:** Young patients with higher GFR at baseline received higher doses of CMS.

There was an increase in plasma levels of colistin when increasing doses of CMS. This suggests that this polymyxin has a linear pharmacokinetics.

Neither clinical outcome nor nephrotoxicity seemed to be related to the CMS dosage regimen.

#### P1619 Population pharmacokinetics of plasma and urine colistin methanesulfonate and colistin in critically patients

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**Objectives:** Colistin, frequently the last line defense therapy against Gram (-) MDR infection, is administered as an inactive prodrug

colistin methanesulfonate (CMS). In healthy volunteers it was shown that CMS is predominantly eliminated unchanged in urine, with renal clearance related to creatinine clearance, while colistin renal clearance is very low. The aim of this study was to assess colistin pharmacokinetics (PK) in critically ill patients from plasma but also urine data to better characterize the effect of renal function.

**Methods:** Patients received multiple doses ranging from 0.5 to 9 MUI of CMS. Blood samples were withdrawn after the first dose and at steady-state while urine samples were collected over one to four dosing intervals. CMS and colistin concentrations in urine and plasma were determined using a validated LC-MS/MS method. Population PK analysis was performed with Monolix software.

**Results:** Fifty-one (51) critically ill patients, 27 males (24 females) median (range) age 61 (21–91) year, weight 74 (36–106) kg, creatinine clearance 80 (10–295) mL/minute were included in the analysis. Plasma samples (n = 867) were collected for every patient while urine samples (n = 34) were collected for 16 of them. A PK model with two-compartment for CMS and one for colistin, distinguishing between renal and non-renal clearances for CMS and assuming negligible renal clearance of colistin, adequately fitted the data. CMS volume of distribution at steady-state was 20 L, its renal and non renal clearances were 59 and 73 mL/minute, respectively. CMS renal clearance was related and slightly lower than creatinine clearance and CMS volume of distribution was related to body weight. Volume of distribution for colistin was 18 L and its total clearance was 57 mL/minute.

**Conclusions:** Urine sampling allowed better estimation of CMS renal clearance in critically ill patients and therefore better evaluation of the impact of renal function on colistin PK.

**Reference:** 1. Clin Pharmacol Ther 89: 875–879.

#### P1620 Formulation of colistin microparticles for passive targeting of lung infection by inhaler

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**Objectives:** Gram negative bacteria (mainly *Pseudomonas aeruginosa*) are often responsible for lung infections and difficult to eradicate using current antibiotics. Therapeutic options for these pathogens are limited, obliging the use of previously discarded drugs such as colistin (COLI). Due to the toxicity associated with parenteral administration of COLI, a less toxic derivative, colistin methanesulfonate (CMS) is administered. However CMS is an inactive prodrug, which converts in-vivo to COLI, responsible for the antibacterial activity. In rats CMS is rapidly absorbed from the lung (F = 70%), and 30% only is converted in COLI in the lung [1]. Then, in order to increase the COLI lung concentration and to enhance the efficacy of treatment and the therapeutic index, COLI microparticles (MP) were formulated for lung inhalation.

**Methods:** Mixtures of inulin (INU) and COLI were spray dried to formulate MP having a 1/1 w/w COLI/INU ratio. INU was selected as the excipient to reduce both the irritating effect and particle surface charging caused by the cationic COLI. MP were characterised by an array of physicochemical methods (particles size, XRD, DSC, NGI). COLI solution and dry MP were intra-tracheally aerosolized in rats lung at a dose of 1.5 mg/kg for systemic pharmacokinetic study (n = 3–6 per group). COLI plasma concentrations were determined by LC-MS/MS assay. Pharmacokinetics parameters were estimated by non-compartmental analysis.

**Results:** MP were monodisperse with a median geometric particle size of 2.3 µm. Their aerosolisation on impactor showed an optimal mass median aerodynamic diameter for deep lung deposition (2.68 µm) and high fine particles fraction (59.5%). Also, their solid state was amorphous, leading to their fast dissolution and so to high diffusion flux. This was interesting as low antibiotic diffusion in bacteria biofilm may be related to their poor efficiency. Lung aerosolisation of COLI as dry particles or solution provided alike plasma profiles, with  $C_{\text{max}}$  and  $t_{\text{max}}$  of 1.26 µg/mL, 24 minutes and 1.63 µg/mL, 25 minutes, respectively, showing rapid absorption. AUCs of 151 and

172 minutes  $\mu\text{g/mL}$  obtained for particles and solution, respectively, were close to AUC obtained after subcutaneous administration (179 minutes  $\mu\text{g/mL}$ ) [1], showing high bioavailability.

**Conclusion:** COLI/INU MP could be of potential use for pulmonary administration by dry powder inhaler.

**Reference:** 1. *Antimicrobial Agents and Chemotherapy*, 2010; 54(9): 3702–3707.

#### **P1621** Characterisation of fluoroquinolones bioavailability in rats after pulmonary delivery

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**Objectives:** Aerosol delivery of antibiotics, including fluoroquinolones (FQs), should provide high drug concentrations directly to the site of infection, and it has therefore recently gained a considerable renew of interest. However no much is known about FQs systemic absorption following aerosol delivery. Recently published in vitro data using Calu-3 cells have shown that this could vary with FQs lipophilicity and affinity for efflux transport systems (1) and a retrospective analysis of the literature based upon FQs diffusion within lung epithelial lining fluid (ELF) has differentiated two groups of FQs (2). The objective of this study was to characterize the bioavailability of FQs representative of each of these two groups: ciprofloxacin (CIP) and moxifloxacin (MXF), plus that of grepafloxacin (GRX) previously described as an outlier (2), after intra-tracheal administration to rats.

**Methods:** CIP, MXF or GRX were administered either intravenously (IV) at respective doses of 7.5, 5 and 5 mg/kg or as an intra-tracheal nebulization (NEB) at respective doses of 7.5, 7.5 and 5 mg/kg for systemic pharmacokinetic study (n = 3–6 per group). MXF and GRX concentrations were determined by HPLC and CIP by LC-MS/MS assay. Pharmacokinetics parameters were estimated by non-compartmental analysis.

**Results:** Following NEB, FQs absorption was always very rapid, with a maximum concentration observed at the first sampling time (5 minutes), and complete with average AUCs ratios (NEB/IV) estimated to 1.14, 0.76 and 0.93 for CIP, MXF and GRX respectively, demonstrating that differences between FQs lipophilicities, responsible for differences in passive diffusion rates across cell barriers in vitro (1), have no consequences on the in vivo absorption rate. The lack of differences between AUCs following IV and NEB administrations suggest that the efflux transport systems controlling FQs passage through Calu-3 cells in vitro are no longer active in healthy rats.

	CIPROFLOXACIN	MOXIFLOXACIN	GREPAFLOXACIN
Dose IV (mg/kg)	7.5	5	5
Dose Neb (mg/kg)	7.5	7.5	5
$t_{1/2}$ IV (h)	1.1 ± 0.1	2.3 ± 0.4	1.8 ± 0.2
$t_{1/2}$ Neb (h)	1.3 ± 0.1	1.6 ± 0.1	1.8 ± 0.1
$C_{max}$ IV ( $\mu\text{g/mL}$ )	1.6 ± 0.4	2.1 ± 0.3	1.5 ± 0.1
$C_{max}$ Neb ( $\mu\text{g/mL}$ )	2.3 ± 1.8	3.4 ± 0.5*	1.7 ± 0.2
AUC Ratios (Neb/IV)	1.14	0.76*	0.93

\* Corrected by the dose

**Conclusions:** Ongoing experiments will soon allow comparisons between FQs ELF concentrations obtained after IV and NEB administrations. However if FQs aerosol delivery may provide an advantage over other routes of administrations by achieving higher concentrations at the infection site, it may not be viewed as an alternative for reducing systemic exposure and toxicity.

**References:** (1) Brillault J. et al. *Antimicrob Agents Chemother* 2010; 54:3–545.

(2) Kiem S and Schentag JJ. *Antimicrob Agents Chemother* 2008; 24–36.

#### **P1622** Calu-3 vs. primary alveolar cells for in vitro evaluation of fluoroquinolone disposition within the lungs

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**Objectives:** Pulmonary drug delivery attracts more and more interest as a treatment of pulmonary diseases or as a new route of administration, leading to the question on whether pulmonary cells form a barrier for drug diffusion. P-glycoprotein (P-gp), an efflux pump responsible for the multi-drug resistance phenomena, has been identified in human alveolar epithelial cells [1]. Recent in vitro studies using a human bronchial epithelial cell line model (Calu-3 cells) expressing P-gp suggested an active transport for fluoroquinolones (FQs) and predicted substantial differences between plasmatic and pulmonary concentrations [2]. To address the influence of possible efflux pump over expression due to their cancerous nature, new investigations were done with primary alveolar rat cells.

**Methods:** Alveolar cells were isolated from male Sprague–Dawley rat lungs with a combination of elastase cell dissociation and differential attachment purification. The cells were seeded on Transwell inserts and cultured for 8 days. Transport studies in apical-to-basal and basal-to-apical directions were performed with moxifloxacin (MXF), ciprofloxacin (CIP), norfloxacin (NOR), grepafloxacin (GRX), pefloxacin (PFX) and levofloxacin (LVX). P-gP expression was evaluated in alveolar epithelial cells and Calu-3 cells by western blotting.

**Results:** The purity of alveolar cells was 80% and the cell viability was 93%. The permeability estimated for FQs through these cells was almost equal in secretory (BL-AP) or absorptive (AP-BL) direction with an efflux ratio  $\sim$ of 1.0 according to the Table 1 suggesting a passive diffusion of FQs through the cell monolayer. Results of western blotting showed an expression of the P-gp protein in alveolar primary cells, but it was substantially lower than in the Calu-3 cell line.

Table 1. Apparent permeabilities ( $10^{-6}$  x cm/s) and efflux ratio (mean  $\pm$  SD), n=3

Drug	AP-BL	BL-AP	ratio
CIP	12.7 $\pm$ 0.4	9.7 $\pm$ 0.7	1.3
NOR	11.0 $\pm$ 0.9	9.3 $\pm$ 0.2	0.8
LVX	17.2 $\pm$ 0.3	14.8 $\pm$ 5.3	0.9
MFX	10.8 $\pm$ 0.5	10.9 $\pm$ 0.2	1.0
GRX	8.9 $\pm$ 0.4	8.7 $\pm$ 0.8	1.0
PFX	11.2 $\pm$ 2.5	10.9 $\pm$ 0.5	1.0

**Conclusion:** Studies with primary alveolar cells showed that the FQs were predominantly transported via a passive diffusion mechanism. Although P-gp was present in these cells, it did not play a significant role in the transport of FQs. The higher expression of P-gp in the Calu-3 cells was correlated with a more active transport through these cells. This over expression of P-gp may be due to the cancerous nature of the Calu-3 cells and could make the primary alveolar cells a better predictive model for pulmonary transport of FQs.

**References:** 1. Endter S et al. *Cell and Tissue Research* 2007; 328: 77–84.

2. Brillault J et al. *Antimicrob Agents Chemother* 2010; 54: 543–545.

#### **P1623** Plasma vancomycin concentrations in patients with *Clostridium difficile* infection taking oral vancomycin

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**Introduction:** Although oral vancomycin is considered to be nonabsorbable, sporadic cases of “red man syndrome” and allergic rash have been observed during treatment of *Clostridium difficile* infection (CDI). A recent phase III trial of fidaxomicin vs. vancomycin

offered the opportunity to systematically assess vancomycin plasma concentrations in CDI patients.

**Methods:** Vancomycin was dosed 125 mg four times daily for 10 days. Plasma was collected pre-dose and post-dose (usually 3–5 hours) on days 1 and 10 of therapy and was assayed using a validated LC-MS/MS assay with a lower limit of quantification of 0.05 µg/mL.

**Results:** One hundred and eighty-nine samples were assayed. Of these, 17 were removed from summarization for being pre-dose (3); more than 24 hours post-dose (5); or from subjects taking IV vancomycin within 30 days (9). The remaining 172 samples came from 102 unique individuals. 25/102 (25%) subjects had plasma levels ranging from 0.052 to 1.71 µg/mL. For those with matching days 1 and 10 samples, no evidence of accumulation after 10 days of oral treatment was noted. Using creatinine levels (available for 101 subjects) as a measure of renal insufficiency, detectable plasma concentrations of vancomycin were found in 11/14 (79%) and in (14/87) (16%) subjects with serum creatinine levels above and below 1.5 mg/dL, respectively ( $p < 0.001$ ).

**Conclusions:** Vancomycin was detected at low concentrations in the plasma of 25% of subjects with *C. difficile* infection who received the drug by the oral route only. Subjects with elevated serum creatinine had measurable plasma vancomycin concentrations more often than subjects with normal creatinine levels, suggesting that renal elimination may be an important consideration when dosing vancomycin orally.

#### P1624 Therapeutic drug monitoring of daptomycin: a 4-year audit of levels from a UK clinical antibiotic service

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**Objectives:** Daptomycin (DAPT) is a lipopeptide antibiotic commonly used for complicated skin and soft tissue infections caused by resistant gram-positive bacteria including methicillin resistant *Staphylococcus aureus* bacteraemia associated with right-sided endocarditis. Elevated DAPT exposures are associated with increased creatine phosphokinase levels, while low exposures are associated with poor clinical response. We have provided a UK DAPT therapeutic drug monitoring (TDM) clinical assay service for 4 years and present a retrospective review of our assay data.

**Methods:** DAPT was assayed using a validated reversed phase HPLC assay. The reasons why many of the samples were sent to the laboratory for DAPT assay are largely unknown but in our experience they are likely to be from renal patients, poor responders or in difficult to treat infection. Data were retrieved from our laboratory computer system and analysed. For TDM we consider pre dose concentrations  $>20$  mg/L as elevated and  $<5$  mg/L as low.

**Results:** Over the last 4 years (2007–2011) we received 760 serum samples for DAPT assay on 168 patients from 50 different hospitals.

DAPT Pre dose concentration mg/L	Neonates	1m-1y	>1y- <18y	>=18y	All
	N=5	N=20	N=18	N=292	N=335
<5	40%	30%	28%	12%	14%
>=5-20	20%	70%	44%	61%	60%
>20	40%	0%	28%	27%	26%

**Conclusion:** Although it is unclear why many of these samples are referred to us, a significant proportion of pre doses (40%) have DAPT concentrations outside of the desirable therapeutic range. Low concentrations were more common in the young (<18 year) but elevated concentrations were equally common (26%) in all age groups. This sub-group may represent a small proportion of patients on DAPT but our audit highlights the clinical value of DAPT TDM in these cases to optimise treatment.

#### P1625 Pharmacokinetics of daptomycin in infants: implications for dosing

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**Objectives:** Daptomycin is approved for use in patients 18 years of age and older. However, use in younger patients has been reported, and clinical studies in paediatric patients are ongoing. The objectives of this study were to evaluate single-dose PK and safety of intravenous daptomycin in infants aged 1–2 years with known or suspected Gram-positive infections receiving standard antibiotic therapy.

**Methods:** Multi-center, open-label, non-comparative study. Following Institutional Review Board approval and written parental consent, five paediatric subjects (age 1–2 year) received daptomycin, 6 mg/kg, over 30 minutes. Daptomycin concentration was measured in blood samples (0.5 mL) obtained at end of infusion and 1, 2, 6 and 12 hours after the start of infusion. Daptomycin PK was analyzed by non-compartmental and compartmental methods using WinNonlin. PK parameters were compared to those of healthy adult subjects [1] and 7-wk old dogs [2].

**Results:** All subjects tolerated daptomycin infusions and no drug-related adverse events were reported. Exposure (AUC) of daptomycin, 6 mg/kg, was lower in 1–2 year old infants compared with adults due to increased clearance (CL) and volume of distribution (Vd). PK simulations predict that 13.85 mg/kg of daptomycin would be required in 1–2 year old infants to achieve similar AUC as adult subjects (632 µg hour/mL) dosed at 6 mg/kg/day, the recommended dose for treatment of bacteraemia (Table 1). This dose is also predicted to produce a  $C_{max}$  70% greater in infants than in adults. In animals, high doses of daptomycin have been shown to cause peripheral neuropathy; the nerve effects are related to  $C_{max}$ , and young animals appear to be more sensitive. No nerve effects were observed in 7-week old dogs following daily daptomycin for 14 days, whereas skeletal muscle and nerve effects were seen after 28 days.

Table 1. PK of daptomycin in 1-2 year old infants and healthy adult volunteers.

Age Group	1-2 yr (n=5)	1-2 yr (simulated)	Adults(1) (n=6)
Dose (mg/kg)	6	13.85	6
$C_{max}$ (mcg/mL)	67.0	161	93.9
AUC (mcg.hr/mL)	281	632	632
$t_{1/2}$ , hr	4.41	4.35	7.9
CL, mL/hr/kg	21.7	21.9	9.1
Vd, mL/kg	122	125	101

**Conclusion:** Exposure of daptomycin in 1–2 year old infants is less than half that of adults. Higher doses of daptomycin may be needed in infants to achieve adequate exposure for treatment of bacteraemia. Clinicians need to be cautious when contemplating use of daptomycin in infants and children, particularly for longer than 14 days, given a theoretical risk of nerve toxicity in children who cannot verbally communicate symptoms or where diagnostics may not detect neurological adverse effects.

**References:** 1. Benvenuto et al. *Antimicrob Agents Chemother* 2006; 50: 3245–49  
2. Cubist Pharmaceuticals, data on file

#### P1626 Anidulafungin pharmacokinetics in critically ill patients

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**Objectives:** Early antifungal treatment with adequate drug exposure reduces mortality in patients with candidemia. Efficacy of

anidulafungin is driven by AUC (area under the curve)/MIC ratio. As anidulafungin clearance is ~30% higher in patients with invasive candidiasis than in patients with oesophageal candidiasis patients at the ICU may be at risk for underexposure. Therefore, insight in anidulafungin pharmacokinetics in a “real-life” population of critically ill patients is important.

**Methods:** We conducted a prospective open-label study in adult patients admitted to an intensive care unit between June 2010 and November 2011. Patients were eligible for inclusion in case of a positive culture for *Candida* from a sterile site – blood or intra-abdominal fluid. Blood samples were taken just before administering anidulafungin and at 1.5, 2, 3, 4, 6, 8, 12 and 24 hours after start of infusion on day 3 or 4 of treatment. Anidulafungin plasma concentrations were measured with a validated LC-MS/MS method. The AUC was calculated using a non compartmental method.

**Results:** Twenty patients with a median age of 71 (IQR: 60–75) were studied; 11 males and 9 females. Cultures for *Candida* were positive in blood for seven patients and in intra-abdominal fluid for 13 patients. In comparison with previously reported data of critically ill patients, we observed an apparent lower exposure and clearance and longer half-life in our patients, see Table. The volume of distribution was similar. MIC values ranged from <0.002 to 0.02 mg/L, and AUC/MIC ratios from 3580 to 32 250. Both anidulafungin  $C_{max}$  and  $C_{min}$  showed a significant correlation with the anidulafungin AUC ( $C_{max}$ :  $R = 0.854$ ,  $p < 0.001$ ;  $C_{min}$ :  $R = 0.884$ ,  $p < 0.001$ ; Spearman correlation coefficient).

	Our data	De Rosa et al. (ECCMID 2010)	Liu et al. (TIMM 2011)	SPC
	ICU (n=20)*	ICU (n=9)*	ICU (n=20)*	Patient model†
PK parameter				
$C_{max}$ (mg/L)	4.7 (3.7-5.8)	6.3 (4.7-9.9)	7.7 (56)	7.2 (23.3)
$C_{min}$ (mg/L)	2.1 (1.8-3.1)	2.4 (1.3-3.2)		3.3 (41.8)
AUC <sub>0-24</sub> (mg·h/L)	65.7 (57.3-85.0)	89.7 (62.2-111.9)	92.7 (41)	110.3 (32.5)
Clearance (L/h)	0.5 (0.3-0.8)	1.1 (0.9-1.6)	1.3 (51)	1.0 (33.5)
Volume of distribution (L)	33.0 (24.5-38.4)	33.5 (27.7-47.4)		
Half-life (h)	33.9 (26.5-67.2)	20.0 (14.5-27.5)		26.5 (28.5)

\* : median (IQR), † : mean (CV)

**Conclusions:** 1 Anidulafungin exposure in our critically ill patients seems lower compared to earlier data. Further analysis is required to determine the factors causing the apparent difference. The effect on outcome has to be evaluated in a prospective study.

2 Trough concentrations correlated very well with total exposure. We propose to use trough concentrations as a surrogate for AUC as this is much easier and practicable in routine practice.

#### **P1627** Penetration of ceftolozane/tazobactam and piperacillin/tazobactam into the epithelial lining fluid of healthy volunteers

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**Objectives:** Ceftolozane/tazobactam, a combination antibacterial with excellent in vitro activity against *Pseudomonas aeruginosa* (MIC<sub>90</sub> = 2 µg/mL) and Enterobacteriaceae (MIC<sub>90</sub> = 1 µg/mL), is currently being developed for the treatment of serious bacterial infections. The steady-state intrapulmonary penetration of ceftolozane/tazobactam was compared with that of piperacillin/tazobactam in this healthy volunteer clinical study.

**Methods:** This was a Phase 1, prospective, randomized (1:1), open-label study of 50 healthy adult volunteers. Each subject received three doses of either 1.5 g ceftolozane/tazobactam (1.0/0.5 g) or 4.5 g piperacillin/tazobactam (4.0/0.5 g). After dosing, a single epithelial lining fluid (ELF) sample was obtained by bronchoalveolar lavage (BAL) from each subject at one of five scheduled time points (5 subjects/time point/treatment group). Serial plasma samples were collected pre- and post-treatment over a 6-hours (piperacillin/

tazobactam) or 8-hours (ceftolozane/tazobactam) time period. Urea levels in the plasma and BAL were used to calculate the ELF drug concentrations. Pharmacokinetic parameters for ELF were calculated by non-compartmental analysis using the mean concentrations at each time point.

**Results:** Ceftolozane/tazobactam and piperacillin/tazobactam penetrated well into ELF, as shown in Table 1.

The mean  $C_{max}$  for ceftolozane and tazobactam was 67.2 and 14.9 µg/mL, respectively, and the mean  $C_{min}$  for piperacillin and tazobactam was 314.6 and 35.0 µg/mL, respectively. The mean ELF concentrations of ceftolozane exceeded 8 µg/mL for 60% of the 8-hours dosing interval. Adverse events were uncommon, mild, and balanced between the two groups. There were no serious adverse events, with only one treatment discontinuation in the piperacillin/tazobactam arm.

Table 1: Summary of ELF to Plasma Penetration Ratios

Analyte	Mean Plasma AUC <sub>0-8</sub> (ug*hr/mL)	ELF AUC <sub>0-8</sub> (ug*hr/mL)	AUC Ratio
Ceftolozane	158.5	75.1	0.48
Tazobactam (with ceftolozane)	19.3	8.5	0.44
Piperacillin	357.3	94.5	0.26
Tazobactam (with piperacillin)	46.1	24.7	0.54

**Conclusions:** The intrapulmonary penetration of ceftolozane/tazobactam compared favorably with that of piperacillin/tazobactam, an agent widely used for treatment of lower respiratory infections. This data suggests that ceftolozane/tazobactam may have potential utility as an agent for treatment of lower respiratory tract infections caused by pathogens with minimum inhibitory concentrations of ≤8 µg/mL.

#### **P1628** Quantitative management of the interaction of ritonavir on itraconazole plasma levels

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**Objectives:** Itraconazole (ITZ) is an antifungal agent extensively metabolized by CYP3A4. It is largely involved in drug-drug interactions (DDIs) as a potent enzymatic inhibitor. Therapeutic drug monitoring (TDM) is required to optimize efficacy and safety, especially because of its very long elimination half-life, of about 40 hours. We describe here the TDM-based management of the effect of ritonavir on ITZ in a HIV patient.

**Methods:** ITZ, and its biologically active metabolite hydroxyl-itraconazole (OH-ITZ), plasma concentrations are assayed by a HPLC-Fluorometry method. ITZ dosage was adjusted to maintain trough levels (C<sub>0</sub>) of (ITZ + OH-ITZ) sum in the range of 1–1.50 mg/L.

**Results:** A 40-year old HIV-infected patient admitted at the Infectious Disease department exhibited skin injuries due to a disseminated *Penicillium marneffei* infection. After 2 weeks of intravenous amphotericin B, oral ITZ 400 mg/day is introduced (day 0). At steady-state (day 10), ITZ and OH-ITZ trough levels were, respectively, 1.00 and 1.30 mg/L (sum 2.30 mg/L). Later on, in the light of general clinical improvement and skin injuries resolution, a highly active antiretroviral therapy (HAART), including darunavir/ritonavir 600 mg/100 mg bid, is started on day 109. A plasma assay realized 10 days after reveals that ITZ plasma level dramatically raised to 4.80 mg/L, associated to an extensive metabolism blockade with OH-ITZ at 0.70 mg/L. A dose reduction of daily ITZ intake by 2-fold (200 mg/day), did not prevent extremely high plasma levels of ITZ and OH-ITZ, respectively at 9.65 and 1.08 mg/L. Under TDM, ITZ was stopped for 36 days and elimination half-life was assessed to be increased at 17 days. ITZ has been reintroduced at a reduced dosage of 50 mg twice weekly, which has resulted in ITZ and OH-ITZ trough

levels of, respectively, 1.62 and 0.24 mg/L (sum: 1.85 mg/L) after 15 days.

**Conclusion:** ITZ is a strong enzymatic inhibitor, but could also be the target of DDIs. In our patient, association with ritonavir, a potent CYP3A4 inhibitor, led to an increase of ITZ elimination half-life by 10-fold. TDM allowed to quantitatively manage the DDI of ritonavir on ITZ, resulting in a dramatically reduced ITZ dosage to 50 mg twice weekly. Early TDM, within the first days of such prescriptions, could be recommended for preventing over-exposure to elevated and unsafe ITZ plasma levels.

**P1629** **Drug interaction: effects of itraconazole on the intestinal pharmaco-metabolising system and ciprofloxacin pharmacokinetics**

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**Objectives:** The intestinal pharmacokinetics of Ciprofloxacin (Cpx) is modified by Itraconazole (Itz), a strong inhibitor of the hepatic metabolism. To better understand the mechanisms involved in the interaction we studied the distribution of Cpx in the gastrointestinal compartment and the expression of the pharmaco-metabolizing system CYP450 and P-gp in the intestine and liver. **Methods:**

Sixteen Wistar outbred female rats were subdivided in four groups and treated orally for three days as follows: (i) Cpx (30 mg/kg/day); (ii) Itz (10 mg/kg/day); (iii) Itz (2 hours before Cpx) and Cpx; (iv) control (no treatment). Serum, small intestine, liver and faeces were collected 2 hours after the last drug administration. Cpx levels were determined by microbiological method. In parallel, the expression of CYP450 3A4 and P-gp was determined in small intestine and liver by Western-blotting.

**Results:** The pretreatment with Itz caused a significant increase of Cpx levels in both serum and liver in comparison to Cpx alone. Concentrations of Cpx in intestinal tissue and in faeces slightly increased after treatment with Itz. Cpx alone induced a slight increase of the hepatic expression of CYP450 3A4 (+8.9%), while Itz alone caused a modest reduction; this became more marked after treatment with their combination (-3.2%). Cpx alone determined slight modifications on the hepatic expression of P-gp (-1.9%). Itz alone reduced P-gp expression (-21.2%), while the Cpx-Itz combination maintained a modest reduction (-11.2%). Cpx and Itz alone induced opposite modifications in the intestinal expression of CYP450 3A4 (+9.4% and -8.9%, respectively), while their combination caused a decrease (-4.2%). Cpx alone, Itz alone and their combination determined an increase of the intestinal expression of P-gp corresponding to 59.2%, 65.3% and 78.4%, respectively, when compared to controls. **Conclusions:**

Itz strongly inhibited the expression of CYP3A4 and P-gp in liver, and only the CYP3A4 in the intestine. The P-gp-CYP3A4 system was inhibited by Itz in liver, while P-gp in the intestine seems highly expressed. The coordination of P-gp-CYP3A4 system appears different in the gastrointestinal compartment (liver or intestinal tissue) in presence of a strong inhibitor such as Itz. These preliminary results confirm data from literature and add new data on the transintestinal elimination of Cpx. The relationship between P-gp-CYP3A4 system and the fluoroquinolones pharmacokinetics need further studies.

## Antimicrobial resistance in isolates of non-human origin

**P1630** **pMLST typing of IncN and IncHI2 plasmids, carrying ESBL-producing genes, isolated from human and animal *Escherichia coli* and *Salmonella enterica* in Germany, the Netherlands and United Kingdom within the SAFEFOODERA ESBLs project**

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**Objective:** To determine the population structure of IncN and IncHI2 plasmids carrying ESBL producing genes isolated from *E. coli* and *S. enterica* from animal and human sources in Germany, the Netherlands and United Kingdom, collected within the EU SAFEFOODERA project "The role of commensal microflora of animals in the transmission of extended spectrum  $\beta$ -lactamases."

**Methods:** Thirty-eight IncN plasmids (23 from *E. coli* and 15 from *Salmonella*), and 21 IncHI2 (nine from *E. coli*, 12 from *Salmonella*), isolated from strains collected at the Institutions BfR and FLI (Germany), HPA and AHVLA (UK), and CVI (the Netherlands), were typed by plasmid Multi Locus Sequence Typing (<http://pubmlst.org/plasmid>).

The IncN plasmids carried blaCTX-M-1 (22 *E. coli*, 15 *Salmonella* from five different serovars, including human and food producing animals), and blaCTX-M-14 (one *E. coli* from cattle). The IncHI2 plasmids carried blaCTX-M-2 (three *E. coli*, 11 *Salmonella* ascribed to four different serotypes; all isolates from poultry/poultry meat), blaCTX-M-1 (four *E. coli* from cattle), and blaCTX-M-9 (two *E. coli* from humans, one *Salmonella* from pig). All blaCTX-M-2-HI2 plasmids carried also replicons for IncP.

**Results:** The population structure of IncN plasmids was very homogeneous with only three different pMLST sequence types detected. Thirty-four plasmids were ascribed to ST1 (repN1-traJ1-korA1). Three blaCTX-M-1 plasmids, isolated from *S. anatum* (one of them isolated in 2006 from meat, two in 2008 from pig) were ST12 (single allele difference in the korA gene, korA9). The blaCTX-M-14 plasmid from one *E. coli* isolated from cattle in UK was the only one showing ST13 (repN2-traJ4-korA1).

The 14 IncHI2 plasmids that could be typed shared the ST2 pDLST profile (smr0018 = 2, smr0199 = 2). Further characterization by multiplex PCR showed the presence of hipA and the lack of smr00092 and smr0183 in these plasmids. The results obtained with the other six HI2 plasmids were not conclusive before the submission of the present abstract.

**Conclusion:** pMLST typing of plasmids is a valuable tool to understand the contribution of IncN and IncHI2 plasmids in the transmission of beta-lactamase genes. The results of this study illustrate that ESBL-genes are linked to specific IncN- and IncHI2-sequence types. Dissemination of sequence types occurs between bacterial species, within different hosts, in different countries, and the analyses give further information how resistance genes are disseminated along the food chain.

**P1631** **Spread of large conjugative plasmids carrying antibiotic, copper and mercury resistance genes among *Enterococcus* from different sources**

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**Objectives:** Antibiotic resistant (ABR) bacteria are a worldwide threat and might be selected by diverse environmental stressors as metals. Copper (Cu) is often used in animal farms (e.g. feed) and mercury (Hg) is a toxic environmental pollutant. Our goal was to assess the occurrence of known CuR/HgR genes among enterococci from several sources, and to characterize the genetic elements linked to their mobilization which often carry ABR genes.

**Methods:** We analyzed 137 *E. faecalis*-Efl, 133 *E. faecalis*-Efm, and 78 *Enterococcus* spp.-Ep from hospitalized (H-47)/healthy humans (HV-67), poultry (P-46), piggeries environment/swine (PE-63), aquacultures (A-73) and sewage (S-52) from Portugal (1997-2011). Genes associated with ABR (vanA, tetM, tetL, ermB, aac6-aph2), CuR (two ATPases; three multicopper oxidases) or HgR (four merA), and platforms (e.g. Tn6009) were searched by PCR. Mating assays (representative CuR/HgR+; 4AB), clonal relatedness (PFGE/MLST) and analysis of plasmid carrying CuR/HgR/ABR genes (S1-PFGE, rep typing, hybridization) were done.

**Results:** Genes coding CuR (trbB-16%, cueO-13%; 41 Efm, 14 Efl, 10 Ep) and HgR (merA1-3%, merA2-3%, merA3-1%; 10 Efm, two Efl, six

Ep) were linked to 39 PFGE types (30 Efm, eight Efl, one Ep) found in several sources (CuR-35%PE, 27%HV, 19%S, 13%P, 10%A, 2%H; HgR-11%PE, 6%S, 4% H/HV). Representative isolates corresponded to Efm CC17 (Cu + Hg-ST393), Efm CC5 (Cu-ST150, ST185), six different singletons in Efm and two in Efl. One merA3 was located in chromosome (Efl-H) while other Hg/CuR/ABR genes were detected on plasmids: (i) tcrB alone (90–300 kb; 2Efm-HV,PE; 1Efl-P); (ii) tcrB + cueO + tetM + tetL + ermB (190–240 kb; Efm-2PE, 2S, 1HV); (iii) tcrB + cueO + merA1 + merA2 + tetM (185–270 kb; Efm-1HV,PE,S each); (iv) tcrB + cueO + merA1 + merA2 + tetM+ermB (200 kb;Efm-1S); (v) merA1 + merA2 + merA3 + ermB+vanA (75 kb; Efl-1H). Erythromycin, tetracycline, streptomycin or ampicillin were co-transferred with Cu and/or HgR genes, and vancomycin with CuR. The repA-PLG1 was identified in 71% of Efm plasmids tested which carry only CuR (20%) or AB, Cu and/or HgR genes (80%). None of merA genes were linked to Tn6009.

**Conclusion:** CuR/HgR genes were often co-located with ABR in large plasmids RepA-PLG1 widely spread. The intensive use of Cu or environmental pollution by Hg might favour the selection of ABR Enterococcus (clonal spread and large mobile plasmids). The diversity of merA sequences among enterococci suggests successful spread of mer genes between bacteria sharing common ecological settings.

#### **P1632** Aquacultures as reservoirs of pathogenic bacteria and clinically relevant antibiotic resistance genes

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**Objectives:** The increase demand for fresh fish is leading the expansion of the aquaculture market and consequently an increase of food safety risks as development or spread of antibiotic resistance (ABR). Our goal was to assess the presence of pathogenic bacteria and clinically relevant ABR genes in aquacultures receiving water from secondary rivers.

**Methods:** Two Portuguese trout aquacultures (TR-A and TR-B) were studied in winter and summer (2010–2011). Samples were collected from river water/sediments located upstream (n = 11) and downstream (11) of both TR, water/sediments from juvenile/adults fish tanks (n = 13), feed (n = 5) and fish (n = 4). They were analysed for Salmonella (standard methods) and for Aeromonas and other Gram negative in selective medium with/without AB (ampicillin, ceftazidime, cefotaxime) after an enrichment step. Clinically relevant genes conferring resistance to  $\beta$ -lactams (blaTEM, blaCTX-M, blaSHV) or fluoroquinolones (qnrA, qnrB, qnrS, qepA, aac6'-Ib-cr) were investigated by PCR/sequencing. Species were identified by ID32GN/16SPCR. ABR was analyzed by agar diffusion (CLSI/EUCAST) and ESBL expression by DDST. Clonality was assessed by PFGE/MLST in specific isolates.

**Results:** Salmonella was detected in 32% of samples from both TR during summer. Eighteen isolates selected based on serogroup and belonging to four PFGE-clones and three MLST types (ST118/ST280/ST508) exhibited resistance to streptomycin (n = 5) and/or kanamycin (n = 1). They were identified upstream (4) and downstream (5) of TR, in the fish viscera (1) and fish tanks (4). Sixty-five Aeromonas isolates were detected in 59% of samples from both TR in the two seasons. Two *A. hydrophila* isolates carrying qnrS2 genes were collected from adult fish tank and downstream river water from TR-A (summer). Gram negative/oxidase negative bacteria (n = 109) were detected in 82% of samples from both TR in winter and summer. SHV12-*E. coli* producers also resistant to streptomycin and tetracycline (n = 19 from seven samples) were isolated from TR-A in winter, including river water collected upstream and downstream of this TR and water from juvenile/adults fish tanks.

**Conclusion:** This study shows that TR may be reservoirs/vehicles for ABR pathogenic bacteria of relevance for human and animal health. The potential risk to spread ABR to humans and animals by water contact and/or food chain is of concern and highlights the need to

establish control policies and surveillance in these food distribution points.

#### **P1633** Prevalence and characterisation of extended-spectrum beta-lactamase (ESBL) and CMY-2 producing *Escherichia coli* isolates from healthy food-producing animals in Tunisia

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**Objective:** To analyse the carriage rate of extended-spectrum beta-lactamase (ESBL)- and/or AmpC-beta-lactamase-producing *E. coli* isolates from faecal samples of food-producing animals in Tunisia, and to characterize the recovered isolates for the presence of other resistance genes and integrons.

**Methods:** Eighty animal faecal samples (23 of sheep, 22 poultry, 22 cattle, six horses, five rabbits and two dromedaries) were obtained from 35 different farms in Tunisia during 2011. Samples were inoculated onto MacConkey agar plates supplemented with cefotaxime (2 mg/L) for cefotaxime-resistant (CTXR) *E. coli* recovery. Characterization of ESBL and AmpC genes, of their genetic environment and of integrons were performed by PCR and sequencing. Detection of associated resistance genes, virulence factors, and phylogroup-typing were performed by PCR. Molecular typing of isolates was determined by MLST and PFGE.

**Results:** CTXR *E. coli* isolates were detected in 11 of 80 faecal samples (13.75%) and one isolate per sample was further characterized (recovered from 10 poultry samples and one dromedary sample). The 11 CTXR isolates were distributed into the phylogroups: B1 (five isolates), A (2), D (3) and B2 (1). The following beta-lactamase genes were detected: blaCTX-M-1 (seven isolates), blaCTX-M-1 + blaTEM-135 (one isolate), blaCTX-M-1 + blaCMY-2 + blaTEM-1b (one isolate) and blaCMY-2 (two isolates). The ISEcp1 and orf477 sequences were found upstream and downstream of blaCTX-M-1 gene, respectively, in all blaCTX-M-1-positive isolates. The nine CTX-M-1 producing strains showed unrelated PFGE-patterns and eight different sequence-types (STs) were identified among them (number isolates/phylogroup/patterns): ST155 (2/B1/P1-P11), ST2255 (1/D/P2), ST57 (1/D/P3), ST2164 (1/A/P5), ST2016 (1/B1/P7), ST58 (1/B1/P8), ST88 (1/B1/P9), and ST10 (1/A/P10). The two blaCMY-2 producing strains were typed each one as ST117/D/P6 and STnew/B2/P4. Seven isolates contained class 1 integrons with four different gene cassettes arrangements: dfrA17-aadA5 (three isolates), dfrA1-aadA1 (2), dfrA15-aadA1 (one isolate) and aadA1 (one isolate). All the isolates showed tetracycline resistance and they contained the genes tet(A) or tet(B). Virulence genes detected were (number isolates): fimA (10), aer (8), papC(2), and papGIII, hly, cnf, and bfp (none).

**Conclusion:** Poultry farms constitute a reservoir of ESBL-producing *E. coli* isolates that potentially could be transmitted to humans via the food chain or by direct contact with them.

#### **P1634** Isolation and identification of multidrug-resistant bacteria from activated sewage sludge

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**Objectives:** Extended-spectrum- $\beta$ -lactamase (ESBL) carrying gram negative bacteria, methicillin resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant enterococci (VRE) are noted for nosocomial infections. Beside the human reservoir these bacteria have spread in the environment.

In this present study, ESBL carrying gram negative bacteria, MRSA and VRE from activated sewage sludge are investigated for their phenotypes and the occurrence of different resistance mediating gene groups.

**Methods:** Activated sewage sludge samples collected from a sewage treatment plant in the area of Graz in Styria (Austria) were screened for the presence of ESBL carrying gram negative bacteria, MRSA and VRE. All specimens were cultured on chromeID ESBL agar, oxacillin resistance screening agar and chromeID VRE agar after enrichment in peptone broth and Enterococcosel broth, respectively. Routine methods for identification (MALDI-TOF, VITEK 2) and susceptibility testing were employed.

Strains were screened for ESBL gene groups (CTX-M, TEM, SHV, VEB, GES, PER1-2) and VRE gene group vanA by PCR. For MRSA spa-typing of protein A was performed.

**Results:** From sewage sludge the ESBL positive species *Enterobacter* sp., *E. coli*, *Pseudomonas* sp., *Klebsiella pneumoniae*, *Acinetobacter* sp. and *Aeromonas* sp. were isolated. First results showed that in ESBL positive strains CTX-M 1 was the most common ESBL gene group. CTX-M 15 was also identified. VRE positive strains carrying vanA gene group were identified for *Enterococcus faecium*. The identified MRSA isolates harboured the spa-types t032 and t6613.

**Conclusions:** All three types of multidrug resistant bacteria could be found in sewage sludge. CTX-M 1 genes were the dominant ESBL group in the analyzed activated sludge samples. Especially the finding of MRSA in waste water is not yet documented for Austria. Identified MRSA spa-type t032 can be assigned to hospital acquired-MRSA (EMRSA15) and spa-type t6613 related to t474 can be assigned to community associated-MRSA.

**P1635** Prospective study on prevalence and characteristics of ESBL/AmpC producing *Escherichia coli* isolated from veal calves at slaughter

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**Objectives:** In the Dutch veal calf industry the majority of animals are imported from many different European countries and are subsequently piled into new herds on veal farms. As a result, on each of these farms, all animals originate from different dairy farms. Therefore these farms act as a melting pot for antimicrobial resistant bacteria and their genes. ESBL/AmpC producing *Escherichia coli* have been reported to occur in many food animal species in different frequencies. The focus of this study was to determine the prevalence and molecular characteristics of plasmid mediated ESBL/AmpC's in *E. coli* isolated from veal calves at slaughter.

**Methods:** As part of an ongoing study in 2011 in 100 slaughter batches originating from different farms, 10 veal calves per batch were screened for the presence of plasmid mediated ESBL/AmpC-producing *E. coli*. So far, 83 flocks are sampled. Faecal samples were collected from 10 individual calves in each farm by rectal swabs. The faecal samples were cultured in LB enrichment broth supplemented with 1 mg/L cefotaxime and incubated over night at 37°C. Subsequently, a MacConkey agar plate supplemented with 1 mg/L cefotaxime was inoculated with the culture from the enrichment broth. The MacConkey agar plates were incubated over night at 37°C. From each flock one isolate was selected for molecular characterization of ESBL/AmpC genes. Chromosomally encoded AmpC's were not taken into account. These isolates were characterized using disk diffusion assays, Identibac AMR-ve micro array, PCR- and sequence analysis.

**Results:** In 66% of the slaughter batches, one or more faecal samples harboured *E. coli* producing ESBLs. Genotypic analysis showed that CTX-M-1, -2, -14, -15, -32 and TEM-52 enzymes were present. In approximately 50% of the isolates harbouring either CTX-M-1 or -15, the isolate also harboured OXA-1. So far, no other plasmid mediated ESBL/AmpC's were detected. From four of the 83 farms sampled so far, <10 individual calves were sampled. These were still included in the study.

**Conclusion:** This study showed that at slaughter 66% of the veal calf slaughter batches were positive for ESBL producing *E. coli*. These were

mainly CTX-M variants. Next to CTX-M variants and TEM-52, no other plasmid mediated ESBL/AmpC's were detected. Molecular characterization will be continued by *E. coli* MLST and plasmid replicon typing.

**P1636** Spread of IncI-blaTEM-52 and IncN-blaCTX-M-1/-32 among *Escherichia coli* isolates from Portuguese piggeries

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**Objectives:** Recent surveys highlighted a piggery reservoir of blaTEM-52 and blaCTX-M genes in Portugal. We evaluated the contribution of clones and plasmids for the spread of these blaESBL genes in this setting.

**Methods:** We analysed 22 ESBL (13 TEM-52, six CTX-M-32, three CTX-M-1)-producing *Escherichia coli* isolates recovered from nine swine samples (faeces, skin) or piggery environment (feed, waste waters) of two geographically distant Portuguese piggeries (2006–2007). Clonal relatedness was investigated by PCR, XbaI-PFGE and MLST. Antibiotic susceptibility testing and conjugation experiments were performed by standard methods. Plasmid analysis included S1-PFGE, identification of incompatibility groups (rep-PCR, hybridization, sequencing) and comparison of RFLP patterns (*EcoRI* and *HincII*). The genetic context of blaCTX-M (ISEcp1, IS26, IS903, ISCR1, orf477) and fluoroquinolone resistance genes [qnr, aac(6')-Ib-cr, qepA] were investigated by PCR and sequencing.

**Results:** Most ESBL producers were recovered from faeces of healthy/sick swine (36%, 8/22) and feed (36%, 8/22). TEM-52-producing isolates (n = 13, piggery F) belonged to diverse A1 (fumC11, fumC84, fumC43), A0 (fumC7) and B1 (fumC29, fumC35) clones, being the blaTEM-52 located on a 90 kb-IncIIR64 plasmid, closely related to those circulating in the hospital setting (2003–2004). CTX-M-1 producers (n = 3, piggery F) belonged to A0 (fumC7), B1 (fumC4) or D1 (fumC224) clones, and CTX-M-32 producers (n = 6, piggery E) belonged to phylogroups A1 (fumC11, fumC27) and B1 (fumC23, fumC175). The blaCTX-M-1 and most blaCTX-M-32 genes were located on identical 40 kb-IncN plasmids. However, the genetic context of such genes was diverse. The orf477 gene was found downstream blaCTX-M in most isolates (n = 6/9), but ISEcp1-bla sequences were only identified in 1 blaCTX-M-1::IncN, 1 blaCTX-M-32::IncN, and 1 blaCTX-M-32::IncFII. ESBL-producing isolates were commonly resistant to sulphonamides (86%), tetracycline (82%), trimethoprim (77%) and streptomycin (72%), and the bla gene was transferred by conjugation (21/22, 96%). Only the qnrS1 gene was identified in a CTX-M-32 producer.

**Conclusion:** This study demonstrated the dissemination of plasmids widespread in different EU countries, as IncI1::blaTEM-52 (humans, poultry and broilers) and IncN::blaCTX-M-1/-32 (humans and swine) also in humans and animals from Portugal, which increases the risk of transmission of antibiotic resistance through the food chain.

**P1637** Colonisation with extended-spectrum and plasmid-mediated AmpC beta-lactamases *Escherichia coli* producers in healthy dogs without antimicrobial pressure – a cross-sectional study

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**Objectives:** To evaluate the frequency of colonization with extended-spectrum beta-lactamases (ESBL) and plasmid-mediated AmpC beta-lactamases (pAmpC) *E. coli* producers among healthy dogs without antimicrobial pressure.

**Methods:** Between November 2010 and January 2011, 142 *E. coli* were isolated from dogs (n = 151), at a private Hospital in the Lisbon



area. The dogs included in the study were healthy with no history of antimicrobial consumption in the previous month. Rectal swabs were inoculated overnight in peptone water and then sub-cultured onto MacConkey agar with a 30 µg cefotaxime disk. *Escherichia coli* typical colonies were selected from the nearest area around the disk and identified by specific *gadA* *Escherichia coli* gene PCR. Susceptibility testing and interpretation was performed using the disk diffusion method according to CLSI guidelines. ESBL production was screened by double-disk synergy test. Genotyping included CTX-M PCR (Edelstein *et al.* 2003), multiplex PCR (Pomba *et al.* 2006) and pAmpC beta-lactamases multiplex PCR (Pérez & Hanson 2002). Beta-lactamases identification was accomplished by nucleotide sequencing.

**Results:** From a total of 142 *E. coli* isolates, full susceptible isolates were 54% (n = 76) and 46% had at least one acquired resistance. Forty-four percent *E. coli* isolates were resistant to amoxicillin, 28% to amoxicillin/clavulanate, 27% to cefoxitin and 19% to cefotaxime. The resistance rate of *E. coli* for other antimicrobials than beta-lactams was 31% to trimethoprim/sulfamethoxazole, 18% to enrofloxacin, and 5% to gentamicin. Among beta-lactam resistant isolates, 12% were pAmpC beta-lactamases producers (one harbouring blaDHA-1, 12 harbouring blaCMY-2; three harbouring blaCMY-type) and 3.5% were ESBL-producers (two harbouring blaCTX-M-1, three harbouring blaCTX-M-15, one harbouring blaCTX-M-32). Multidrug-resistance defined by resistance to three or more antimicrobial classes was present in 11 out of 16 pAmpC beta-lactamases producer isolates and in four out of five ESBL-producers.

**Conclusion:** This study demonstrates that extended-spectrum and pAmpC beta-lactamases isolates are present in the healthy dog population, even without the selective pressure of antimicrobial therapy. Our findings are relevant, as they show dogs as reservoirs for bacteria resistant to oxyimino-cephalosporins and cephamycins - critically important antimicrobials, as defined by the WHO criteria 1 and 2. This may have impact on human health due to the close and direct contact between pets and owners.

#### **P1638** Concentrations and remediation of cephalosporin residues in waste milk from dairy farms in England and Wales

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**Objectives:** The objectives of this study were to determine the concentrations of cephalosporin residues in waste milk (milk unfit for human consumption, fed to calves) from dairy farms in England and Wales and to investigate potential methods for remediation by either heat treatment or adjustment of pH to decrease cefquinome residue concentrations prior to feeding to calves.

**Methods:** Samples of waste milk were collected from 103 dairy farms in England and Wales, together with information on the antibiotics administered to the cows. Samples were stored frozen prior to quantitative analysis for cephalosporin residues by liquid chromatography-tandem mass spectrometry (LC-MS/MS). To investigate possible methods for remediation of waste milk which might be suitable for use on dairy farms to reduce residues, experiments were conducted on raw, unpasteurised milk that had been spiked with cefquinome sulphate (final cefquinome concentration 2 µg/mL). The effects of temperature and pH on the degradation of cefquinome were studied over a period of 10 days. Spiked milk samples were maintained at either 5, 18, 36 or 50°C for the temperature stability study, and adjusted to either pH 1, 4, 7, or 10 for the pH stability study. In each study aliquots of spiked milk were taken for analysis by LC-MS/MS after 0, 2, 4, 6, 8, 24, 48, 72, 120, and 240 hours. Samples were stored frozen at -80°C prior to analysis.

**Results:** Cefquinome (a 4th generation cephalosporin) was the most prevalent cephalosporin detected in waste milk, being detected in 21% of samples tested. Cefalonium, cefalexin and cefapirin (all first generation cephalosporins) were detected in 7.7%, 5.8%, and 2.9% of

samples respectively. Overall, there was good agreement with the reported use of cephalosporins and the detection of cephalosporin residues (agreement for 95% of samples tested).

**Conclusion:** This survey shows that cephalosporins are present in waste milk, a product often used for feeding calves on dairy farms in England and Wales and that cefquinome was the cephalosporin most frequently detected. It has been suggested that cephalosporins in waste milk may select for cephalosporin-resistant bacteria in the intestine of calves that receive this milk. Practical measures to achieve enhanced degradation of cefquinome residues such as alteration of the milk storage temperature or pH adjustment are currently being investigated and will be presented at the meeting.

#### **P1639** Potential zoonotic transmission of CMY-2 producing *E. coli* between pet animals and humans

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**Objectives:** The aim of the study was to investigate transmission of CMY-2 producing *Escherichia coli* between pet animals and humans.

**Methods:** Human and pet animal (canine and feline) clinical *E. coli* isolates displaying the CMY-2 resistance phenotype were collected between 2008 and 2011 in defined geographical areas in Denmark and Wisconsin, US. The strain collection included 73 pet animal isolates and 42 human isolates in Wisconsin, and 12 pet animal isolates and 13 human isolates in Denmark. PCR and sequence analysis for identification of blaCMY-2 were carried out. Confirmed CMY-2 producing isolates were further characterised by PCR-based replicon typing of *E. coli* transformants or transconjugants. Selected IncI1 and IncA/C positive plasmids were compared by restriction fragment length polymorphism (RFLP). Limited to the Danish isolates, we performed multilocus sequence typing (MLST).

**Results:** Altogether we identified blaCMY-2 in 62 animal and seven human isolates from the US, and in 12 animal and 13 human isolates from Denmark. PCR-based replicon typing of *E. coli* transformants or transconjugants is presently still in progress. blaCMY-2 was placed on plasmids belonging to a wide range of incompatibility groups. The most prevalent plasmid replicons detected were II and A/C. Most IncI1 plasmids from *E. coli* in Danish humans and dogs were indistinguishable by RFLP (8/11). IncI1 plasmids from the US were more diverse. Seventeen different RFLP patterns were found among 24 isolates. However, indistinguishable plasmid patterns were found in a group of isolates originating from humans and dogs (5/24). The IncA/C plasmids from US dogs were indistinguishable (6/6). By MLST a total of 20 distinct sequence types (ST) were detected among 23 isolates. ST963 was found both in a dog and a human isolate.

**Conclusion:** The location of blaCMY-2 on indistinguishable IncI1 plasmids in Danish dogs and humans combined with the finding of mainly genetically unrelated *E. coli* STs indicates that this resistance determinant primarily spreads between hosts by plasmid-mediated horizontal transfer and not by clonal dissemination.

#### **P1640** Diversity of blaCTX-M-like genes among polluted and unpolluted rivers

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**Objectives:** The main goal of this study is to perceive compositional traits of the blaCTX-M environmental gene pool by comparing the diversity and genetic context of blaCTX-M genes from polluted and unpolluted streams.

**Methods:** Polluted (P) and unpolluted (UP) rivers from a Portuguese lotic system were inspected for the presence of extended spectrum beta-lactamases (ESBL) producers. Cefotaxime-resistant strains were isolated from stream water and ESBL production was inspected by double disc synergy test. The occurrence of blaCTX-M genes and their genomic environment was inspected by PCR and sequencing. Also,

antimicrobial susceptibility testing against 16 antibiotics of six different classes was performed for blaCTX-M positive strains. Two clone libraries of blaCTX-M gene fragments amplified from polluted and unpolluted environmental DNA were constructed and analyzed.

**Results:** Of the 225 cefotaxime-resistant strains isolated from P and UP streams, 39 were identified as ESBL producers, with 18 carrying a blaCTX-M gene (15 from P and three from NP). Among blaCTX-M-positive strains, multiresistance was detected in 16 strains (14P and 2UP). Analysis of CTX-M nucleotide sequences showed that 17 isolates produced CTX-M from group 1 (CTX-M-1, -3, -15 and -32) and 1 gene belonged to group 9 (CTX-M-14). The genetic environment study revealed the presence of different genetic elements previously described in clinical strains. ISEcp1 was found in the upstream region of all isolates examined in the present study, but disrupted in eight isolates by IS26 and in 1 by IS5. All presented downstream an Orf477 except for one isolate with an IS903-like element. The distance between ISEcp1 and the start codon of blaCTX-M genes was as previously described for the same genes. Culture-independent blaCTX-M-like libraries comprised 16 CTX-M gene variants, 14 types in the P library and four types in UP library, varying from 68–99% similarity between them. Besides the much lower diversity among UP CTX-M-like genes, the majority are similar to chromosomal ESBLs such as blaRAHN-1 and blaFONA-5.

**Conclusion:** Results here presented show clear differences between polluted and unpolluted lotic ecosystems concerning the occurrence and diversity of blaCTX-M genes, which support the hypothesis that natural environments are reservoirs of resistant bacteria and resistance genes that persist and potentiate the dissemination of clinical-relevant genes mainly due to anthropogenic-driven selective pressures.

**P1641 Isolation and characterisation of extended-spectrum beta-lactamase CTX-M bacteria from waste milk samples from dairy farms in England and Wales in 2011**

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**Objectives:** The aim of this study was to analyse waste milk samples from farms in England and Wales for the presence of Extended Spectrum Beta-Lactamase (ESBL) bacteria.

**Methods:** Waste milk due for feeding to calves that day was collected from 103 farms and stored chilled for up to 24 hours whilst transported to the laboratory. Counts of bacteria in raw waste milk samples were made on blood agar (total aerobic count), CHROMagar ECC (total Enterobacteriaceae count), CHROMagar CTX (presumptive ESBLs) and CHROMagar ECC + 16 mg/L cefoxitin (mainly AmpC-producing bacteria). Milk samples were then incubated overnight in buffered peptone broth before plating on the above agars to detect presence/absence of bacteria. Presence of ESBL and AmpC phenotypes in isolates from selective agars was determined using appropriate MAST sensitivity disks. Isolates with an ESBL phenotype were tested for blaCTX-M, blaOXA, blaSHV and blaTEM genes, isolates with a blaCTX-M gene were sequenced to determine CTX-M sequence type and most ESBL phenotype presumptive *E. coli* isolates were serotyped. Bacteria were identified by MALDI-ToF. Additionally, MICs of nine antibiotics were performed against representative ESBL isolates.

**Results:** Bacteria [presumptive *E. coli*] were isolated from 99%, 84.5% [43.7%], 62.14% [4.85%] and 20.39% [1.9%] of the 103 milk samples, on blood agar, CHROMagar ECC, CHROMagar ECC + 16 mg/L cefoxitin and CHROMagar CTX respectively before enrichment. Mean total [presumptive *E. coli*] counts per ml in raw milk were 1.42 x 10<sup>7</sup>, 5.30 x 10<sup>5</sup> [1.94 x 10<sup>4</sup>], 1.75 x 10<sup>4</sup> [3.22 x 10<sup>2</sup>], 3.80 x 10<sup>4</sup> [5.10 x 10<sup>2</sup>] on blood agar, CHROMagar ECC, CHROMagar ECC + 16 mg/L cefoxitin and CHROMagar CTX respectively. Maximum total aerobic counts per ml and total Enterobacteriaceae counts per ml were in the order of 10<sup>9</sup> and 10<sup>7</sup> respectively. A total of six milk samples was positive for CTX-M bacteria (Table 1). CTX-M sequence types 1, 14, 14b and 15 were present in isolates of

*Citrobacter*, *Enterobacter cloacae*, *E. coli* and *Kluyvera intermedia* (Table 1). Some of the CTX-M isolates were highly resistant to cephalosporins (Table 1).

**Conclusions:** Waste milk samples from farms in England and Wales can have high bacterial counts. About 6% of waste milk samples in this study contained bacteria which carried the blaCTX-M gene. These results show that, if waste milk is fed to calves it may expose them to CTX-M bacteria.

Table 1. Characteristics of representative ESBL isolates (to include all CTX-M isolates) from waste milk

ID	Bacterial species	Serotype	Presence of genes by PCR				CTX Type	MICs (μg/ml) of antibiotics for which the									
			CTX	OXA	TEM	SHV		AMC	CAZ	CEQ	CTX	LEX	HEO	OOX	STR	SUL	
A	<i>E. coli</i>	ST1	+	+	+	+	10	120	2.33	0.03	0.06	4	16	2	0.4	1000	
B	<i>E. coli</i>	081	+	+	+	+	33	120	33	66	120	120	1	2	16	16	
C	<i>E. coli</i>	081	+	+	+	+	33	120	33	66	120	120	1	2	16	16	
D	<i>E. coli</i>	081	+	+	+	+	33	120	33	66	120	120	1	2	16	16	
E	<i>E. coli</i>	ST	+	+	+	+	1	120	4	8	64	1	1	120	120	32	
F	<i>E. coli</i>	ST	+	+	+	+	1	120	4	8	64	1	1	120	120	32	
G	<i>E. coli</i>	ST	+	+	+	+	1	120	4	8	64	1	1	120	120	32	
H	<i>E. coli</i>	ST	+	+	+	+	1	120	4	8	64	1	1	120	120	32	
I	<i>Enterobacter cloacae</i>	ST	+	+	+	+	1	120	2	8	32	64	32	120	64	4	
J	<i>Acinetobacter baumannii</i>	ST	+	+	+	+	1	120	1	4	2	32	8	120	120	32	
K	<i>Acinetobacter baumannii</i>	ST	+	+	+	+	1	120	2	4	8	64	64	120	64	16	
L	<i>Acinetobacter baumannii</i>	ST	+	+	+	+	1	120	1	2	4	32	32	120	32	32	
M	<i>Acinetobacter baumannii</i>	ST	+	+	+	+	1	120	1	4	8	64	120	120	32	32	
N	<i>Enterobacter cloacae</i>	ST	+	+	+	+	14b	120	8	64	120	120	2	2	3	32	
O	<i>Enterobacter cloacae</i>	ST	+	+	+	+	14b	120	8	64	120	120	2	2	3	32	
P	<i>E. coli</i>	ST	+	+	+	+	34	120	1	4	16	32	1	1	120	32	32
Q	<i>E. coli</i>	ST	+	+	+	+	34	120	1	4	16	32	1	1	120	32	32
R	<i>E. coli</i>	081	+	+	+	+	80	120	16	23	8	64	1	1	120	32	1000
S	<i>E. coli</i>	081	+	+	+	+	80	120	16	23	8	64	1	1	120	32	1000
T	<i>E. coli</i>	081	+	+	+	+	80	120	16	23	8	64	1	1	120	32	1000
U	<i>E. coli</i>	ST	+	+	+	+	+	120	16	23	8	64	1	1	120	32	1000
V	<i>E. coli</i>	ST	+	+	+	+	+	120	16	23	8	64	1	1	120	32	1000
W	<i>Enterobacter cloacae</i>	ST	+	+	+	+	10	120	120	4	32	120	1	120	120	3000	
X	<i>Enterobacter cloacae</i>	ST	+	+	+	+	10	120	120	4	32	120	1	120	120	3000	
Y	<i>Enterobacter cloacae</i>	ST	+	+	+	+	10	120	120	4	32	120	1	120	120	3000	
Z	<i>Citrobacter</i>	ST	+	+	+	+	10	120	4	2	32	64	32	120	32	1000	

ND, not determined; AMC, amoxicillin; CAZ, ceftazidime; CEQ, cefepime; CTX, cefotaxime; LEX, ceftoloxime; HEO, meropenem; OOX, oxytetracycline; STR, streptomycin; SUL, sulfabactam

**P1642 Differences in antibiotic susceptibility of *E. coli* isolates with poultry-associated and non-poultry-associated extended-spectrum beta-lactamases**

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**Objectives:** Recent findings from the Netherlands demonstrated genetic similarity between ESBL genes, plasmids and bacterial genotypes among *E. coli* isolates from poultry, retail chicken meat and humans. Of human *E. coli*, 35% contained poultry-associated (PA) ESBL genes, 86% of which were blaCTX-M-1 or blaTEM-52. This suggests that PA ESBL genes were acquired by humans through the food-chain. Presence of two distinct compartments could be suspected; one in the community fuelled by food contamination and one in hospitals fuelled by cross-transmission. This would be supported if PA ESBL genes would be especially prevalent among community-acquired (CA) infections, most notably urinary tract infections (UTIs). Moreover, one would expect that bacteria harbouring PA ESBL genes would differ in resistance pattern from those considered to be hospital-acquired due to differences in antimicrobial selective pressure in human medicine and poultry industry.

Aim was to determine whether human *E. coli* isolates with a PA ESBL gene were more prevalent in CA than nosocomial UTIs and whether presence of PA ESBL genes can be predicted upon the susceptibility profile.

**Methods:** In total 133 ESBL positive *E. coli* urine isolates from 2009, obtained in 31 clinical microbiology laboratories, were included (57 nosocomial, 59 community, 12 LTCF, five missing). Genotyping of ESBL genes was performed sequencing. CTX-M-1 and TEM-52 positive isolates were considered PA, all others NPA. MICs for tobramycin, ciprofloxacin, trimethoprim/sulfamethoxazole, gentamicin, amikacin, nitrofurantoin and fosfomicin were determined using broth microdilution. Statistical analyses were performed using chi-square.

**Results:** PA ESBL genes were not more prevalent in isolates obtained in general practice (17/59) than in the hospital (17/57; n.s.). Isolates with PA ESBL-genes were more frequently susceptible to ciprofloxacin (51% vs. 26%; p = 0.0015), gentamicin (86% vs. 63%; p = 0.0079),

tobramycin (PA: 91% vs. NPA: 34%;  $p < 0.0001$ ), and amikacin (98% vs. 67%;  $p < 0.0001$ ), while for other (combinations of) drugs tested no difference was found.

**Conclusions:** Since PA ESBL genes had the same prevalence among ESBL positive *E. coli* in CA and nosocomial UTIs, existence of two different compartments for ESBLs was not supported. Compared to *E. coli* with a NPA ESBL gene, *E. coli* with a PA ESBL gene were more susceptible to ciprofloxacin and aminoglycosides. Presence of PA ESBL genes could not be predicted upon susceptibility profiles.

**P1643** Presence of extended-spectrum beta-lactamase producing Enterobacteriaceae in wastewaters, Kinshasa, the Democratic Republic of the Congo

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**Objective:** Extended spectrum beta-lactamase (ESBL) producing Enterobacteriaceae are a public health concern worldwide, but few data are available from Central Africa. The aim of this study was to assess the presence of ESBL-producing Enterobacteriaceae in wastewaters of Kinshasa, capital of the Democratic Republic of the Congo (DRC).

**Method:** Enterobacteriaceae were recovered from environmental water samples and screened for ESBL production by disk diffusion using cefotaxime, ceftriaxone and ceftazidime disks. Confirmation of ESBL production was done by the combined double-disk method using cefotaxime and ceftazidime alone and in combination with clavulanic acid, according to CLSI guidelines (CLSI M100-S21). Double-disk confirmed ESBL producers were further identified up to species level and tested for antimicrobial susceptibility using Microscan NBC42 panels. Detection and identification of ESBL producing bla genes was performed by a commercial multiplex ligation PCR microarray Check-MDR CT101.

**Results:** In February 2011, water was sampled at 11 sewer and nine river sites in nine municipalities (both residential quarters and slums) of the city of Kinshasa. A total of 194 non-duplicate Enterobacteriaceae were recovered. Eighteen isolates were positive for ESBL screening, of which 14 (7.2% out of 194) were confirmed by disk diffusion. They were recovered from eight sampling sites (five sewers and three rivers) in five different municipalities. The main species were *Enterobacter* spp. (46.6%) and *Klebsiella pneumoniae* (40.0%). Co-resistance to both aminoglycoside and fluoroquinolone antibiotics was observed in 10 isolates, the remaining isolates showed co-resistance to either aminoglycoside ( $n = 3$ ) or fluoroquinolone antibiotics ( $n = 1$ ) respectively. All but one isolates carried blaCTX-M genes belonging to CTX-M1 group. For one isolate, no putative bla gene was detected.

**Conclusion:** A recent study from India showed tap water samples to be contaminated with carbapenemase bla NDM-1 producing organisms. The present results demonstrate that multiresistant bacteria are contaminating wastewater systems. This finding suggests a widespread dissemination of ESBL producing bacteria in the community of Kinshasa. Cities in Central Africa should be added to the map of potentially ESBL-contaminated environments.

**P1644** Zoonotic potential of multidrug-resistant *Escherichia coli* clonal groups in Portugal

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**Objectives:** Multidrug-resistant (MDR) plasmids together with clonal dissemination of resistant isolates are possibly the most successful combination of factors contributing to the spread of antibiotic resistance genes. Our aim was to identify clones associated with multidrug-resistance among human and dolphin isolates, in order to evaluate zoonotic potential and risk.

**Methods:** Sixty two MDR human *Escherichia coli* isolates were randomly selected from NIH collection, being previously isolated from different clinical specimens in seven geographically apart Portuguese hospitals from 2004 to 2009. Two *E. coli* isolated from dolphin's respiratory exudates in 2009 and 2010, at the National Laboratory of Veterinary Research, were also included in this study for their zoonotic potential analysis. Antimicrobial susceptibility was performed by broth-microdilution method (EUCAST). PCR and sequencing were used to screen and identify beta-lactamase and Aac(6')-Ib-cr encoding genes, while PCR-based replicon typing was used to characterize plasmids from MDR isolates. Genetic relatedness of human and dolphin isolates was examined both by PFGE and MLST. Mobile genetic elements were also investigated through PCR mapping assays.

**Results:** Regarding the human isolates, 48 (77%) were CTX-M producers. We detected blaCTX-M-1 ( $n = 4$ ), blaCTX-M-3 ( $n = 3$ ), blaCTX-M-14, blaCTX-M-15 ( $n = 34$ ), blaCTX-M-32 ( $n = 3$ ), ( $n = 4$ ), blaTEM-1 ( $n = 39$ ), and blaSHV-12 ( $n = 8$ ) genes as well as aac(6')-Ib-cr ( $n = 26$ ). Concerning the isolates recovered from the dolphins, one of them produced TEM-1, OXA-30, CTX-M-15 and Aac(6')-Ib-cr and the other TEM-1, Aac(6')-Ib and Aac(6')-Ib-cr. Replicon-typing revealed a severe predominance of IncF plasmids in both animal and human isolates; IS26 and ISEcp1 were also detected in both groups, being associated with blaCTX-M-15 and Aac(6')-Ib-cr plus OXA-30, respectively, in one of the dolphin isolates. Genetic relatedness analysis by PFGE revealed one major cluster corresponding to a single epidemic clone A, which included 22 (35%) of all human isolates and both dolphin isolates. They exhibited the same combination of MLST alleles, corresponding to ST131.

**Conclusion:** This study illustrated the dominance of common antibiotic resistance genes, plasmids and clonal groups, specifically blaCTX-M-15, aac(6')-Ib-cr, IncF plasmids and ST131 in both human and animal isolates, reflecting their linkage and enhancing their zoonotic potential. Studies should be performed to further deepen their role as hotspots of resistance.

## Automation of the microbiology laboratory

**P1645** Evaluation of manual vs. automated plate spreading techniques

H.L. Jones\* (Slough, UK)

**Objective:** A new techniques which has very recently been introduced within the microbiology laboratory is the idea of automated plate spreading techniques aimed at producing more single colonies than the original manual method of plate spreading, reducing time from the sample being taken to results and treatment, and producing more standardised results for a more accurate measure of bacterial growth on the agar plate. For this study the Inoqula<sup>®</sup> from Kiestra<sup>®</sup> was used which demonstrates a unique bead rolling technology used in the acquisition of single colonies.

**Methods:** For the acquisition of single colonies five dilutions to McFarland standard of an ATCC strain of *Staphylococcus aureus* were inoculated onto blood agar plates and streaked using a sterile loop and the Inoqula from Kiestra and single colonies counted, a selection of patient samples were also used. The time trials were conducted with pre-prepared batches of a set number of specimens. Faeces samples were also used, the manual plates were inoculated straight from the sample the automated method used maximum recovery diluents (MRD) approximately 1 g a pea sized lump was inoculated into the MRD and left to stand at room temperature for an hour. Because of the nature of MRD a second study using faeces samples was also conducted using samples known to contain a pathogen but also contain a lot of normal gut flora to see how long to you could leave the MRD before the normal gut flora is too over grown.

**Results:** As the concentration of bacteria increased the numbers of single colonies fell, the coefficient of variation for each McFarland standard concentration was considerably lower for the automated method. During the time trial the automated method showed to be up to

37% quicker. The MRD study showed that after 2 hours the target organisms could no longer be retrieved due to gut flora overgrowth.

**Conclusion:** The results of this particular study have shown that the Inoqula from Kiestra did in fact create the greatest number of single colonies throughout the McFarland dilution specimens and the patient samples. The automated system is very user friendly and has added benefits such as creating audit trails for media batches and time/date recording of all samples run through the system as well as being quicker and requiring fewer staff to run the system than inoculating and spreading of patient samples manually, all of these benefits add up to a good edition to an NHS laboratory.

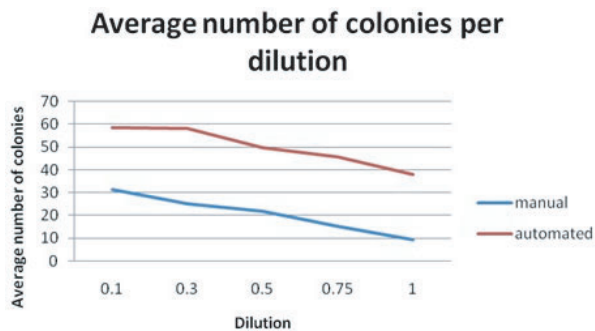


Fig. 1. Average number of colonies per dilution.

#### P1646 Evaluation of an automated digital image analyser for the screening of urine samples submitted for culture

A. Tessari\*, M.A. Giroto, A. Astolfi, P. Gotto, M. Scarin (Rovigo, IT)

**Objectives:** Urine samples constitute a large proportion of the specimens processed in clinical microbiology laboratories but up to 80% are usually negative. Several methods for screening out the culture negative samples have been proposed. Instruments able to detect both bacteria and leucocytes have demonstrated to be suitable to identify patients suspected for urinary tract infections (UTIs). We evaluated the sediMAX (A. Menarini Diagnostics) urinary analyzer to screen for positive samples and to reduce the number of urines requiring culture.

**Methods:** In this study 879 consecutive urine samples from hospitalized (29%) and outpatients (71%), representing all the age groups, were collected during a 3 months period and analysed in parallel by routine culture and the sediMAX instrument. Semi quantitative culture was performed inoculating urines by a calibrate loop on CPS chromogenic and COS sheep blood agar plates (bioMérieux), then incubated at 37°C for 24 hours. The sediMAX uses digital imaging and an automatic recognition software to classify urine particles and report quantitative results. A common set of cut-off values was adopted, with both leukocytes and bacteria counts used for the screening. Results were initially analysed as a single population and then evaluated in subgroups divided by gender, hospitalization and age.

**Results:** Two hundred forty-three (28%) samples resulted positive for culture. The sediMAX compared to culture showed 96% sensitivity, 79% specificity and 98% negative predictive value (NPV). Analysis of male and female hospitalized patients subgroups revealed high sensitivity performance (100% and 99%) but lower specificity (62% and 52%), in many cases due to antimicrobial treatment. Outpatient's females samples gave high sensitivity (97%) and NPV (99%) values but lower specificity (78%), mostly related to the large amount of false positive results observed in urine of pregnant women. Specimens collected from male patients revealed high NPV (98%) and specificity (93%) but lower sensitivity (84%), probably because bacterial cut-off values were not appropriate.

**Conclusions:** The high sensitivity values and NPV observed in this study show that the sediMAX could be a suitable instrument for speed up microbiological screening for suspected UTIs. Data analysis evidenced that a single set of cut-off values is not appropriate to process all the samples and emphasizes the need to adopt patient-specific values in order to further improve the diagnostics performances.

#### P1647 Evaluation of automation and lean methodologies on the urine workflow in a microbiology laboratory

J.B. Laughlin\* (Reading, UK)

**Objective:** Urinalysis remains one of the three major in vitro diagnostic screening tests after serum chemistry profiles and complete blood counts. When it comes to improving efficiency, the greatest benefits often come from revamping high-volume manual processes. Traditional, manual urine screening methods are time-consuming, outmoded and inefficient and open to significant errors that negatively impact patient care and drive up costs. Inaccurate microscopy results may also lead to unnecessary cultures and a subsequent cost. Estimates have shown that 25% of urine cultures are unnecessary, which not only waste resources, but delays other results

A "top to bottom" assessment of urinalysis testing, that is, pre-analytical, analytical, and post-analytical work processes and outcomes were evaluated and addressed, using Lean Six Sigma methods and automation.

**Methods:** A baseline measure of turnaround time (TAT) defined as time of booking in to time the report is released and staff productivity was established. This was compared to TAT performance and staff productivity after implementing Lean process modifications, included the creation of a urine work cell, automation for the analytical and post-analytical stages. Five LEAN principles were applied to the urine workflow:

**Results: 1** Average time required for a technician to process a urinalysis specimen was reduced by 50%, from 150 to 75 second. This was predominately due to elimination of non-value steps in the process. **2** Prior to Lean Six Sigma and automation average turnaround times were 36.77 hour and post it improved dramatically to 0.72 hour (Fig. 1). This equates to a 5000% improvement.

**3** Staffing mix on the urine bench changed substantially. This enabled the redeployment of more skilled staff, improving overall lab performance.

**Conclusion:** Implementation of Lean Six Sigma methodologies in the urine work flow of a microbiology lab resulted in significant improvements in both productivity and TAT. This was accompanied by more accurate results, reduction in the number of enquires regarding specimen status and an enhanced ability in the lab to meet peaks in demand. Implementation of automation and Lean demonstrated synergy between the two. Lean improved the processed while automation standardized the process. Lean eliminated the waste while automation automated the processes that helped reduce the waste. Ultimately the two significantly helped improve patient care pathways.

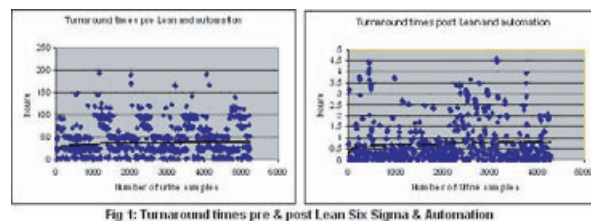


Fig. 1. Turnaround times pre & post Lean Six Sigma & Automation

Fig. 1. Turnaround times pre and post Lean Six Sigma & automation.

**P1648** How liquid-based microbiology can change the workflow in microbiology laboratories

C. Fontana\*, M. Favaro, C. Favalli (Rome, IT)

**Background:** The Liquid based Microbiology (LBM) concept has been introduced in the microbiology laboratories by the advent of the ESwab system. From the development of the ESwab tube collection system to date many other devices have been added to the list, so that presently there is no branch of the microbiology that has not been improved by the introduction of these devices. In our laboratory we have adopted the LBM system since 2008 and currently are used from Gram stains to molecular platforms.

**Method:** In this study we have used ESwab, Fecalswab and SL-solution (Copan Italia, Brescia). ESwab is used for the microbiological specimen usually collected with a swab (like genital, nasal, oropharyngeal, ocular, ear, and wound specimens); Fecalswab is for gastrointestinal sample collection (stool or rectal swab). Both systems were used to perform traditional culture (either manual or automated using the WASP system), Gram stain smear preparation, direct detection of bacterial antigen and toxins and molecular assays. SL-Solution, a new device generation, used to pre-treat mucous rich specimens for gram smear, culture and molecular test for the detection of many pathogens. Samples in ESwab medium are used to detect pathogens using the following assays: Direct sequencing with Rip Seq Mixed (Isentio); Duplicate Real Time CP, MP, LP on the Smart Cycler, (Euroclone); *M. tuberculosis* complex with the MTB GeneXpert and GeneXpert C.difficile (Cepheid); MOTT (Mycobacteria other than tuberculosis) with the Genotype Mycobacteria Direct (Hain); BD CT/GC with ProbeTec™, bacteria vaginosis with the Affirm™ VP8; Duplica Real Time PRC for Mycoplasma genitalium and Ureaplasma urealyticum (Euroclone).

**Results:** Our 3 years experience and extensive utilization of LBM devices demonstrates that a laboratory operating in manual-mode can automate several processes, by changing specimens collection from traditional Transystems to liquid phase ones. The same specimen collected or treated with such devices can be used for Gram stain, culture, antigen/toxins detection and numerous molecular assays, without affecting the sensitivity of the assays, but improving the work flow by reducing processing time, like in the case of direct sequencing.

**Conclusion:** The Copan LBM device family allows us to optimize the workflow in the laboratory being suitable for Gram stain smear preparation, culture, with manual and automated inoculation methods, and for molecular assays.

**P1649** Optimised integration of new technologies (VITEK® MS and PREVI™ Isola) in a microbiology laboratory using the Lean 6 Sigma methodology

J. Djapo-Tiani\*, L. Van Helleputte, G. Habib, J. Collard, H. Palumbo (Liège, BE; Marcy L'Etoile, FR)

**Objectives:** With the recent availability of new technologies for the microbiology laboratory, it becomes more and more important to integrate them in the most efficient way. Our laboratory has decided to use the Lean 6 Sigma process prior to the implementation of new technologies with as objective:

- 1 a reduction of the non-added-value tasks in order to increase productivity,
- 2 the reorganization of the flow and processes in order to integrate two new automated instruments (VITEK® MS and PREVI™ Isola)
- 3 improve TAT using mass spectrometry for rapid identification

**Methods:** An audit has been performed during 5 days in February 2011 by a team specialized in Lean 6 Sigma methods. This analysis has lead to a number of recommendations:

- 1 reorganization of the workflow in functional working cells for day 0 and day 1, automation and standardization of the plate streaking process and introduction of mass spectrometry for bacterial identification,
- 2 process and methods standardization.

**Results:** Six months after the laboratory workflow modification and installation of the new automated systems, the first conclusions show:

- 1 a reduction of TAT for the more complex samples
- 2 a reduction of the workload and stress for laboratory technicians
- 3 the ability to integrate 10% additional samples in the current organization
- 4 the reallocation of a 1.5 FTE on dispatching tasks and on quality assurance

**Conclusion:** The integration of new technologies (mass spectrometry for rapid identification and automated plate streaking) utilizing the Lean 6 Sigma process enabled us to optimize the whole microbiology workflow. This enabled us to also decrease the workload, the TAT, facilitate introduction of the accreditation process and handle an increased volume of samples.

**P1650** Application of the Fungitell® assay on a fully automated coagulation analysing system allows for STAT testing of (13)-beta-D-glucan in serum samples

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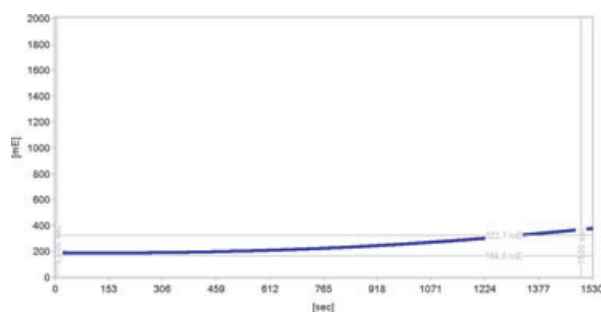
**Objectives:** Testing for (13)-beta-D-glucan (1-3BDG) in serum samples with the Fungitell® assay is used for detection of invasive fungal disease independent of culture.

However, this assay is presently based on a 96 well microplate batch format having major limitations regarding automation and time-to-result. Because the principle of this assay is based on the Limulus Amebocyte pathway, the method was applied on a fully automated coagulation analysing system allowing for both, immediate single sample- and large scale-testing.

**Methods:** The standard manual protocol of the Fungitell® assay (Associates of Cape Code, MA) was applied on the fully automated BCS XP® coagulation analysing system (Siemens Healthcare Diagnostics, Germany). After calibration and generation of the standard curve (Fig. 1) analytical measuring range, limits of quantitation and imprecision were evaluated. For linearity testing dilution series using serum samples of known concentrations of 1-3BDG obtained from a patient with culture confirmed Candidaemia was performed in triplicates. Imprecision was tested using three serum samples with low, intermediate and high concentrations of 1-3BDG 10 times in a row.

Results were compared with those obtained using the standard manual protocol outlined by the manufacturer.

**Results:** The automated assay is linear with a lower limit of quantitation of 15 pg/mL up to an upper limit of quantitation of 4000 pg/mL and yielded sufficient correlation when archived standard curves were used ( $R^2 = 0.97-0.99$ ). Imprecision testing showed CVs within 7% in all three concentrations tested. When standard samples were tested in parallel with both, the automated and the standard manual protocol, CVs were found to be within 6% indicating sufficient concordance. Time to result took 50 minute with the manual protocol and 35 minute with the automated protocol.



**Conclusion:** This new application of the Fungitell® assay on an automated coagulation analyser allows STAT testing in addition to large scale testing for 1-3BDG and is therefore suitable for the clinical

routine. This may offer an early and reliable decision support for clinicians regarding start of antifungal therapy.

Commercial systems for detection of antimicrobial resistance in Gram-positive organisms

Fig. 1. Extinction (mE) vs. time (second) standard measuring curve generated by the automated BCS XP coagulation analysing system showing 1-3BDG specific kinetic of a 25 pg/mL standard concentration.

## Commercial systems for detection of antimicrobial resistance in Gram-positive organisms

### P1651 Screening for methicillin-resistant *Staphylococcus aureus* and selective broth enrichment of colonisation samples by TPX MRSA assay

T. Stenholm\*, A. Hakanen, E. Hakanen, H. Härämä, K. Rantakokko-Jalava, P. Kotilainen (Turku, FI)

**Objectives:** Rapid high-throughput screening tools are needed to limit the spread of methicillin-resistant *Staphylococcus aureus* (MRSA). The TPX MRSA assay is based on two-photon excitation of fluorescence detection (TPX) technology originally developed to replace existing enzyme-linked immunosorbent assays with a point-of-care compatible automated device. In our previous experiments 97.9% of MRSA isolates were detectable by the assay. The aim of this study was to test the use of the assay in phenotypic screening of clinical colonization samples for MRSA.

**Methods:** A total of 125 colonization samples were collected from 14 MRSA carriers and 16 healthy subjects. Nose, throat, perineum, groin and axillae were sampled in all cases with up to two additional sites. Each site was swabbed twice to produce samples for testing by both the TPX MRSA assay and by conventional MRSA culture. The TPX MRSA assay allowed up to 96 samples simultaneously screened in a microtiter plate. Ready-to-use microtiter plates enabled a highly simplified assay protocol. After an 18 hour test run the selectively enriched reaction mixtures were recovered for further testing.

**Results:** Thirty-eight samples were MRSA positive, i.e. MRSA culture was positive or MRSA was isolated from the sample well after a TPX test run. The TPX MRSA assay sensitivity was 87.9% and specificity 85.9% in detection of MRSA. The standard MRSA culture including a broth enrichment step had a comparable sensitivity of 86.8%. Positive predictive value of the TPX MRSA assay was 72.5% while negative predictive value was 94.4%. On average a true positive TPX MRSA test result was confirmed after 2 hours of measurement. 11.2% of TPX MRSA test results were inconclusive, a vast majority of these were throat samples containing viscous mucus.

**Conclusions:** We propose that initial use of the TPX MRSA assay instead of a conventional broth enrichment step would provide useful preliminary results without any further delay to final results. An increase in laboratory workload would be minimal and the assay is suitable for wide-scale screening. Expensive isolation measures could be discontinued early for the screening test negative patients suspected of MRSA colonization. An increase in laboratory workload would be minimal even if all TPX MRSA negative samples were also subjected to confirmation by conventional methods.

### P1652 Comparison of gradient strips for use in detection of *Staphylococcus aureus* isolates with reduced susceptibility to glycopeptides

J. Richards\*, C. Estrada, L. Davies, M. Wootton, R.A. Howe (Cardiff, UK)

**Objectives:** Reduced glycopeptide resistance in *Staphylococcus aureus* is an important clinical problem. Both homogenous and heterogeneous intermediate resistance to glycopeptides in *S. aureus* (GISA/hGISA) is difficult to detect using most standard disc

susceptibility testing. MIC determination aids detection of GISA and hGISA and can be performed with many techniques. Although microbroth dilution (MBD) is the reference method, gradient testing is quick and easy to perform. This study aims to compare the different commercial gradient strips of vancomycin and teicoplanin against a known set of hGISA/GISA and glycopeptide susceptible *S. aureus* (GSSA).

**Methods:** Eight GISA, 48 hGISA and 59 GSSA were used. MIC determination was performed using Etest (BioMerieux), MICE (Oxoid) and MIC test (Liofilchem/Launch diagnostics) strips plus MBD. All tests were performed on Mueller Hinton agar (MHA) as advised by the manufacturer. All gradient strips were compared on both MHA and Isosensitest agar (ISA) in control strains on 15 occasions; Mu3 (hGISA), Mu50 (GISA), ATCC25923 (GSSA) and ATCC29213 (GSSA). Geometric mean MICs (GeoMeanMIC) were calculated, and sensitivity (sn), specificity (sp) for the detection of GISA were compared for both vancomycin (VAN) and teicoplanin (TEIC).

**Results:** The results are summarised in the Table 1. VAN GeoMeanMICs were 1.3/2.4/0.9 and 2.5/4.1/1.6 respectively for hGISA and GISA respectively compared with 1.9 and 3.1 for MBD. Sn & sp for detection of GISA were 50% and 100% for both Etest and MBD, while sn was higher for MICE at 87.5% with reduced sp at 93.3%. Comparison of replicate testing of the control GSSA strain ATCC25923 showed similar results for ISA and MHA. However, MICE gave noticeably higher readings than Etest or MIC test for VAN.

	VAN			TEIC		
	GeoMean	sn	sp	GeoMean	sn	sp
GSSA/hGISA/GISA						
Etest	0.6/1.3/2.5	50	100	0.4/2.1/4.3	62.5	100
MICE	1.4/2.4/4.1	87.5	93.3	0.3/1.8/3.9	62.5	100
MIC test	0.4/0.9/1.6	37.5	100	0.7/1.8/4.2	62.5	100
MBD	0.7/1.9/3.1	50	100	0.4/2/4	62.5	100
	VAN			TEIC		
	Control ATCC 25923	sn	sp	sn	sp	sp
MHA/ISA						
Etest	1.4/1.4	50	100	1.7/1.2	62.5	100
MICE	2.6/2.8	87.5	66.6	1.6/1	62.5	100
MIC test	1.2/1.2	37.5	100	1.1/1.1	62.5	100

**Conclusions:** No method, including the reference MBD method gave good sensitivity for detection of GISA. Of the gradient tests examined, Etest gave equivalent results to MBD, while MICE had increased sensitivity, but reduced specificity. Results for control strains were not affected by media. Further work is required to establish an appropriate method for establishing reduced glycopeptides susceptibility in *S. aureus*.

### P1653 Ability of automated susceptibility testing instruments to detect glycopeptide intermediate resistance in *Staphylococcus aureus*

M. Wootton\*, J. Andrews, J. Richards, J. Bradley, L. Davies, V. Kundalia, R.A. Howe (Cardiff, UK)

**Objectives:** Reduced glycopeptide susceptibility in *Staphylococcus aureus* is an important clinical problem. It is now current practice not to disc test for detection of homogenous and heterogeneous intermediate resistance to glycopeptides in *S. aureus* (GISA/hGISA). BSAC & EUCAST recommend MIC determination. With automated susceptibility testing now common in laboratories it is important to evaluate their use in detecting hGISA/GISA.

**Methods:** Eleven hGISA & nine GISA plus three *S. aureus* susceptible controls were used in this study. MIC determination was performed in two centres (Cardiff & Birmingham) by two technicians in each centre using vancomycin (V) Etest (BioMerieux) & MICE (Oxoid) plus Vitek (Birmingham) & Phoenix (Cardiff). All gradient tests were performed on Mueller Hinton agar (MHA) as advised by the manufacturer. MIC & interpretation was compared for all methods using BSAC guidelines.

**Results:** Of 11 hGISA 11.3%, 38.7%, 0% and 9.1% were detected successfully by Etest, MICE, Phoenix and Vitek respectively. Of nine GISA 60.9%, 58.6%, 33% and 59.6% were detected successfully by

Etest, MICE, Phoenix and Vitek respectively (Table 1). 0% of two control strains exhibited MICs of >2 mg/L using both Etest & MICE. MICs of >2 mg/L were seen in ATCC 25923 on 75% of occasions using MICE and 0% using Etest.

**Conclusions:** Detection of hGISA/GISA was poor for all methods, with approximately 40% of GISA missed by most methods. Gradient strips detected more hGISA than any automated instrument, with MICE detecting more hGISA than Etest but suffering false positives with the ATCC 25923 sensitive control strains. Further work is required to determine a satisfactory method for diagnostic laboratories to detect reduced glycopeptides susceptibility in *S. aureus*.

Table: Percentage of resistance detected

	Mean % detected	
	hGISA	GISA
Etest	11.3	60.9
MICE	38.7	58.6
Phoenix	0	33
Vitek	9.1	59.6

**P1654** Evaluation of Vitek 2 AST-P612 card for daptomycin susceptibility testing of *Staphylococcus aureus* and coagulase-negative staphylococci

B.M. Willey\*, L. Mazzulli, A. Mazzulli, A. Mazzulli, C. Porter, P. Lo, K. Wong, S.M. Poutanen (Toronto, CA)

**Background:** DAP is a useful agent to treat Gram-positive infections including those due to methicillin-resistant SA (MRSA). Thus, it is important for laboratories to report accurate DAP MIC. As DAP depends on precise calcium ion content in the test medium to produce accurate MIC, testing is complex. This study evaluated Vitek 2 DAP testing of SA and CNS.

**Methods:** Of 370 study isolates, 270 were genetically and geographically diverse MRSA and methicillin-susceptible SA (MSSA) and 100 were highly characterized CNS blood isolates. The MSSA/MRSA respectively, included 72/69 community-acquired and 52/13 hospital-acquired strains (consecutive blood, other clinical sites, and surveillance screen isolates), 6/38 NARSA VISA and 1/19 Cubist DAP non-susceptible (NS; >1 mg/L = NS) isolates. As per Cumitech 31A, AST-P612 card DAP MIC was compared to CLSI broth microdilution (BMD) MIC.

**Results:** By BMD, 28 (10.4%) SA and no CNS were DAP NS. Among SA, the essential agreement (EA) was 80.7% (95%CI: 75.6–85), categorical agreement (CA) was 95.4% (95%CI: 90.5–96.4), while very major (VME) and major (ME) error rates were 10.7% (95%CI: 2.9–28) and 5.4% (95%CI: 3.1–9.1), respectively. By Vitek2, DAP MIC values were significantly higher than BMD in 55.6% SA by 1–2 log dilutions (two-tailed p-value < 0.0001). For CNS, EA was 98% (95%CI: 92.6–

99.9), CA was 99% (95%CI: 94–99.9), ME rate was 1% (95%CI: <0.01–6), and the VME was unable to be determined as there were no DAP NS CNS available for study.

**Conclusions:** While the AST-P612 generally compared well to BMD MIC for CNS, for SA, it overestimated DAP MIC as evidenced by high ME (>3%) and low EA (<90%). As well, true DAP NS was overlooked in three MR-VISA leading to an unacceptable VME rate of >3%.

**P1655** MIC determination for daptomycin using VITEK2: the DaVIT multicentre study

J. Knobloch\* on behalf of the DaVIT study group

**Objectives:** Daptomycin is a potent antibiotic for the therapy of infections caused by Gram-positive organisms. Antimicrobial susceptibility test (AST) panels for the VITEK2 able to perform Daptomycin testing were introduced recently. As MIC determination for Daptomycin can be strongly influenced by the growth conditions the performance of Daptomycin AST using VITEK2 was compared to the MIC determination using Etest in a multicenter study.

**Methods:** Thirty-two laboratories tested up to 20 clinical isolates of *S. aureus*, *E. faecalis* or coagulase negative staphylococci using the VITEK2 AST panel P-611. MIC determination using Etest was performed on MH-Agar (MH) of different manufacturers. Control strains *S. aureus* ATCC29213 and *E. faecalis* ATCC29212 were tested at least twice during the study period with both methods in each laboratory.

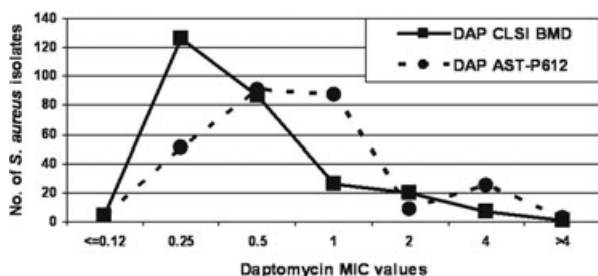
**Results:** In total 629 clinical Gram-positive isolates were analyzed, including 437 *S. aureus*, 64 *E. faecalis*, 86 *S. epidermidis*, and 42 other coagulase-negative staphylococci (CNS). Seventy-nine and 76 AST results of control strains ATCC29213 and ATCC29212 were included, respectively. In average AST performed by VITEK2 displayed significantly higher MIC values compared to AST using Etest. However, only few major errors were observed for both methods. Using VITEK2 all quality control strains were correctly classified as susceptible. Three *S. aureus* and two *S. epidermidis* isolates from patients displayed MIC values >1 µg/mL interpreted as resistant in VITEK2. For *S. aureus* isolates this phenotype was confirmed by Etest, whereas *S. epidermidis* isolates were tested susceptible by Etest. No significant differences in the performance of Etest were observed between MH-agar from most manufacturers. However, using Etest on MH from OXOID several major errors with incorrect classification (false resistant) of clinical *S. aureus* isolates as well as strain ATCC29213 were observed.

**Conclusion:** Resistance against Daptomycin is a rare event in the investigated study population (<1%). The VITEK2 AST for Daptomycin revealed correct SIR determination for almost all tested clinical isolates and control strains. Significantly higher MIC values were observed using VITEK2 compared to Etest resulting in two cases of false interpretation for two *S. epidermidis* isolates. Therefore, results with low level resistance determined with VITEK2 should be confirmed by an alternative test method prior reporting. Not all MH could be used for AST using Etest as confirmation test.

**P1656** EPI-V™, a rapid, inexpensive detection test for vancomycin-resistant enterococci directly from stool specimens

C. Kontnick, L. Post, C. Edberg\* (New Haven, US)

**Objective:** Vancomycin resistant enterococci (VRE) have been reported throughout the world and have become a major and pervasive clinical problem, especially in tertiary care hospitals and nursing homes. EPI-V™ (Pilots Point LLC, Sarasota, Florida) was developed to provide a simple, low cost test of VRE carriage directly from stool specimens. It is based on classical biochemical parameters formulated and optimized as a stable powder. Specificity of EPI-V™ resides in its detection of beta-glucosidase (b-glu) and L-tryptophanyl arylamidase (PYR). Sensitivity resides in the optimization of defined



nutrients according to the Defined Substrate principal. The EPI-V™ is in powder form, ready to use by adding water. Distinct colour changes denote the presence of VRE.

**Methods:** Specimens consisted of 400 sequential human rectal/perirectal surveillance specimens obtained as part of the ongoing surveillance program. Two swabs in tandem were used. Conventional processing included plating one swab on an aerobic campylobacter plate (6 mg/L vancomycin) and identifying and performing an antibiotic susceptibility test on colonies consistent with enterococci. For EPI-V™, water is added to the test tube to the line, the swab twirled, and incubated at 35°C. A distinct blue/black color and simultaneous red PYR are specific for VRE.

**Results:** See Table 1.

The average time to a positive for EPI-V™ was 4.8 hour. The average time to a positive test/result for the conventional method was 44 hour (2.8 days).

There were no false positive EPI-V™ tests, either for identification or vancomycin susceptibility.

Results:	Conventional Pos	Conventional Neg
EPI-V™ Pos	128	15
EPI-V™ Neg	8	249

**Conclusions:** The EPI-V™ is a complete one step system for the direct detection of VRE directly from stool specimens. It combines the identification (ID) and antibiotic AST) tests in an optimized, stable powder. The ID and AST are long used standards in a new, optimized format. EPI-V™ was somewhat more sensitive than an agar based standard and both were 100% specific. The rapid results available from EPI-V™ [an average of 4.8 hour] and low cost make it very attractive for epidemiology use.

#### **P1657** Discrepancies between MicroScan, Etest and broth microdilution for the determination of daptomycin susceptibility in enterococci

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**Objectives:** Daptomycin is a lipopeptide with bactericidal activity against enterococci, however, the emergence of resistance to daptomycin during therapy has been described. Our laboratory routinely determines daptomycin susceptibility of enterococci using an automated microdilution method (MicroScan). Recently, we have observed a decrease in the susceptibility to daptomycin, particularly in *E. faecium* (91% susceptible), using the MicroScan panel PC32. In this study we compare the results obtained by MicroScan, Etest and the standard broth microdilution (BMD) methods for the determination of the in vitro susceptibility of enterococci to daptomycin.

**Methods:** From December 2010 to October 2011 a total of 1673 enterococcal isolates (1311 *E. faecalis* and 362 *E. faecium*) were recovered in our laboratory and tested for daptomycin susceptibility using the MicroScan panel PC32. All isolates that tested nonsusceptible (NS) to daptomycin according to CLSI breakpoints (MIC > 4 mg/L), were further tested by the Etest method in Mueller-Hinton agar, following the recommendations of the manufacturer, and by BMD according to CLSI guidelines. *E. faecalis* ATCC 29212 and *S. aureus* ATCC 29213 were used as control strains.

**Results:** A total of 37 isolates (32 *E. faecium* and five *E. faecalis*) tested NS to daptomycin by the MicroScan method. Most patients had no prior exposure to daptomycin. All isolates were fully susceptible to vancomycin. By the Etest method, all isolates tested susceptible to daptomycin (range 1.5–4 mg/L) as well as by the BMD method (range

2–4 mg/L). Etest reported in general MICs two-fold dilutions lower than MicroScan and 0.5 to one-fold dilutions lower than BMD.

**Conclusion:** The results of this study show variability in the results obtained with different methods for the determination of the in vitro susceptibility of enterococci to daptomycin and also show a high rate of false nonsusceptible results obtained with the MicroScan method compared to Etest and to the standard BMD. Laboratories using the MicroScan panel PC32 should confirm daptomycin nonsusceptible results by Etest or by BMD before reporting.

#### **P1658** Agreement of the MIC test strip vs. Etest in MIC determination of *Streptococcus pneumoniae*

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**Objectives:** In this study we examined the agreement between MIC-values for commonly used antibiotics obtained by the MIC Test Strip (Liofilchem) and the Etest (bioMérieux) in a well characterized collection of *Streptococcus pneumoniae* strains.

**Methods:** The MIC values of nine antimicrobials (benzylpenicillin-PG, ampicillin-AM, cefotaxime-CT, meropenem-MP, ciprofloxacin-CI, erythromycin-EM, clindamycin-CM, tetracycline-TC, and trimethoprim-sulfamethoxazole-TS) were determined using MIC Test strip (Liofilchem) and Etest (bioMérieux). A total of 93 characterized *S. pneumoniae* strains belonging to 26 different serotypes and 56 sequence types, including penicillin non-susceptible (PNSP)- (n = 31), macrolide resistant- (n = 30) and wild-type isolates (n = 32) were included. The MIC Test strips and Etests were applied on MH-agar with 5% horse blood inoculated with a 0.5 McFarland suspension in MH-broth and incubated for 20–24 hours at 35 ± 2°C in 5% CO<sub>2</sub>. *Streptococcus pneumoniae* ATCC 49619 was used as quality control strain. The numbers of strains with a MIC Test strip MIC-value greater than two-fold different from that obtained by the Etest were recorded. The MIC Test Strip MICs were categorized as a very major, major or minor error if the recorded MIC resulted in a change in clinical categorization (S-susceptible, I-intermediate, or R-resistant); R to S, S to R, or S to I or R to I, respectively, using clinical breakpoints as defined by EUCAST.

**Results:** The MIC Test Strip did result in a MIC-value difference more than two dilution steps compared to the corresponding Etest MIC for seven antibiotics (n = number of strains); PG (n = 3), AM (n = 1), CT (n = 7), MP (n = 4), CI (n = 24), CM (n = 9), and TS (n = 16). The divergent MIC Test Strip MIC-values did not result in any very major or major errors. However, for five drug-strain combinations minor errors occurred; for PG (n = 1), AM (n = 1), MP (n = 2), and CI (n = 1), changing susceptibility categorization from S to I (n = 3; PG and MP), or from I to R category (n = 2; CI and AM). All PNSP-strains were categorized correctly by both methods.

**Conclusion:** We found good agreement between the two different gradient methods. For seven antibiotics (PG, AM, CT, MP, CI, CM, and TS) a greater than two-fold divergence was observed, while for EM and TC all test results were within two-fold dilution. The divergent MIC-values obtained by the MIC Test Strip resulted in only five minor errors in clinical categorization of susceptibility.

#### **P1659** A CE-marked automated molecular test on the Abbott m2000 for the detection of vancomycin resistance genes *vanA* and *vanB* most commonly found in vancomycin-resistant enterococci

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**Objectives:** Vancomycin-resistant enterococci (VRE) infections are a major cause of health care-associated infections, leading to increased



treatment cost and longer hospital stays. Longer hospital stays increase a patient's risk for infections such as those of the blood stream, urinary tract, wounds, or sepsis. We have addressed the underserved need for a rapid and sensitive test for the detection and differentiation of the *vanA* and *vanB* genes most commonly found in VRE in patients at risk for colonisation or infection through development of the IMDx VanR for Abbott m2000 assay.

**Methods:** IMDx has developed a proprietary bioinformatics platform, NGENix™, which utilises iterative analytic processes to rapidly design oligonucleotide solutions to regions of a sequenced genome, assess the thermodynamics of the DNA multiplex, and review potential cross-reactivity within the solution and other genomes. We applied NGENix™ to design a qualitative multiplexed real time PCR-based

**Table 1. IMDx VanR for Abbott m2000 Performance Characteristics**

<b>Limit of Detection</b>	115 CFU/mL for <i>E. faecium</i> MMC4 ( <i>vanA</i> ) (95% CI: 104 – 128)
	223 CFU/mL for <i>E. faecalis</i> V583 ( <i>vanB</i> ) (95% CI: 204 – 243)
<b>Diagnostic Sensitivity</b>	96.4%
<b>Diagnostic Specificity</b>	98%

assay targeting the *vanA* and *vanB* genes in VRE strains from *E. faecium* MMC4 and *E. faecalis* V583, respectively.

**Results:** The newly designed oligonucleotide solutions, formatted for the Abbott m2000 system, were tested for analytical performance by analysing raw stool, rectal or peri-rectal swab samples. The IMDx VanR for Abbott m2000 assay demonstrated no cross reactivity when tested against 60 viruses and microorganisms. No deleterious effect was observed in the presence of potentially interfering substances, such as over the counter medications frequently used at or around the sampling site, as evidenced by non-significant deviations in FCN and MR values in the presence of these substances. A total of 576 tests were performed in precision studies and demonstrated <4% CV of FCN for all variables. Performance characteristics are listed in Table 1.

**Conclusions:** Development of the IMDx VanR for Abbott m2000 assay demonstrates the power of our in silico model to design a robust, multiplexed real time PCR-based test. When coupled with the Abbott m2000 system, the IMDx VanR for Abbott m2000 assay can produce results for up to 94 patient samples in <4 hours, providing a cost-effective solution to the need for rapid and accurate diagnostic tests that will help control the spread of VRE. The IMDx VanR for Abbott m2000 assay is currently CE-marked.

Table 1. IMDx VanR for Abbott m2000 performance characteristics

## ESBLs

### **P1660** Epidemiological characterisation of CTX-M-producing *Escherichia coli* using the DiversiLab system

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**Objectives:** During the last decade an increasing prevalence of extended-spectrum beta-lactamase (ESBL) producing Enterobacteriaceae has been detected worldwide, mainly due to dissemination of *Escherichia coli* and *Klebsiella pneumoniae* producing CTX-M-type ESBLs. In Örebro County, Sweden CTX-M-15 and CTX-M-14 are the dominant ESBL-types in *E. coli*.

Epidemiological typing of clinical isolates of CTX-M-producing *E. coli* isolated in Örebro County has previously been performed using a phenotypic method (PhenePlate system). The results showed a heterogeneous population with only a few minor clusters. The aim of this study was to genotypically characterize these bacteria using a commercial semi-automated rep-PCR (DiversiLab system).

**Methods:** Consecutive clinical isolates of CTX-M-producing *E. coli* (n = 152) collected from 1999 to 2008 were included in the study. The majority of the isolates were collected from urine, and the rest from blood, wounds, and the respiratory tract. DNA was isolated using the UltraClean Microbial DNA isolation kit and the DiversiLab Escherichia kit was used for rep-PCR amplification. DNA fragments were separated by electrophoresis in microfluidic DNA LabChips on Agilent 2100 Bioanalyzer.

**Results:** We identified 81 DiversiLab (DL) types, of which 20 comprised two or more isolates and 60 were singletons. The DiversiLab system was less discriminatory than the PhenePlate system where we identified 18 minor clusters but the majority of the isolates were singletons. One of the DL types contained 29 isolates that was identified as five distinct clusters by the PhenePlate system.

**Conclusion:** The results obtained with both DiversiLab and PhenePlate indicates that the population of CTX-M-producing *E. coli* is quite diverse. In general the results were relatively similar with the two methods; however, since the DiversiLab system is less discriminatory than the PhenePlate system it may be a better method to use for initial screening in outbreak situations.

### **P1661** High diversity of extended-spectrum beta-lactamases among clinical isolates of *Escherichia coli* from Portugal

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**Objectives:** ESBL-producing *E. coli* are currently amplified in hospitals throughout the world, including in Portugal, from which last surveys include isolates from 2002 to 2004. Our goal is to characterize current epidemiological features of ESBL-producing *E. coli* from diverse Portuguese hospitals.

**Methods:** One hundred and sixty-nine percent (2006–2007 and 2009–2010) ESBL-producing *E. coli* from three Portuguese hospitals (n = 2 Centre; n = 1 North) were studied. Bacterial identification and antibiotic susceptibility testing were performed by standard methods. ESBL characterization included DDST, PCR (blaCTX-M, blaSHV and blaTEM) and sequencing. Clonal relatedness was established by XbaI-PFGE. ST131-B2 clone and *E. coli* phylogroups were identified by PCR.

**Results:** A high clonal diversity was observed, with isolates belonging mainly to B2 (66%), but also A (13%), B1 (12%) or D (8%) phylogroups. They produced mostly CTX-M-I (80%; CTX-M-1, -15, -32) enzymes, although CTX-M-IV (9%; CTX-M-9, -14), SHV (8%; SHV-12) and TEM (3%; TEM-10, -52, -116) were also detected. An epidemic B2-ST131 clone (n = 112, four PFGE-types) harbouring mostly blaCTX-M-15 frequently associated with blaOXA-1 and/or blaTEM-1/-10/-116 (96%; 2006–2007, 77%; 2009–2010, 48%), and occasionally blaCTX-M-1 (2%), blaSHV-12 (1%) or blaCTX-M-14 (1%) was detected in all hospitals since 2006. *E. coli* belonging to phylogenetic group D (n = 14, 14 PFGE-types) or A (n = 22, 18 PFGE-types) produced a diversity of CTX-M enzymes (78%; CTX-M-1, CTX-M-14, CTX-M-15 or CTX-M-32) and less frequently SHV-12 (14%) or TEM-52 (8%). Both phylogroups were identified in all clinical settings during the studied periods. B1- *E. coli* isolates (n = 21, 18 PFGE-types) from 2006 to 2007 harboured blaCTX-M (56%; CTX-M-14, CTX-M-1-like), blaSHV-12 (33%), or blaTEM-52 (11%), while those from 2009 to 2010 carried mostly blaCTX-M (67%; CTX-M-9, -14). Isolates exhibited resistance to tetracycline (86%), streptomycin (83%), ciprofloxacin (79%), kanamycin (74%), sulphonamides (69%), tobramycin (64%), and gentamycin (62%).

**Conclusion:** We report current widespread of CTX-M-15-ST131 *E. coli* in different hospitals and a diversity of A, B1 and D clones producing mostly CTX-M (CTX-M-15, -14) and SHV-12 enzymes, which represents a shift of ESBL types comparing with previous surveys (2002–2004) where TEM-type enzymes were the most prevalent.

**P1662** Duration of faecal carriage of ESBL-producing *E. coli* and *K. pneumoniae* following first-time clinical infection

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**Objectives:** Determine prospectively duration of fecal carriage of ESBL-producing Enterobacteriaceae following clinical infection, and identification of factors associated with prolonged carriage.

**Materials and methods:** Sixty-one patients treated for infection with ESBL-producing *E. coli* (n = 56) and *K. pneumoniae* (n = 5) were included (women n = 37; median age 61 years). Patients had bloodstream infection (BSI) (n = 7), urinary tract infection (UTI) (n = 46), abdominal infection (AI) (n = 2), soft tissue infection (n = 5) or UTI and AI (n = 1), and were primary care outpatients (n = 12), hospital outpatients (n = 29) or inpatients (n = 20). Fecal samples and questionnaires on antimicrobial treatment and risk factors for ESBL (antimicrobial treatment, hospitalisation, urinary catheter, travel and hospitalisation abroad 6 months before infection, and abnormalities in the urinary tract) were self-collected at 1, 3, 6 and 12 months. ESBL-producing strains were detected with ESBL ChromID agar (bioMérieux), followed by susceptibility testing and ESBL confirmation with VITEK2 (bioMérieux). Epidemiological typing with pulsed-field gel electrophoresis is on going. Phylogrouping of *E. coli* was based on triplex PCR (chuA, yjaA, TspE4.C2). ST131 was screened for with pabB PCR. Variables that might influence the duration of carriage were compared for carriers and non-carriers at 12 months using Fisher's exact test.

**Results:** Carrier rate after three months was 66% (n = 40), after 6 months 55% (n = 34) and after 12 months 44% (n = 26). Of the carriers at 12 months, 21 were positive during the entire follow-up and five had a varying status. Among non-carriers at 12 months, 11 were negative during the entire follow-up, seven were positive for 3 months and 12 positive for 6 months, and five had a varying status. In 10% (n = 6) ESBL was found in another species during the follow-up than at inclusion. Thirty-five percent (18/51) of retrievable *E. coli* isolates belonged to phylogroup B2, and 27% (14/51) to ST131. B2 occurred more frequently among carriers at 12 months than non-carriers (52% vs. 21%, p = 0.04). ST131 was also more frequent among carriers (39% vs. 17%), but not statistically significant. BSI was the only other factor that significantly was associated with prolonged carriage (23% vs. 2.9%, p = 0.03), but carbapenem use was also higher among carriers (19.2% vs. 2.9%, p = 0.07).

**Conclusions:** Fecal carriage is common 12 months after clinical infection. Negative samples within the first year do not imply that the carriage is eliminated.

**P1663** High acquisition rates of ESBL-producing Enterobacteriaceae among Dutch travellers

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**Background:** The increasing rate of resistance in Enterobacteriaceae is a major cause of concern. Unprecedented human air travel and migration allow multiresistant clones and plasmids to be transported rapidly between countries and continents. Foreign travel has been demonstrated to be a risk factor for colonization with ESBL-producing Enterobacteriaceae.

**Objective:** To study the fecal acquisition of ESBL producers and risk factors among Dutch travelers.

**Study Design:** From March 2011 to September 2011, a prospective follow-up study was conducted at the travel clinic of Leiden University Medical Center (LUMC) and Municipal Health Service Leiden, the Netherlands. Healthy Dutch volunteers travelling outside Europe were enrolled. Data on potential travel-associated risk factors and rectal swabs were collected before and after traveling. Rectal swabs were cultured on chromID ESBL agar after preculturing in selective broth. ESBL confirmation was performed with combination disk synergy

testing of ceftazidim with clavulanic acid. Isolates are currently analysed with a DNA microarray Check-MDR CT103 for the presence of ESBLs, plasmid mediated ampCs and carbapenemases.

**Results:** A total of 473 travelers were included. Thirty-three participants carried ESBLs before travel and were excluded from the analysis. The data of the first 307 Dutch travelers, who completed the study, are described here. A total of 107 participants with negative pre-travel samples were colonized with ESBL-producing *Escherichia coli* (n = 100), *Klebsiella pneumoniae* (n = 4) or both (n = 2), or *Enterobacter cloacae* (n = 1) after the trip. Despite the small number of travelers to India (n = 16), multivariate analysis showed that this was associated with the highest risk factor for the acquisition of ESBLs (OR 8.4). Travel to other destinations was associated with the following rates of posttravel ESBL colonization: 42% for Asia (India excluded), 26% for Africa, 25% for the Middle-East and 17% for Southern/Middle America. Staying in budget hotels showed a positive trend for acquiring ESBLs. Gastroenteritis during the trip was not a significant risk factor.

**Conclusion:** This study found a very high fecal carriage of 35% ESBLs among Dutch travelers. The highest acquisition rate was found in travelers to India. In this study population, gastroenteritis during the trip was not associated with the acquisition of ESBLs. We found a higher pretravel fecal ESBL carriage of 11% than we had expected from earlier data.

**P1664** Molecular characterisation of clinical isolates of Enterobacteriaceae resistant to extended-spectrum cephalosporins in Modena, Italy

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**Objectives:** The aim of this study was to investigate the molecular mechanisms of resistance to extended-spectrum cephalosporins in clinical isolates of Enterobacteriaceae collected in the Laboratory of Microbiology and Virology of Modena University Hospital during a survey performed between 2008 and 2009.

**Methods:** Identification and susceptibility tests of bacterial isolates were performed by Vitek 2 (Biomérieux) and interpreted according to Clinical and Laboratory Standards Institute guidelines. Phenotypic production of Extended Spectrum b-lactamases (ESBLs) and AmpC-type b-lactamases (CBLs) was assessed by double-disk synergy test and E-test (cefotetan/cloxacillin). Clonal relatedness was investigated by Randomly Amplified Polymorphic DNA (RAPD) and Arbitrarily Primed-PCR (AP-PCR). The presence of genes encoding ESBLs (TEM, SHV and CTX-M) and CBLs (CMY) was investigated by PCR. Restriction analysis were used to assign the CTX-M amplicons to group 1, 2, 8, 9 or 25. Selected amplicons were sequenced in order to determine the b-lactamases variants.

**Results:** Bacterial isolates (n = 138) were selected from a total of 1119 ESBL/ampC producers identified by phenotypic methods (*Escherichia coli* n = 73/680, *Klebsiella pneumoniae* n = 23/153, *Proteus mirabilis* n = 42/286). RAPD/AP-PCR allowed to identify 80 different clonal lineages. One isolate of each clone was investigated by PCR to reveal the presence of ESBL or ampC encoding genes. Among the ESBL producers, blaCTX-M-type genes were overall the most prevalent (n = 47) with a predominance of distinct variants belonging to group 1 (n = 39). However blaCTX-M of group 9 (n = 7) and 2 (n = 1) were also detected. blaCTX-M alleles were detected in *E. coli* (n = 37) and *K. pneumoniae* (n = 10). The remaining alleles were blaTEM (n = 42) and blaSHV (n = 15). Acquired AmpC of the CMY lineage were detected in *E. coli* (blaCMY-2, n = 4) and *P. mirabilis* (blaCMY-16, n = 8; blaCMY-2, n = 2). Sequencing results revealed an high variability among CTX-M-type b-lactamases (CTX-M-1, CTX-M-14, CTX-M-15, CTX-M-24, CTX-M-32, CTX-M-66). Regarding TEM- and SHV-type beta-lactamases, variants TEM-1, TEM-52, TEM-177 and SHV-1, SHV-2a, SHV-12 were detected, respectively.

**Conclusion:** The high incidence of ESBLs and CBLs in clinical specimens is becoming common in our hospital as in the rest of Italy and requires surveillance. CTX-M-type enzymes are the predominant ESBLs in *K. pneumoniae* and *E. coli*, while TEM-52 and the CMY-2-like CBLs are prevalent in *P. mirabilis*.

**P1665** Emergence and genetic environment of CTX-M enzymes produced by clinical *Escherichia coli* isolates in Germany, 2005–2009

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**Objectives:** CTX-M type ESBLs are the predominant ESBL enzymes in Enterobacteriaceae isolates worldwide. The objective of this study was to evaluate the prevalence of CTX-M type ESBLs among German *Escherichia coli* (ECO) isolates between 2005 and 2009. Additionally, CTX-M producing isolates collected in 2005 were analysed regarding the transferability and the presence of ISEcp1 that have apparently involved in dissemination and expression of the enzymes.

**Methods:** Three hundred, 292 and 297 ECO recovered during three multicentre studies conducted in 2005, 2007 and 2009, respectively, were studied. ESBL-producing organisms were identified according to CLSI criteria and characterized by isoelectric focusing (IEF), amplification of blaCTX-M genes and sequencing. Conjugation experiments with CTX-M producing isolates collected in 2005 were done with ECO J53 as recipient strain. Transconjugants were selected using cefotaxime (4 mg/L) and sodium azide (200 mg/L). Plasmids of non-conjugative strains were transformed into competent ECO DH5-alpha cells by electroporation. Promotor regions of blaCTX-M genes were investigated by PCR and sequencing.

**Results:** The percentage of CTX-M producing ECO was 4.7% (14/300) in 2005, 11.6% (34/292) in 2007 and 11.4% (34/297) in 2009. In 2005, 50% (7/14) of the CTX-M producing ECO harboured CTX-M-1, 35.7% (5/14) CTX-M-15 and 14.3% (2/14) CTX-M-14, while 26.5% (9/34), 58.8% (20/34), 11.8% (4/34), 2.9% (1/34) and 2.9% (1/34) of the CTX-M positive isolates produced CTX-M-1, -15, -14, -9 and -2, respectively, in 2007. In 2009, 44.1% (15/34), 50% (17/34) and 5.9% (2/34) of isolates expressed CTX-M-1, CTX-M-15 and CTX-M-14, respectively. Six CTX-M-1 and 1 CTX-M-14 enzyme expressed by 7/14 CTX-M producing strains collected in 2005, were located on conjugative plasmids. CTX-M enzymes of the remaining seven strains were not transferable. ISEcp1 was found in 6/7 strains (one expressed CTX-M-1, one CTX-M-14 and four CTX-15) with non-transferable plasmids upstream the respective blaCTX-M gene as well as on conjugative plasmids of two strains expressing CTX-M-1 and -14, respectively.

**Conclusions:** Our data suggest that the rate of CTX-M producing strains among ECO isolates doubled between 2005 and 2007, but remained unchanged between 2007 and 2009. CTX-M-1 was the primary CTX-M type in 2005, while CTX-M-15 predominated in 2007 and 2009. CTX-M-1 enzymes were mainly disseminated by conjugative plasmids whereas the spread of CTX-M-15 enzymes seems to be associated with ISEcp1.

**P1666** Epidemiology, antimicrobial susceptibility, treatment and outcomes of health care-associated and nosocomial urinary tract infections caused by extended-spectrum beta-lactamase producing *Escherichia coli*

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**Objectives:** To evaluate the activity of ertapenem and other antimicrobial agents and to describe the epidemiology, clinical presentation, and outcomes of urinary tract infections (UTI) caused

by extended spectrum beta lactamase (ESBL) producing *Escherichia coli* in health care associated or nosocomial infections.

**Materials:** One hundred and seventy-three ESBL-positive *E. coli* isolates from patients with clinically confirmed urinary tract infection were collected between January 2009 and January 2011 in infectious disease laboratory. Susceptibility testing was performed with agar diffusion and E test methods. All results were evaluated according to CLSI guidelines. Antimicrobial susceptibility testing was carried out using the antibiotics fosfomicin, nitrofurantoin, ertapenem, aminoglycosides, quinolones, co-trimoxazole. The risk factors for acquisition and outcomes of UTI caused by ESBL producing *E. coli* was reviewed retrospectively by using Infectious Diseases consultation chart.

**Results:** Of 173 patients with UTI 43.4% were attending ambulatory care and 56.6% were hospitalized. All of them used antimicrobial therapy in the previous 30 days. Predisposing factors, co-morbidities, treatment, type of severity of UTI, and outcomes are shown in table.

We found no resistance against ertapenem and fosfomicin in all tested isolates. Nitrofurantoin exhibited high in vitro activity (97.6%). Susceptibility rate was determined as 89% for aminoglycoside. High rates of resistance to ciprofloxacin (80.1%) and cotrimoxazole (70.6%) were observed. Mortality (11%) was determined in patients associated with urosepsis and comorbid diseases.

**Conclusion:** Our study results may aid to clinician select appropriate antibiotic therapy suspected of having UTI in health care associated or hospitalized patients caused by ESBL producing *E. coli*. Ertapenem may be a first choice of treatment for upper UTI and fosfomicin for lower UTI.

Table. Characteristic of patients with ESBL-producing *E. coli*

	N (173)	%
Age (years) median	58(15-90)	
Number of patient age >65 yr	65	37.8
Female gender	120	74
Predisposing factors		
Presence of urinary catheter	46	26.5
Previous antimicrobial use	173	100
Urinary problems		
Previous urological operation	28	15
(Prostate ca, bladder ca, renal transplantation, double j op, Nephrectomy, etc.)		
Anomaly of urinary tract	18	10
Prostatic hypertrophy	7	4
Orchiditis	11	7.5
Neurological impairment of the bladder	7	4
Comorbidities		
Renal transplantation	9	5.2
Renal insufficiency	15	8.6
Diabetes mellitus	35	20.2
Immunosuppression	35	20.2
Non urologic Neoplasia	50	28.9
Genitourinary neoplasia	8	4.6
Misc	60	46.2
Attending service		
Ambulatory care	75	43.4
Hospitalized	98	56.6
Clinical presentation		
Asymptomatic bacteriuria	28	16.2
Lower urinary tract infection	14	8.1
Upper urinary tract infection	76	43.9
Chronic cystitis	27	15.6
Urosepsis	28	16.2
Antimicrobial Therapy		
Ertapenem	31	18
Imipenem	34	19.6
Miprofloxacin	37	21.3
Ciprofloxacin	9	5.2
Fosfomicin	35	20.1
Nitrofurantoin	1	0.6
Coligiparone/colibactam	2	1.1
Piperacillin/tazobactam	5	2.9
None (Asymptomatic bacteriuria)	22	12.7
Outcome		
Cure	154	89
Death	19	11

Table 1. Characteristic of patients with ESBL-producing *E. coli*

**P1667** Characterisation of infection with community-acquired extended spectrum beta-lactamase producing pathogens: an observational cohort study

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**Objectives:** The increasing worldwide prevalence of Extended Spectrum Beta-Lactamase (ESBL)-producing organisms has posed a challenge for antimicrobial therapy over the last two decades. The spread of this resistance to the community has had major public health implications. We aim at identifying the characteristics of patients with ESBL-producing *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*) from the community and determining the outcomes of these infections.

**Methods:** Adult patients infected with ESBL-producing *E. coli* and *K. pneumoniae* and admitted to the American University of Beirut Medical Center were prospectively enrolled between January 2009 and September 2011. We excluded patients who had an infection with the same organism during the preceding year. Patients were classified as having a community-acquired (CAI), healthcare-associated, or hospital-acquired infection. For the purpose of this analysis, the two latter groups were combined under the general heading of healthcare-associated infection (HAI). Patients were followed for complications and outcome during their index admission.

**Results:** A total of 209 patients were included in the study; 56 (26.8%) had CAI, of which 82.1% were secondary to ESBL-producing *E. coli*. Although the urinary tract was the most common primary site in both CAI and HAI (78.6% and 44.4%, respectively,  $p < 0.001$ ), respiratory tract and wound infections were higher in the latter group (5.4% vs. 13.7% and 7.1% vs. 15.7%, respectively). On multivariable analysis, patients with CAI were more likely than those with HAI to have diabetes (OR 2.0, 95% CI 1.0–4.0), but were less likely to have received immunosuppressive therapy (OR 0.3, 95% CI 0.1–1.0) or antibiotics (OR 0.3, 95% CI 0.1–0.6) within 30 days of the infection, and were less likely to have a history of malignancy (OR 0.2, 95% CI 0.1–0.6). Patients in the CAI group had a more favorable outcome than those in the HAI group: sepsis (41.1% vs. 57.5%, respectively,  $p = 0.03$ ), respiratory failure (3.6% vs. 14.0%,  $p = 0.033$ ), hospital stay (11.2 vs. 19.2 days,  $p = 0.036$ ), and mortality (3.6% vs. 19.9%,  $p = 0.04$ ).

**Conclusion:** Emerging antimicrobial resistance and more importantly its community spread prompt careful detection of patients in whom empirical coverage for ESBL producing organisms should be considered. Diabetic patients seem to be at increased risk of acquiring ESBL-producing *E. coli* or *K. pneumoniae* from the community. CAI tends to be a milder disease.

**P1668** Genetic diversity and emergence of clone ST131 among lower respiratory tract isolates of extended-spectrum beta-lactamase producing *Escherichia coli* from Slovenia

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**Objectives:** *Escherichia coli* (*E. coli*) exhibits considerable genetic diversity due to a wide range of virulence associated genes and is consequently implicated in a variety of diseases. However, *E. coli* is rarely associated with human respiratory tract infections. Since *E. coli* infections are becoming a serious health concern due to the emergence of highly virulent and antimicrobial-resistant clones, we decided to elucidate the genetic characteristics of extended-spectrum beta-lactamase (ESBL) producing *E. coli* isolates from respiratory tract specimens and the emergence and prevalence of clone ST131.

**Methods:** A total of 160 non-duplicate *E. coli* ESBL isolates from respiratory tract specimens consecutively collected between the years 2002 and 2010 at a tertiary hospital in Slovenia were included in the study. The phylogenetic group of the isolates was determined by the

triplex PCR method as described by Clermont. Genes coding for SHV, TEM, CTX and plasmid-mediating quinolone resistance (PMQR) were also screened by PCR using specific primers. Multilocus sequence typing (MLST) was performed according to the instructions at the *E. coli* MLST website. Additionally, all isolates were screened by PCR for the presence of 10 virulence genes.

**Results:** Among 160 isolates 56.5%, 23%, 18% and 2.5% segregated to phylogenetic group B2, D, A and B1, respectively. Whereas the majority of the isolates (85%) possess blaCTX group I genes, blaCTX-M group nine genes were detected only in 18% of all isolates. Additionally 57% and 11% of isolates were positive for TEM and SHV types of ESBLs, respectively. One isolate was positive for PMQR gene qnrA and four isolates, all from the year 2010, were positive for qnrB. MLST and virulence gene analysis revealed that 52% of all isolates were ST 131. All of them were associated with the presence of virulence genes iha, sat, usp, fyuA, iutA and flu.

**Conclusions:** Our study revealed that respiratory tract isolates of ESBL producing *E. coli* predominantly belong to the highly virulent and resistant phylogenetic group B2. The first ST131 isolates were collected in 2006. Since then their prevalence increased from 26% to 54% in 2010. Whereas 87% of ST131 isolates belonged to phylogenetic group B2, and were positive for blaCTX-M group nine genes, 13% were from group D and positive for blaCTX-M group nine genes. All detected ST131 isolates shared virulence genes iha, sat, usp, fyuA, iutA and flu.

**P1669** The open door clonal ESBL-producing *Escherichia coli* in the acute hospital and the community

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**Objectives:** *Escherichia coli* that produce extended-spectrum beta-lactamases (ESBLs) are a major cause of healthcare-associated infection and are often multidrug resistant (MDR). In this study 100 clinical isolates of *E. coli* collected in a major hospital and in community healthcare facilities in Dublin, Ireland during 2009 and 2010 were retrospectively studied.

**Methods:** *Escherichia coli* phylogenetic groups were determined by the PCR method of Clermont et al. Pulsed-field gel electrophoresis (PFGE) was used to determine the genetic relatedness of the isolates. Patient demographic data, clinical data and antimicrobial susceptibility data were collected by retrospective analysis of electronic medical records to investigate the epidemiology of specific clones.

**Results:** Phylogenetic groups B2 (62%) and D (18%) were the most common and were associated with non-urinary isolates ( $p < 0.0001$  by Fisher's exact test). PFGE revealed considerable diversity within the collection with 87 distinct types found. Twelve clusters were identified based on a similarity of  $\geq 80\%$ , the largest of which contained 34 isolates and clustered with the epidemic UK strain A. The pandemic ESBL-producing *E. coli* clone O25b-ST131 comprised 56% of all isolates. Residents of healthcare facilities in the community exclusively carried clonal strains belonging to O25b-ST131 and phylogenetic group D.

**Conclusion:** A number of virulent *E. coli* clones may be largely responsible for healthcare-associated ESBL-producing *E. coli* in Dublin.

**P1670** Rep-PCR analysis (DiversiLab) indicates ESBL plasmid transmission from verotoxin producing enteroaggregative *Escherichia coli* O104:H4

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**Objectives:** In Germany a large outbreak of Verotoxin producing enteroaggregative *Escherichia coli* (VTEC O104:H4) expressing a

CTX-M extended spectrum beta-lactamase (ESBL) has recently been experienced. During the course of infection, ESBL producing *E. coli* strains were observed which lack Verotoxin expression in several patients, indicating genetic rearrangement in the gut of patients. In this study we analyzed the pattern stability of rep-PCR as well as clonal relationship of paired ESBL producing Verotoxin-positive and -negative *E. coli* using the DiversiLab System.

**Methods:** *Escherichia coli* isolates grown on on McConkey Agar containing 8 mg/L cefotaxime were subcultivated over night in enrichment broth and Verotoxin expression was analyzed using a specific ELISA. Presence of stx2, int11, and blaCTX-M was confirmed by PCR. Rep-PCR was performed using the DiversiLab *E. coli* kit for confirmed O104:H4 isolates as well as follow up isolates from patients carrying phenotypic Shiga-toxin negative ESBL expression *E. coli*.

**Results:** Sixteen confirmed O104:H4 strains including a long term carrier isolate (87 days) could be clustered together but separated from non-O104 VTEC strains by rep-PCR. For six patients strain pairs of Verotoxin-positive and -negative *E. coli* were analyzed. Presence of the phage and the CTX-M plasmid could be confirmed with PCRs positive for stx2, int11, and blaCTX-M in all primary and follow up strains. In five patients the follow up strains could be clearly separated by rep-PCR from the primary isolates with 78.0–81.4% similarity compared to the respective primary isolates. These isolates were negative for stx2 but positive for int11, and blaCTX-M indicating an ESBL plasmid transfer. One follow up strain pairs displayed clonal identity compared to the rep-PCR cluster of O104:H4 strains. This strain displayed a positive PCR for stx2 despite the lack of phenotypic Verotoxin expression, indicating regulatory defects in this strain.

**Conclusions:** During infection with the ESBL producing STEC/VTEC O104:H4 different genetic rearrangements can occur in affected patients resulting in ESBL producing *E. coli* lacking phenotypic expression of Shiga-toxin/Verotoxin. Rep-PCR using the DiversiLab System is a suitable tool for the analysis of the clonal relation of *E. coli* strains to elucidate transfer of genetic elements in independent clonal lineages.

#### **P1671** Prevalence of ESBL-producers causing urinary tract infections, in Aveiro, Portugal

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**Objectives:** Extended spectrum beta-lactamases (ESBLs) are an increasing cause of resistance in nosocomial and community isolates. The aims of the present study were to detect the most prevalent organisms causing urinary tract infection (UTI) in patients, in the emergency room (ER) and among those to screen for ESBL-producers.

**Methods:** During a 10 months period, isolates were included in this study according to the following criteria: belonging to a patient >65 years old, with a primary diagnostic – UTI and collected in the ER. The identification and susceptibility profile of the isolates were performed with the Vitek2 system (according to CLSI guidelines) and Advanced Expert System (VITEK 2 AES) (BioMérieux, Marcy L'Étoile, France). ESBL producers were confirmed by Etest™ (AB Biodisk) ESBL with Cefotaxime/Cefotaxime + Clavulanic acid and Ceftazidime/Ceftazidime + Clavulanic acid strips, according to manufacturer's instructions.

**Results:** From a total of 6406 urine samples, 272 isolates were selected to this study according to the inclusion criteria. There was no significant difference in the number of isolates collected from male and female patients. The most prevalent species were *Klebsiella pneumoniae*

(35.7%), *Escherichia coli* (29.8%) and *Pseudomonas aeruginosa* (14.3%). *Klebsiella pneumoniae* and *Escherichia coli* were further investigated for the presence of ESBL. An ESBL phenotype was detected in 82.5% of the *Klebsiella pneumoniae* and 44.4% of the *Escherichia coli*.

**Conclusions:** The presence of ESBL in clinical specimens is becoming a very serious problem in our region. The percentage of ESBL-producing *K. pneumoniae* in the ER of our hospital is extremely high and indicates a spread of these isolates into the community settings. The occurrence of ESBL-producing *E. coli* is also considerable and requires surveillance. The presence of ESBL's among the isolates highlights the importance of routine detection of ESBL producers.

#### **P1672** Molecular characterisation of beta-lactamases of Enterobacteriaceae in Europe

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**Objectives:** The Study for Monitoring Antimicrobial Resistance Trends (SMART) has tracked global susceptibility patterns of gram-negative pathogens of intra-abdominal infections (IAI) since 2002. Enterobacteriaceae (ENT) collected in Europe during 2008–2009 which were either phenotypically extended spectrum beta-lactamase (ESBL) positive or non-susceptible to ertapenem (ETP, MIC > 0.5) were further studied to determine molecular mechanisms of resistance. This report summarizes types of beta-lactamases found, and evaluates the ability of the EUCAST ETP breakpoint to identify carbapenemase-producing isolates.

**Methods:** Five thousand nine hundred and eighty-three ENT were collected in 2008–2009 from 44 hospitals in 13 European countries. Isolates with ETP MICs > 0.5 using MicroScan broth microdilution panels, or ESBL+ using the CLSI broth microdilution method, were analyzed for ESBL (SHV, TEM, CTX-M), ampC (ACC, CMY, DHA, FOX, MIR-ACT, MOX), and KPC using the Check-Points assay, and VIM, OXA, IMP, and NDM carbapenemases by multiplex PCR. Chromosomal ampC was assumed to be present in *Enterobacter*, *Citrobacter*, and *Serratia* spp., and was not characterized. All genes were sequenced and analyzed using SeqScape software.

**Results:** 5827/5983 (97.4%) ENT were susceptible to ETP; 298 of these were phenotypically ESBL+, comprised of 59 SHV+ (one coproduced CTX-M), seven TEM+ (two coproduced CTX-M), and 232 CTX-M+. Additionally, 38 were plasmidic AmpC+, and two VIM+ (ETP MICs 0.5 and 0.25, the latter coproducing CMY-2 AmpC).

One hundred and fifty-six ENT were non-susceptible to ETP; of these 41 were SHV+ (one coproduced CTX-M, seven coproduced plasmidic AmpC), 17 CTX-M+ (one coproduced SHV, two coproduced AmpC), 20 KPC+, 23 VIM+, six OXA-48+, 53 plasmidic AmpC (almost all ACT/MIR; 51/53 were *Enterobacter* spp.), and 21 were negative for all tested beta-lactamases (21/21 were *Enterobacter* spp.).

**Conclusions:** 1 The vast majority of ENT in Europe were susceptible to ETP; however, *Enterobacter* spp. – especially those producing plasmidic AmpC – tended to be less susceptible than other species.

2 CTX-M is by far the most prevalent ESBL found in IAI isolates in Europe.

3 Carbapenemases were very rare, with the exception of Greece which accounted for 20/20 KPC and 16/23 VIM.

4 EUCAST ETP breakpoints (0.5/1/2) performed well with regard to separation of carbapenemase-producing from non-producing isolates.

**P1673** Duration of colonisation with extended-spectrum beta-lactamase-producing enterobacteria

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**Objectives:** The emergence of ESBL-producing enterobacteria is becoming an ever-increasing problem, calling for successful preventive measures. The aim of our study was to determine the duration of colonisation and risk factors for prolonged colonisation with ESBL-producing *Escherichia coli* or *Klebsiella pneumoniae*.

**Methods:** Patients colonised or infected with ESBL-producing bacteria were included in a prospective study from November 2009 to May 2011. Rectal swab, urine culture, and throat swab were performed in each patient. Sample collection was repeated every 3 months. A questionnaire was filled out by a researcher at every sample collection to gather data about potential risk factor for prolonged colonisation. Collected samples were inoculated on chromogenic agar selective for ESBL-producing bacteria. Disc diffusion method was done for *K. pneumoniae* and/or *E. coli* isolates to assess antimicrobial susceptibility profile for each isolate. To evaluate potential risk factors for prolonged colonisation with ESBL-producing bacteria Fisher's exact test was used.  $p < 0.05$  was considered significant. Statistical analysis was performed with SPSS version 17.0 (SPSS, Chicago, IL, USA).

**Results:** Thirty-three patients (23 male, 10 female) completed the 6 months follow-up. Patients were 24–94 years old (60 years on average). Two patients were diabetics on insulin, one patient had chronic kidney failure and three were on the immunosuppressive therapy. Eighteen patients were colonised with *K. pneumoniae*, eight patients with *E. coli*, in seven patients both were isolated. Ninety-nine sample collections were done altogether. Three months after the initial sampling 22/33 (66.7%) patients had an ESBL-producing enterobacteria isolated from at least one clinical sample. Six months after the initial sampling 17/33 (51.5%) patients were ESBL positive. Urine catheter, chronic wound, immobility and percutaneous gastrostomy tube were associated with ESBL positive cultures after 6 months of follow-up.

**Conclusion:** Our study showed that 6 months after the initial sampling more than half of patients included in the study were still ESBL positive. The high percentage of ESBL positive patients calls for caution and need for isolation at readmission. Patients with urine catheters, gastrostomy tubes, chronic wounds and immobile patients are at particular risk for prolonged ESBL carriage.

**P1674** Clonal structure, ESBLs and acquired AmpCs of *Escherichia coli* populations colonising patients in rehabilitation centres in four countries

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**Objectives:** To compare the ESBL/AmpC-producing *Escherichia coli* from patients in four rehabilitation centres in different countries.

**Methods:** This multinational, prospective study (EU Project MOSAR) was conducted in four rehabilitation centres from 2008 to 2010: BM (Berck, France; two wards, 80 beds), FS (Rome; two wards, 106 beds), GI (Barcelona; one ward, 38 beds), and TA (Tel-Aviv; two wards, 50 beds). All patients were screened for colonization with Enterobacteriaceae resistant to expanded-spectrum cephalosporins (ESCs). Patient-unique *E. coli* isolates were checked for ESBL and AmpC production phenotypically. Typing was done by PFGE and MLST; beta-lactamases were identified by PCR and sequencing.

**Results:** A total of 311 isolates (BM,  $n = 30$ ; FS,  $n = 108$ ; GI,  $n = 32$ ; TA,  $n = 141$ ) were analyzed. The overall number of *E. coli* clones (sequence types, STs) was 55 (164 PFGE types), with the clonal diversity degree varying from 2.0 to 4.8 isolates per ST in particular populations (GI and TA, respectively). ST131 was predominant, with prevalence of 25.0% in GI, 41.4% in TA, 55.6% in FS and 66.7% in

BM, and was the only clone observed in all centres. Other wide-spread clones, identified in three sites each, were ST10, ST38, ST354, ST405 and ST648. Of the more specific clones, a notable role was played by ST57, ST156 and ST393 in GI, and ST372 and ST398 in TA. Ten new STs were found. 95.5% of the isolates produced ESBLs and 4.8% had acquired AmpCs. All these were dominated by CTX-Ms (83.2%; 11 variants), followed by SHVs (12.0%). CTX-M-15,-14 and CMY-2 were observed in all centres, and CTX-M-15 was the most prevalent in general (39.8% isolates; 36.4% clones), though in particular sites it did not prevail either among isolates (TA) or clones (FS) or both (GI). CTX-M-14 (11.3% isolates; 25.5% STs) was predominant in GI whereas CTX-M-1 (9.4%) had spread into many clones in FS. ST131 had different enzymes in each centre (nine enzymes altogether), and CTX-M-15 was identified in 54.5% of its isolates, ranging from 93.3% in FS to only 25.9% in TA, where CTX-M-27 was prevalent too (24.8% of isolates). Almost none of the clones of wider distribution or higher prevalence strictly correlated with a single beta-lactamase.

**Conclusions:** This study shows the current, complex picture of ESC-resistant *E. coli* populations in rehabilitation centres. Although specific clones and enzymes were identified in different countries, ST131 was predominant overall but produced a variety of beta-lactamases.

**P1675** The epidemic multi-resistant *Escherichia coli* ST131 clonal group is prevalent in Copenhagen and differs significantly from other Danish extended-spectrum betalactamase (ESBL)-producing *E. coli*

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**Objectives:** *Escherichia coli* sequence type 131 (ST131) is a pandemic clone, causing antimicrobial-resistant infections. We assessed the prevalence of *E. coli* ST131 among 115 consecutive ESBL-producing *E. coli* isolates from 115 patients (2008–2009) from three hospitals and 100 general practitioners' offices in Copenhagen, and compared ST131 and non-ST131 ESBL isolates for multiple host and bacterial characteristics.

**Materials and methods:** O:K:H serotyping was done according to Ørskov and Ørskov. Antimicrobial susceptibility was assessed using the Sensititre system. Phylogenetic group, virulence genotypes (for >60 markers of extraintestinal pathogenic and diarrheagenic *E. coli*), and ESBL-encoding genes were determined by using established PCR-based methods and DNA sequencing.

**Results:** Among 5473 *E. coli* isolates from 5473 patients (81% urine, 5% blood, 14% other sources; 68% hospital-source, 32% community-source), 115 (2.1%) produced ESBLs. Of these, 44 (38%) were ST131. Neither ESBL production nor ST131 status varied significantly in relation to specimen type or hospital vs. community origin. Compared with non-ST131 isolates, ST131 isolates were positively associated ( $p < 0.05$ ) with CTX-M-15, naladixic acid and ciprofloxacin resistance, phylotype B2, and virulence genes *afa/dra* (Dr family adhesins), the F10 *papA* allele (P fimbriae variant), *fyuA* (yersiniabactin receptor), *iha* (adhesin-siderophore), *itutA* (aerobactin receptor), *kpsmII* (group 2 capsules), *malX* (pathogenicity island), *ompT* (outer membrane protease), and *sat* (secreted autotransporter toxin), and negatively associated with CTX-M-1, CTX-M-14, gentamicin and chloramphenicol resistance, and virulence genes *hra* (heat resistant agglutinin) and *kpsMTIII* (group 3 capsules). The 44 ST131 isolates exhibited 2 O antigens (O25, O16), two flagellar antigens (H4, H5), and 7 K antigens (K2, K5, K16, K22, K20 K23, K98, K100). In contrast, the 71 non-ST131 isolates exhibited 31 O, 28 K, and 20 H antigens. ST131 was significantly associated with the O25, K100, and H4 antigens (each,  $p < 0.001$ ). Prevalent ST131-associated O:K:H serotypes included O25:K100:H4 ( $n = 18$ ), O25:K5:H4 ( $n = 5$ ), O25:K:H4 ( $n = 5$ ), Orough:K100:H4 ( $n = 3$ ).

**Conclusions:** ESBL-producing *E. coli* ST131 strains are prevalent in Copenhagen in the hospital and community, are associated with the O25, K100, and H4 antigens, and differ significantly from other Danish

ESBL-positive *E. coli* regarding ESBL enzymes, serotypes, virulence genes, and antibiograms.

**P1676** **Characterisation of resistance mechanisms and epidemiology of Enterobacteriaceae collected during a phase II clinical trial for ceftazidime avibactam**

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**Objectives:** This study aimed to screen for extended-spectrum beta-lactamase (ESBL)-encoding genes and to assess the epidemiology of *Escherichia coli* strains from patients enrolled in a Phase II trial for Ceftazidime Avibactam (CAZ AVI) in adults with complicated urinary tract infections (cUTIs).

**Methods:** Antimicrobial susceptibility testing was performed on 238 strains according to CLSI (2009) methodologies. CLSI and EUCAST breakpoint criteria were applied for MIC interpretations. Enterobacteriaceae displaying ceftriaxone (CRO) and/or CAZ MIC values of  $\geq 2$  mg/L and non-fermenters with CAZ MICs at  $\geq 16$  mg/L were screened for beta-lactamase genes by PCR and sequencing. Relative transcription levels of ampC were determined. All *E. coli* strains (186) were screened for two single nucleotide polymorphisms known to be unique to the ST131 lineage by Real-Time PCR.

**Results:** The following strains were evaluated: 76 *E. coli*, one *Enterobacter cloacae*, one *Klebsiella pneumoniae* and, among non-fermenters, one *Acinetobacter baumannii* and three *Pseudomonas aeruginosa*. Enterobacteriaceae showed CAZ AVI MIC results of  $\leq 0.25$  mg/L, except for one *E. cloacae* (MIC, 2 mg/L). Non-fermenters exhibited higher CAZ AVI MICs (4–16 mg/L). A total of 71 (93.4%) *E. coli* harboured blaCTX-M-like genes, most commonly (68/71; 95.8%) blaCTX-M-15. Three (4.2%) *E. coli* carried blaCTX-M-14 and 5 (6.6%) possessed blaCMY-2-like genes, which also harboured blaCTX-M-15. blaSHV-12-like genes were detected in 3 (4.2%) *E. coli*, including one CTX-M-15 producer. blaOXA-1/30 was noted in 38 (53.5%) *E. coli*, which also harboured blaCTX-M-15. Only three *E. coli* had overexpression of AmpC. The *K. pneumoniae* strain carried blaSHV-like, while the *E. cloacae* overexpressed AmpC. All *P. aeruginosa* tested had upregulated AmpC expression, whereas the *A. baumannii* strain possessed blaOXA-2- and blaKLUA-like genes. Among all *E. coli*, 35/186 (19%) were ST131, among which 22 (62.9%) met the screening criteria for ESBL genes and all harboured blaCTX-M-15.

**Conclusions:** Nearly all *E. coli* that met the screening criteria for ESBL genes produced CTX-M-15 alone or in combination with other ESBLs. All *E. coli* ST131 with higher CAZ and/or CRO MIC values ( $\geq 2$  mg/L) produced CTX-M-15, supporting the role of this clone in blaCTX-M-15 dissemination in cUTI. This is the first report of blaKLUA-like genes in *A. baumannii*.

**P1677** **Molecular characterisation of extended-spectrum beta-lactamases produced by community-acquired *Escherichia coli* causing urinary tract infections in Tunis**

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**Background and aims:** Recently, extended spectrum beta-lactamases (ESBL) producing *Escherichia coli* have emerged as responsible for community acquired (CA) infections leading to serious therapeutic problems. A particular *E. coli* virulent clone ST131 belonging to B2 phylogenetic group and producing CTX-M-15 enzyme has been broadly disseminated in the world. The aims of the present study were to investigate ESBL types and phylogenetic groups of CA-ESBL producing *E. coli* causing urinary tract infections in Tunisia and to detect a clonal relationship between them.

**Methods:** Fifteen CA-ESBL producing *E. coli* were isolated between January 2007 and December 2009 at the microbial laboratory of Charles Nicolle Hospital. Microbial identification was done by conventional

methods. Antimicrobial susceptibility was determined by disk diffusion method and detection of ESBL production was performed using double disk synergy test. The presence of blaCTX-M, blaSHV and blaTEM genes was detected by PCR and sequencing. Genomic typing was determined by pulsed-field gel electrophoresis (PFGE) analysis. Phylogenetic groups and identification of ST131 clone were performed by multiplex PCR.

**Results:** Two strains were resistant only to beta-lactams, four were resistant to all aminoglycosides and six were resistant to fluoroquinolones and cotrimoxazole. No resistance was detected to carbapenems or to colistin. The most common type of ESBL was CTX-M-15 (N = 13), followed by SHV-12 (N = 2). PFGE results showed 13 different clones. Ten isolates were clustered within phylogenetic group B2, two within groups A, one within group B1 and one within group D. Fourteen strains were assigned to the sequence type ST131.

**Conclusion:** In conclusion, this is the first detailed documentation of CA-ESBLs producing *E. coli* in Tunisia. Of particular concern is the predominant presence of the CTX-M-15 enzyme in CA- *E. coli* corresponding to ST131.

**P1678** **ESBL and VRE colonisation in attendees of an infection control symposium**

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**Objectives:** To evaluate the prevalence of colonization with extended spectrum beta-lactamase producing enterobacteriaceae (ESBL) and of vancomycin resistant enterococci (VRE) in infection control personnel and to look for risk factors.

**Study design:** Cross-sectional study

**Methods:** Participants were recruited at the 2011 symposium of the German National Nosocomial Infection Surveillance System (KISS). Participation was voluntarily and anonymous. Volunteers were asked to perform a rectal swab and to fill in questionnaires on risk factors of ESBL or VRE carriage (report on diet, recent travel, and antibiotic use). We will analyze the risk factors by logistic regression analysis. Rectal swabs were inoculated onto ESBL and VRE Chromogenic agar; species identification and susceptibility testing was done by using a VITEK 2 system.

**Results:** Two hundred thirty people participated i.e. 36% (230/639) symposium attendees. Participants were mainly infection control nurses and doctors from hospitals in Germany and Austria. In total, only 1.3% of the participants stuck to a vegetarian diet.

No VRE faecium or faecalis were isolated whereas ESBL were isolated from eight out of 230 individuals (3.5%). All ESBL producing bacteria were identified as *E. coli*. All of the ESBL positive persons reported consumption of meat. Only two of the eight ESBL-colonized individuals had a history of antibiotic use in the last year and but three out of eight had a recent travel history to Greece.

**Conclusion:** The relatively high colonization rate of 3.5% with ESBL producing enterobacteriaceae among infection control personnel is of concern and reflects probably less an occupational health risk but the reservoir of and the expansion into the community.

## Carbapenemases from all over the world

**P1679** **Prevalence of metallo-beta-lactamases in Enterobacteriaceae from the SMART programme, and detection of a new variant: blaVIM-33**

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**Objectives:** The aim of the study was to investigate the prevalence and characterize metallo-beta-lactamase (MBL) genes in ertapenem non-susceptible Enterobacteriaceae from the SMART program (2008–2009).

**Methods:** Eight hundred and fifty-five ertapenem non-susceptible (MIC  $\geq 0.5$  mg/L) isolates of Enterobacteriaceae from the worldwide Study for Monitoring Antimicrobial Resistance Trends (SMART)

2008–2009 program were screened using a multiplex-PCR for the presence of blaIMP, blaVIM, blaNDM genes and identified using DNA sequencing. Genetic relatedness was determined with PFGE using XbaI and multi-locus sequencing typing (MLST).

**Results:** One hundred and four isolates (12.2%) comprising *K. pneumoniae* (n = 51, 49%), *E. coli* (n = 31, 29.8%) and the remaining (n = 22, 21.2%) including *Enterobacter* spp., *C. freundii*, *K. oxytoca*, *P. rettgeri*, *P. vulgaris* and *M. morgani* carried a MBL gene: 69 (8.1%), 28 (3.3%), and seven isolates (0.8%) were positive for blaNDM, blaVIM and blaIMP, respectively. Sequencing identified the following variants: blaNDM-1, -4, -6, blaIMP-1, -26 and blaVIM-1, 2, 5, 26, 27 and a novel variant, blaVIM-33. The geographical area of collection was diverse: India for the blaNDM-positive isolates, South Pacific for the blaIMP-positive, Europe for the blaVIM-positive isolates except 2 collected outside of Europe (North America, blaVIM-1, and Latin America, blaVIM-2). Of the 28 blaVIM-positive isolates, 13 isolates (46.4%) possessed only blaVIM while the remaining isolates also produced extended-spectrum and AmpC beta-lactamases. The following sequence types were identified among the VIM-producing *K. pneumoniae*: ST1, ST17, ST29, ST43, ST147 (with four different but related pulsotypes) and ST278.

**Conclusions:** Our study highlights the importance of surveillance programs using molecular techniques in providing insight into characteristics and distribution of sequence types among Enterobacteriaceae that produce MBLs and documents the regional differences of our findings and one novel variant, blaVIM-33.

**P1680** First description of the metallo-beta-lactamase GIM-1 in *Acinetobacter pittii* (formerly *Acinetobacter genomospecies 3*)

M. Kaase\*, N. Pfennigwerth, F. Szabados, S. Gatermann (Bochum, DE)

**Objectives:** Multidrug-resistance in species of the *Acinetobacter baumannii*-calcoaceticus complex is an increasing problem since respective strains might cause difficult to control outbreaks in hospitals. Multidrug-resistance in those species is mainly observed in *A. baumannii*, whereas *A. pittii* (formerly *Acinetobacter genomospecies 3*) tends to be more susceptible. Carbapenem resistance in *Acinetobacter* spp. is mainly caused by class D carbapenemases like OXA-23 and in some strains by metallo-beta-lactamases like IMP, SIM or NDM. The metallo-beta-lactamase GIM-1 has never been described in *Acinetobacter* spp.

**Methods:** *Acinetobacter pittii* strains with reduced susceptibility to imipenem were referred to the German reference laboratory for multidrug-resistant gram-negative bacteria. Species identification was checked by MALDI-TOF analysis and rpoB sequencing. Carbapenemases were tested for by a combined test using EDTA, a bioassay based on cell-free extracts as well as PCRs for OXA carbapenemases and metallo-beta-lactamases. The integron structure was sequenced with specific primers. Strain typing was done by pulsed-field gel electrophoresis (PFGE) and identification of a GIM-1 harbouring plasmid was performed by S1 nuclease restriction followed by Southern Blot hybridization.

**Results:** GIM-1 was found in four *A. pittii* strains all of which displayed EDTA synergy both in the combined disk test and the bioassay based on cell-free extracts. No other carbapenemase gene was found in those strains. GIM-1 was always part of a class 1 integron. Although strains showed no relatedness when tested by PFGE, all of them harboured GIM-1 encoding plasmids of the same size (~60 kb).

**Conclusion:** The GIM-1 carbapenemase has spread to the species *A. pittii* (formerly *Acinetobacter genomospecies 3*) and is obviously transferred by an identical plasmid. The further spread of carbapenemases in this species needs to be monitored carefully.

**P1681** Carbapenemases arrived in Germany: report for 2011 of the national reference laboratory for multidrug-resistant gram-negative bacteria

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**Objectives:** Multidrug-resistance in Enterobacteriaceae, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* is of utmost therapeutic importance since no innovative antimicrobial drugs against gram-negative bacteria will be introduced into clinical practice within the next five years. Among all resistance mechanisms the worldwide spread of carbapenemases is the most worrisome development. However, the correct identification of carbapenemases is challenging for the microbiological laboratory.

**Methods:** The National Reference Laboratory for Multidrug-Resistant Gram-negative Bacteria offers the free service of carbapenemase detection in bacterial isolates with elevated carbapenem MICs. All isolates are tested by a wide array of phenotypic and molecular methods. A bioassay based on cell-free extracts allows the detection of still unknown beta-lactamases.

**Results:** A total of 1074 isolates were sent to the National Reference Laboratory in 2011 between January 1st and October 11th mainly for investigation for carbapenemases, but also for clarification of the resistance mechanism to 3rd generation cephalosporins or molecular strain typing. Several different carbapenemases could be detected, including OXA-48 (n = 79), OXA-162 (n = 4), OXA-181 (n = 1), OXA-204 (n = 1), KPC-2 (n = 41), KPC-3 (n = 9), VIM-1 (n = 47), VIM-2 (n = 28), VIM-4 (n = 4), VIM-26 (n = 1), IMP-7 (n = 1), IMP-8 (n = 7), IMP-13 (n = 2), IMP-31 (n = 1), NDM-1 (n = 16), GIM-1 (n = 2), OXA-23 (n = 134), OXA-72 (n = 7) and OXA-58 (n = 2). In Enterobacteriaceae most Carbapenemases were found in *K. pneumoniae*, especially OXA-48, KPC-2 and KPC-3. VIM-2 was the most frequent carbapenemase in *P. aeruginosa* and OXA-23 in *A. baumannii*.

**Conclusion:** Almost all carbapenemases found worldwide have arrived in Germany. However, the molecular epidemiology in Germany with a predominance of OXA-48 differs significantly from observations made in other countries like Greece, Israel, USA or the United Kingdom. An ongoing surveillance of resistance determinants is necessary, especially for infection control and diagnostics.

**P1682** Acquisition of carbapenem resistance favours expansion of a limited number of successful *Acinetobacter baumannii* clonal lineages

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**Objectives:** To test the hypothesis that the acquisition of carbapenem resistance determinants favours the clonal expansion of a few successful *Acinetobacter baumannii* clonal lineages and therefore reduces strain heterogeneity among nosocomial *A. baumannii* isolates.

**Methods:** From an international collection comprising ~10 000 *Acinetobacter* isolates recovered from hospitalised patients between 2007 and 2010, 912 were preselected to represent maximal diversity among both carbapenem-susceptible (CARB-S) and carbapenem-resistant (CARB-R) isolates. Identification to species level was determined by gyrB multiplex PCR. Imipenem and meropenem susceptibility was determined by Etest. For the final analysis, 200 isolate pairs were selected (174 hospitals in 48 countries). An isolate pair was defined as a CARB-S and a CARB-R isolate recovered from the same center or hospital unit, with a time span of ≤1 year between isolation. Molecular epidemiology was investigated using rep-PCR (DiversiLab). Isolates were typed to determine their clonal relationship and their belonging to one of the previously described eight worldwide clonal lineages. The presence of OXA- and metallo-carbapenemases, and ISAbal1 upstream of blaOXA-51-like was investigated by PCR.

**Results:** Overall, 151 CARB-R isolates (76%) harboured acquired OXAs with OXA-23 the most prevalent (42% of isolates). ISAbal1 was upstream of blaOXA-51-like in 37 resistant isolates. Metallo-carbapenemases were not detected.



Table 1. Epidemiological background of 200 isolate pairs by DiversiLab

Epidemiological background of 200 isolate pairs by DiversiLab				
Clonal lineage	Carbapenem-resistant		Carbapenem-susceptible	
	No. isolates	%	No. isolates	%
WW1	21	10.5	15	7.5
WW2	92	46	49	24.5
WW3	8	4	1	0.5
WW4	5	2.5	6	3
WW5	28	14	25	12.5
WW6	2	1	3	1.5
WW7	5	2.5	8	4
WW8	3	1.5	2	1
Unclassified	37	18.5	90	45

Note: Boldface indicates clonal lineage in the two sets.

Among the 200 CARB-S isolates there were 62 different strain types, whereas among the 200 CARB-R isolates there were only 34 different strain types as assessed by rep-PCR. Molecular analysis showed that 55% of CARB-S vs. 81.5% of CARB-R isolates belonged to one of the eight worldwide (WW) clonal lineages. WW2 was the clonal lineage most frequently detected among both CARB-S and CARB-R isolates but was more frequent (46 vs. 24.5%) among CARB-R isolates (Table 1).

**Conclusion:** Carbapenem-resistance was mainly associated with the clonal lineages WW2, WW5 and WW1. Carbapenem-susceptible isolates were less clonal, with almost half the isolates not clustering with previously defined clonal lineages. Acquisition of carbapenem resistance determinants reduces strain heterogeneity among clinical *A. baumannii* isolates.

**P1683 Carbapenemase-producing Enterobacteriaceae strains in Hungary**

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**Objectives:** Carbapenem resistance among Enterobacteriaceae strains due to the production of carbapenemase enzyme is a growing clinical problem all over the world. The aim of our work was the complex characterization of carbapenemase-producing Enterobacteriaceae isolates submitted to the National Center for Epidemiology between January 2009 and August 2011.

**Methods:** The putative production of a carbapenemase was tested by modified Hodge test. The presence of carbapenemase- and extended spectrum beta-lactamase (ESBL) encoding genes was verified using PCR followed by sequencing. The possible clonal relationships were investigated by PFGE analysis and in some cases by MLST.

**Results:** During the study period 122 carbapenemase-producing Enterobacteriaceae isolates were investigated. Of these, 12 *Klebsiella pneumoniae* (KP) isolates produced KPC-enzyme, and 110 strains (43 *Enterobacter cloacae* (EbC), 41 KP, 20 *Serratia marcescens*, three *Citrobacter freundii*, two *K. oxytoca* and one *Escherichia coli*) produced VIM-type MBLs. All the KPC-producers and half of the MBL-producers proved to be ESBL-producers, as well. Ten KPC-2-producers isolated in 2009 and 2011 belonged to the ST258 KP clone. Class-1 integron carrying the blaVIM-4 gene was detected in all MBL-producers. In many isolates, the structure of integron (In238b) was found to be identical with an integron previously characterized in Hungary from *Pseudomonas aeruginosa* and *Aeromonas hydrophila* isolates. Thirty-four KP strains isolated from three nosocomial outbreaks in 2011 belonged to the Hungarian epidemic clone designated ST15, and they carried the same integron.

**Conclusion:** Our results highlight the fact that carbapenemase-producing Enterobacteriaceae strains have emerged rapidly in our country. The increasing number of these strains demonstrates the need of a continuous surveillance and forceful activities of infection control in Hungary.

**P1684 Carbapenem-resistant Acinetobacter baumannii in Lithuanian hospitals**

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**Objectives:** To perform phenotypic, genotypic and molecular characterization of carbapenem-resistant clinical *A. baumannii* isolates collected in three regional hospitals in Lithuania.

**Methods:** One hundred and eight nonduplicate clinical isolates resistant to carbapenems were collected in Clinics of Kaunas University of Medicine (N = 32), Vilnius Emergency Hospital (N = 51) and Siauliai Hospital (N = 25) during the year 2011. Their antimicrobial susceptibility was determined by the disc diffusion. The molecular characterization of carbapenem resistant isolates was performed by using PFGE, PCR assays and sequencing.

**Results:** The majority of isolates were resistant to imipenem, meropenem, piperacillin, ciprofloxacin, ceftazidime and tazobactam (97–100%), while significantly lower resistance to aminoglycosides was observed among isolates from Clinics of Kaunas University of Medicine (Table 1).

Analysis of PFGE-*ApaI* macrorestriction patterns revealed that the isolates from all hospitals belonged to 10 distinct genotypes, which all except one, were grouped into a major cluster (at 85% Dice similarity cutoff) and belonged to European clone II (EC II). Notably, the emergence of *A. baumannii* isolates belonging to European clone I (EC I) was observed in Vilnius Emergency Hospital.

The carbapenem resistance of EC II isolates from all hospitals was related to OXA-carbapenemases with OXA-40-like enzyme, while OXA-23-like carbapenemase was identified in EC I isolates from Vilnius Emergency Hospital.

EC II isolates shared the common aminoglycoside and tetracycline resistance determinants strA, strB, tetB. However a high incidence of aacC1 gene was found in one EC II genotype group from Vilnius and aadB was prevalent among isolates from Siauliai Hospital.

Isolates of EC I presented a distinct pattern of resistance genes including aacC1, aphA6, aphA1, aadA1, tetA as well as catI associated with chloramphenicol resistance.

96% of isolates were intl-positive. The only gene cassette array identified was aacC1-ofX'-orfX''-aadA1 and it was found present in isolates of EC I from Vilnius Emergency Hospital.

Table 1. The characteristics of A. baumannii isolates from three hospitals.

Hospital	No. of isolates	Enterase type	BCP	MEM	TEP	SAM	CAR	GM	AN	CIP	IPM	PIP	OXA	Other resistance genes
Clinics of Kaunas University of Medicine	32	EC II	22%	100%	97%	28%	87%	19%	22%	100%	100%	100%	osa-40 like	strA, strB, tetB, aacC1
Vilnius Emergency Hospital	51	EC II	100%	100%	100%	0%	100%	100%	100%	100%	100%	100%	osa-40 like	strA, strB, tetB, aacC1
Siauliai Hospital	25	EC I	100%	100%	100%	9%	100%	100%	100%	100%	100%	100%	osa-23 like	aacC1, aphA6, aphA1, aadB, catI, tetA

BCP - carbapenemase II aptin, MEM - meropenem II aptin, TEP - tetracycline II aptin, SAM - ampicillin II aptin, AN - ampicillin II aptin, CIP - ciprofloxacin II aptin, IPM - imipenem II aptin, PIP - piperacillin II aptin.

**Conclusion:** The molecular survey of carbapenem-resistant *A. baumannii* in Lithuanian hospitals showed that they were predominantly presented by OXA-40 producers belonging to EC II, while OXA-23 producers of EC I emerged in Vilnius Emergency Hospital. We observed the isolates from distinct hospitals carried the diverse patterns of genes conferring to aminoglycoside resistance.

**P1685** Molecular analysis of nosocomial isolates of *Escherichia coli* expressing multiple beta-lactamases in a tertiary referral hospital of northern India

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**Objective:** The co-existence of different classes of beta lactamases has emerged worldwide as a cause of growing antimicrobial resistance. Genes for these drug resistant determinants are often carried on mobile elements thus facilitating their horizontal transfer to other related as well as unrelated microbes. Present study was designed to investigate the co-existence of different class of plasmid mediated beta lactamases as well as their characterization and transmission dynamics among nosocomial isolates of *E. coli* in a tertiary referral hospital in northern India.

**Methods:** A total of 455 consecutive, non-duplicate clinical isolates of *E. coli*, collected during 2009–2011 in SS Hospital, BHU, India, were investigated for the presence of extended-spectrum beta-lactamases (ESBL), AmpC and metallo-beta-lactamase (MBL) by phenotypic as well as PCR assay. *E. coli* harbouring different ampC, ESBL and MBL gene (CMY-2, CTX-M and NDM-1) and resistance to other groups of antibiotics were selected for mating experiments. All the isolates were typed by random amplification of polymorphic DNA (RAPD).

**Results:** Phenotypically, 294/455 (59.5%) isolates were confirmed as AmpC beta-lactamase producer, 219/455 were ESBL positive and 9/455 were MBL producers. Presence of both AmpC and ESBL was demonstrated in 140 isolates while AmpC along with MBL was reported in seven isolates. None of the isolates showed simultaneous production of all the three enzymes. On performing PCR, 103 isolates were harbouring different ampC gene, 165 were harbouring different ESBLs while five isolates were harbouring NDM-1 gene. Among different classes of ampC, ESBL and MBL genes, blaCMY, blaCTXM, and blaNDM type was found to be the prevalent ones. The study also revealed strong association between integrons and different beta lactamases. Most isolates carried the blaCMY-2 and blaCTX-M gene on a conjugatively transferable plasmid while plasmid carrying blaNDM-1 was non-transferable. Forty-one clonal types of *E. coli* were found by RAPD profiling.

**Conclusion:** In light of our current struggle against MBLs, the presence of multiple mechanisms of beta lactamases mediated resistance expressed by these organisms highlights the importance their continuous surveillance in this part of the world and rapid detection of these drug resistant determinants to formulate the treatment strategy and infection control policy to prevent or slow down their dissemination in the hospital environment as well as in community.

**P1686** Carbapenemase-producing Enterobacteriaceae in Irish critical care units: results of a national pilot study

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**Objectives:** Carbapenemase producing Enterobacteriaceae (CPE) was first reported in Ireland in 2009 with eight further CPE cases reported in late 2010, many of whom had history of critical care unit admission. A multi-disciplinary working group convened in April 2011 to plan a pilot study to screen patients admitted to Irish critical care units for rectal carriage of CPE. The study aimed to establish a baseline prevalence of CPE, raise awareness of CPE and ensure that all microbiology laboratories gained experience of processing CPE screening swabs.

**Methods:** Critical care units and microbiology laboratories were invited to participate in a voluntary 4 week study to systematically screen patients admitted to critical care units weekly for rectal carriage of CPE. Screening swabs were processed in accordance with a laboratory protocol adapted from that recommended by the US Centers for Disease Control & Prevention (CDC). Suspected CPE isolates were referred to the Antimicrobial Resistance & Microbial Ecology (ARME) Laboratory for confirmatory testing.

**Results:** Forty critical care units served by 27 microbiology laboratories representing all regions of Ireland participated in the study in June 2011, which captured 84% of public hospital critical care beds. Routine CPE screening was already carried out in eight hospitals, six of which had already reported patients with CPE in the previous year. Seven microbiology laboratories had not yet processed any patient CPE screening swabs. During the study, there were 839 opportunities to take a screening swab for rectal carriage of CPE and 760 screening swabs were taken, reflecting 91% compliance with the study protocol. On average, 28 swabs (range 6–126) were processed by participating laboratories over the study period. Five suspected CPE isolates (all *Enterobacter* sp.) were referred to the ARME Laboratory but none were confirmed as carbapenemase producers.

**Conclusion:** CPE was not detected in 40 Irish critical care units during this short pilot study. There was a high level of participation in this study with 84% of public critical care beds captured, representing all regions of Ireland and a high level of compliance with the study protocol (91%). This study establishes an important baseline for CPE in Irish critical care units and should be repeated periodically to monitor the emergence of these multi-drug resistant organisms in a highly vulnerable patient population.

**P1687** Prevalence of MBL genes in meropenem non-susceptible *Pseudomonas aeruginosa* isolates from a Belgian tertiary ICU, 2003–2009

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**Objectives:** (i) To study the prevalence of MBL-genes in meropenem non-susceptible *Pseudomonas aeruginosa* isolates; (ii) To determine the genetic relatedness of MBL positive strains.

**Methods:** Between 2003 and 2009, a total of 135 meropenem non-susceptible clinical isolates of *P. aeruginosa* were collected from the 39 bed Intensive Care Unit (ICU) of the Antwerp University Hospital, Belgium. Only one isolate per patient was included. All identifications were confirmed by MALDI-TOF MS (Bruker). Meropenem susceptibility testing was performed by disk (Neosensitabs, Rosco Diagnostica) diffusion testing according to Rosco guidelines (2010). PCR assays were performed to amplify the sequences of the bla-IMP, bla-VIM1, and bla-VIM7 genes (primer sequences are shown in the Table 1). Quality control was performed for the amplification of the MBL gene using two positive controls (a VIM positive *P. aeruginosa* and an IMP positive *Acinetobacter junii* which were obtained from the Belgian reference centre for Gram negative aerobic bacteria), and a negative control (*P. aeruginosa* ATCC27853). Pulsed field gel electrophoresis was performed on all MBL positive strains. Pulsotypes were defined following the criteria as described by Tenover et al. (J. Clin. Microbiol. 1995; 33:2233-9)

**Results:** MBL genes were found in 58 of the 135 (43%) meropenem non-susceptible strains: 27 were positive for bla-IMP, and 31 for bla-VIM. The MBL positive strains belonged to four different pulsotypes: three VIM pulsotypes, and 1 IMP pulsotype. The relative number of MBL positive strains did not differ significantly between 2003 and 2009 ( $p = 0.56$ ).

Primer used for bla<sub>IMP</sub>, bla<sub>VIM1</sub>, and bla<sub>VIM7</sub>

Primer	Sequence	PCR product size (bp)
IMPPB1FW	5'-GGCGTTTATGTTTCATACTTCGTT-3'	235
IMPPB1RV	5'-TCGAGAATTAAGCCACTCTATTCC-3'	
VIM1-437FW	5'-TGTCCTGATGGTATGAGT-3'	437
VIM1-437RV	5'-ATTGAGCCAGATCGGCATC-3'	
VIM7FW	5'-GTCCGCGATGCTGATGAGT-3'	437
VIM7RV	5'-CATTCTGCCAAATTGGCATCG-3'	

**Conclusion:** MBL genes were highly prevalent in meropenem non-susceptible isolates obtained from our Belgian ICU in the period 2003–2009. MBL genes were found in four different pulsotypes of *P. aeruginosa*.

**P1688** Prevalence of carbapenemase producing Enterobacteriaceae in Moroccan community

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**Objective:** The increasing frequency of carbapenemase producing Enterobacteriaceae is a major cause of concern in human health, as this significantly limits treatment options for life-threatening infections. Therefore detailed understanding of the molecular basis and epidemiology of carbapenem-resistance is needed. This study is designed to assess, for the first time in Morocco, the current prevalence of community carbapenemase-producing Enterobacteriaceae, and to characterize their resistance genes.

**Methods:** A total of 119 extended spectrum beta-lactamases (ESBLs) producing Enterobacteriaceae isolates collected from seven Moroccan cities during January 2010–December 2011 were included in this study. Antibiotic susceptibility testing was performed as recommended by the CA-SFM. Carbapenem MICs and modified Hodge test were obviously performed. The presence of genes encoding carbapenemases, AmpC, ESBLs, plasmid mediated quinolone resistance (PMQR), aminoglycoside resistance, tetracycline resistance and class 1 integrons were investigated by PCR and sequencing. Conjugation experiments were done to determine the mobility of carbapenemase genes. The clonal relatedness among isolates was analyzed by pulsed field gel electrophoresis (PFGE).

**Results:** Seventeen of 119 strains (14.2%) were carbapenem-resistant. The Hodge test has detected carbapenemase production in nine isolates, that were positive for blaIMP-1 (n = 3) and blaOXA-48 (n = 6). The MIC values of imipenem showed that 2 carbapenemase producing isolates were resistant to imipenem. The ESBL genes, blaCTX-M-15 (n = 8), blaSHV-12 (n = 1) and blaSHV-28 (n = 1) were co-produced with the carbapenemase genes detected. Three isolates harboured AmpC genes; blaDHA-1, blaACT-like and blaCMY-2. The PMQR was identified in six isolates; qnrB1 and aac(6′)-Ib-cr type alleles were detected. The class1 integrons were detected in six isolates, with amplicons ranging from 0.6 to 2.1 kb in length. The results of conjugation experiments indicated that blaOXA-48, blaCTX-M-15, blaTEM-1, aac(3)-II and aac(6′)-Ib-cr genes were carried by a conjugative plasmid of high molecular weight. PFGE analysis showed genetic diversity among isolates with divergent DNA banding patterns.

**Conclusion:** The frequency rate of carbapenemase producing Enterobacteriaceae found should not be underestimated, so the detection of carbapenemases and the implementation of appropriate strategies are necessary to limit the spread of carbapenem resistant Enterobacteriaceae.

**P1689** Prevalence and characterisation of carbapenemase producing Enterobacteriaceae in a university hospital centre, Casablanca, Morocco

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**Objectives:** The objective of this study is to report the prevalence of carbapenemase producing Enterobacteriaceae in the Ibn Rochd University Hospital Center (CHUIR) in Casablanca – Morocco, during a 3 months period and to characterize their resistance genes.

**Methods:** Since 1 June 2011, all strains of Enterobacteriaceae isolated in the microbiology laboratory of the CHUIR, were tested routinely for carbapenemase producing. Antibiotic susceptibility testing was performed and interpreted as recommended by CLSI 2011 criteria. A modified Hodge test was performed on all isolates showing reduced

susceptibility to ertapenem. The presence of encoding carbapenemase resistance genes was investigated by multiplex and simplex PCR for the detection of blaOXA-48, blaKPC, blaNDM-1 and blaVIM.

**Results:** During a 3 months period (1 June–31 August 2011), of 704 non-repetitive strains of enterobacteriaceae isolated (620 from samples referred for diagnostic and 84 from rectal samples for digestive tract colonization screening [DTCS]), 28 (4%) were non-susceptible to ertapenem (16 from clinical specimens and 12 from DTCS). All had a positive Hodge test and 26 of them were extended spectrum beta-lactamases (ESBLs) producing. The results of the multiplex and simplex PCR were as follows: 19/28 (67.8%) strains were oxa-48 carbapenemase producing (14 *K. pneumoniae*, one *K. oxytoca*, one *K. terrigena*, two *E. cloacae* and one *E. coli*), five (17.8%) strains (all *K. pneumoniae*) were NDM-1 producing, while four (14.2%) strains showed absence of the four genes tested.

**Conclusion:** These results show a high prevalence of carbapenemase producing Enterobacteriaceae in the CHUIR in Casablanca: the blaOXA-48 was the most common genes followed by blaNDM-1 described for the first time in our university hospital. This situation requires intensification of hospital hygiene measures.

**P1690** Emergence of carbapenemase-producing Enterobacteriaceae and other gram-negatives in Scotland

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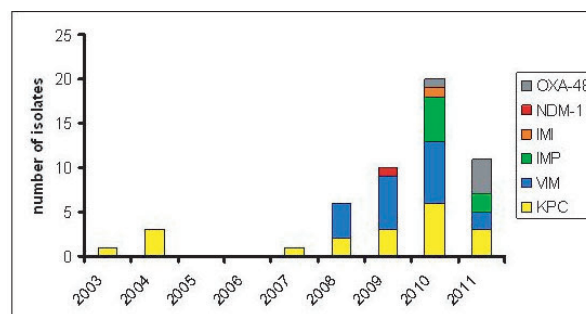
**Objectives:** To document the rapid emergence of carbapenemase producers among Enterobacteriaceae and other Gram-negatives in Scotland.

**Methods:** Microbiology laboratories in Scotland routinely test all Enterobacteriaceae and non-fermenting Gram-negative rods for susceptibility to carbapenems. An MIC  $\geq 1$  mg/L of imipenem/meropenem (or ertapenem for Enterobacteriaceae only) prompts laboratories to investigate the presence of a carbapenemase. Confirmatory methods to detect carbapenemase producers include VITEK 2 testing (MICs and mechanisms inferred by the Advanced Expert System), Modified Hodge Test, Etest (+/-EDTA) and KPC/MBL disc-testing. The Health Protection Agency's Antibiotic Resistance Monitoring and Reference Laboratory (ARMRL) carries out further characterisation, including MIC determination using BSAC agar dilution methodology and PCR detection of carbapenemase genes.

**Results:** The first isolation of a carbapenemase producer was a KPC-producing *Enterobacter cloacae* collected during a survey in 2003. In the following year three similar isolates were reported from the same laboratory, but there was no further spread of this strain and no carbapenemase producers were reported in Scotland in the following 2 years.

In 2007, a *Klebsiella pneumoniae* producing a KPC carbapenemase was reported from a catheter urine specimen.

In the period 2008–2011 reporting of carbapenemase producers increased with six isolates reported in 2008, 10 in 2009, 20 in 2010



Carbapenemases detected in Enterobacteriaceae and non-fermenters in Scotland, 2003- October 2011

and 11 in the first 10 months of 2011 (see Fig. 1). Most of these isolates were multidrug-resistant and were resistant to all first-line antimicrobial agents.

Since 2003 a total of 52 Scottish isolates (35 Enterobacteriaceae, 13 *Pseudomonas aeruginosa* and four *Pseudomonas fluorescens*) have been confirmed to produce a carbapenemase, including KPC (n = 19), VIM (19), IMP (7), OXA-48-like (5), NDM-1 (1) or IMI (1) enzymes. A single isolate of *Citrobacter freundii* produced NDM-1 enzyme. No history of foreign travel was reported for any of the affected individuals.

**Conclusion:** The rapid emergence of diverse carbapenemases in multiple Gram-negative genera in Scotland mirrors experience in the rest of the UK and highlights the need for strengthened local and national surveillance in order to prevent further spread of these multidrug-resistant organisms.

**P1691** **Dissemination of blaVIM in Greece at the peak of the epidemic 2005–2006: clonal expansion of *Klebsiella pneumoniae* clonal complex 147**

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**Objectives:** Determine the clonal diversity of blaVIM-carrying *K. pneumoniae* isolated in various parts of Greece years 2005–2006 by using multi-locus sequence typing (MLST).

**Materials and Methods:** Between the years 2005 and 2006 21 isolates were sampled from the following geographical areas in Greece: Athens (n = 11), Thessaloniki (n = 3), Piraeus (n = 3), Crete (n = 2) and North-West Greece (n = 2). One hospital from each area was represented, except Athens (five hospitals) and Thessaloniki (two hospitals). The isolates were sampled from a larger collection of isolates available at the national public health institute to represent distinct pulsed-field gel electrophoresis (PFGE)-patterns. The isolates were subjected to MLST, PCR-based plasmid replicon typing, screening for the virulence genes allS, rmpA and wcaG, and screening for capsular serotypes K1, K2, K5, K20, K54 and K57 (all associated with invasive disease). Additionally, capsular serotypes K1, K2, K5, K20, K54 and K57 (all associated with invasive disease). Seven of the isolates were derived from blood, whereas the rest of the isolates were isolated from urine, wound cultures or sputum. Clonal complexes were defined as isolates with maximum one divergent allele in MLST (single-locus variants; SLV).

**Results:** The far most frequently detected sequence type was ST147 (n = 7). Additionally two SLVs of this ST were detected (ST675 and ST677), making the total number of isolates in clonal complex (CC) 147 9/21 (43%). CC147-isolates were detected in all regions except Piraeus. Other detected CCs or STs were CC18 (n = 5), CC14 (n = 3), ST36 (n = 2), ST323 (n = 1) and ST674 (n = 1; double locus variant of ST18). None of the three virulence factors were detected in any of the isolates. Capsular type K2 was detected in the two ST14 isolates. CC18 was significantly more often associated with bloodstream infection (BSI) (p = 0.01). IncA/C was the only plasmid replicon type found in eight of the isolates, whereas both IncA/C and IncN was found in one isolate. The rest of the isolates were non-typable with the PCR-based plasmid replicon typing. There was no correlation between STs and Inc-types.

**Conclusions:** The study points to a limited number of clones disseminating blaVIM among *K. pneumoniae* during the peak of the epidemic, several of them being examples of epidemic clones. Later studies from Greece have shown that ST147 and ST323 are also responsible for simultaneous dissemination of blaVIM and blaKPC.

**P1692** **Genetic study of carbapenem-resistant Enterobacteriaceae in Hong Kong**

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**Objective:** Carbapenems such as imipenem and meropenem are important agents for the treatment of infections caused by multidrug-

resistant Enterobacteriaceae clinical isolates. The recent emergence of carbapenem-resistant Enterobacteriaceae (CRE) has become a serious public health issue. The present study is aimed to characterize the CRE isolates in clinical settings in Hong Kong.

**Method:** A prospective study was conducted from September 2010 to August 2011. Clinical isolates of *E. coli* and *Klebsiella pneumoniae* with reduced susceptibility to carbapenems in accordance with the Clinical and Laboratory Standards Institute (CLSI), were collected from nine hospitals in Hong Kong. Combined-disk tests and molecular methods were applied to screen for the presence of carbapenemase. All carbapenem resistant *E. coli* and *K. pneumoniae* isolates were subject to Multilocus-sequence Typing (MLST) to characterize the population structure. PCR are also performed to detect the already known carbapenemase genes such as blaKPC, blaIMP, blaNDM, blaSMB, blaVIM, blaGES, blaVEB and also blaOXA.

**Result:** A total of 17 non-duplicated *E. coli* and 52 non-duplicated *K. pneumoniae* isolates from 67 patients were found to meet criteria (inhibition zone diameter of either meropenem, imipenem or ertapenem  $\leq 23$  mm) for CRE. Among the 69 carbapenem resistant isolates, five isolates were found to produce carbapenemase (three KPC, one IMP and one NDM). MLST results showed that the *E. coli* isolates are mainly ST131 (12%), ST354 (12%), ST405 (12%) and ST744 (12%) while ST11 (33%) are the most frequent type among *K. pneumoniae*.

**Conclusion:** The dissemination of carbapenem resistant *E. coli* and *K. pneumoniae* has increased over the past few years in Hong Kong. However, only three kinds of carbapenemase (KPC, IMP and NDM) have been found so far. MLST results show that these carbapenemase resistant isolates are genetically diverse. The resistance mechanism of those non-carbapenemase producing isolates is still unclear. Further study is needed and will be carried out soon.

**P1693** **First report of metallo-beta-lactamase producing *Pseudomonas aeruginosa* from Tanzania**

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**Objectives:** Carbapenemases are now disseminating worldwide among clinical important Gram-negative pathogens. Epidemiological data from Africa and particularly from Sub-Saharan Africa are limited. The aim of this project was to investigate the presence of carbapenemases among clinical isolates of *Pseudomonas aeruginosa* in Tanzania.

**Materials and methods:** The study included 90 isolates of *Pseudomonas* sp. isolated from pus, blood and urine specimens from May 2010 to July 2011 at Muhimbili National Hospital in Dar-es-Salaam, Tanzania. Susceptibility testing was performed by disc diffusion and Etest. Carbapenem-resistant isolates were characterised using PCR assays for carbapenemase-genes, pulsed-field gel electrophoresis (PFGE), multi-locus sequence typing (MLST), and serotyping.

**Results:** Out of the 90 isolates, 30 (33.3%), 16 (17.7%) and 15 (15.5%) were from outpatient clinics, burn unit, and surgical ward, respectively. The remaining specimens (33.3%) came from the paediatric, medical, ICU, ENT, EMD and psychiatric wards. Eight isolates (8.9%) were carbapenem-resistant. All carbapenem-resistant isolates were obtained from children and confirmed positive for the metallo-beta-lactamase (MBL) VIM by PCR. All eight isolates were also resistant or intermediate susceptible to gentamicin, tobramycin, amikacin, and trimethoprim-sulfamethoxazole but susceptible to ciprofloxacin and colistin. PFGE showed four different pulsotypes with two isolates belonging to each type. MLST of four isolates corresponding to each pulsotype showed that three isolates belonged to sequence type (ST) 244 and one isolate to ST640. None of the isolates were typeable by serotyping.

**Conclusion:** This study is to our knowledge the first report of MBL-producing *P. aeruginosa* from Tanzania and further expands the global dissemination of carbapenemases.

**P1694 Investigation of carbapenem resistance in MDR isolates from bacteraemic patients**

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**Objectives:** The aim of this study was to analyze the emergence, mechanisms of resistance and molecular epidemiology of carbapenem-resistant *A. baumannii* (Ab) and *K. pneumoniae* (Kp) isolates from bacteraemic patients in a tertiary teaching Hospital, from January to August 2011.

**Methods:** Identification and susceptibility tests were performed by Vitek2 automated system and E-test. Carbapenem resistance mechanisms were investigated by phenotypic methods (imipenem/imipenem + EDTA, imipenem/imipenem + Boronic acid disks) and the genetic basis of resistance was investigated by PCR, targeting blaVIM, blaKPC, blaNDM, blaOXA. Rep-PCR was performed for investigation of clonal spread of isolates.

**Results:** A total number of 26 Ab and 51 Kp were isolated from bacteraemic patients in our Hospital, during the study period. All Ab (100%) and 23/51 Kp (45.1%) were carbapenem-resistant. Twenty-one of 26 Ab (80.8%) and 12/23 (52.2%) of Kp were isolated from ICU patients. Reduced susceptibility or resistance to tigecycline occurred in 18/26 (69.2%) of Ab and 13/23 (56.5%) of Kp strains. Pan-drug resistance, including resistance to colimycin, occurred in 5/23 (21.7%) of MDR Kp isolates. Phenotypic methods were in accordance with molecular methods for detection of resistance mechanisms for Kp, while imipenem + EDTA method showed false positive results for MBL production in Ab. PCR revealed that among Kp, 18/23 (78.3%) harboured the blaKPC gene, 6/23 (21.7%) the blaVIM, while no blaNDM was detected. All Ab harboured blaOXA genes, while for the first time to our knowledge, blaOXA-23 was detected in our country, in five Ab strains. Rep-PCR revealed multi-clonal diversity of isolates.

**Conclusion:** Results indicate that resistance to carbapenems of *A. baumannii* and *K. pneumoniae* is of great concern among bacteraemic patients. The excessive use of antibiotics, in addition to the endemic spread of MDR strains, results in a serious threat of infections. Urgent application of infection control measures is necessary to avoid further dissemination.

**P1695 High rates of non-susceptibility to non-carbapenem antibiotics for extended-spectrum beta-lactamase producing Enterobacteriaceae in Toronto, Canada**

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**Objective:** To determine susceptibility rates of non-beta-lactam antibiotics for extended-spectrum beta-lactamase producing Enterobacteriaceae (ESBL-E) in Toronto, Canada.

**Methods:** Retrospective review of clinical cultures positive for ESBL-E Ambler Class A (*Escherichia coli* and *Klebsiella pneumoniae*) at 12 hospitals (five academic and seven community) between 2004 and 2009. Only the first clinical isolate from a patient was considered. Antibiotic susceptibilities were based on the VITEK2 (BioMérieux) or Phoenix2 (Becton Dickinson). ESBL-E were confirmed with the double disk diffusion test according to CLSI standards.

**Results:** The incidence per 1000 patient days for ESBL-E increased from 0.16 in 2004 to 0.49 in 2009. There were a total of 2270 isolates (1969 *E. coli* and 301 *K. pneumoniae*) over 6 years. Susceptibilities were as follows for *E. coli* and *K. pneumoniae*: ciprofloxacin (11.2%, 31.8%), gentamicin (51.5%, 38.1%), tobramycin (32.3%, 28.5%), amikacin (97.1%, 94.5%), trimethoprim-sulfamethoxazole (TMP-SMX) (34.2%, 21.0%), imipenem (99.7%, 100%) and meropenem (99.9%, 100%). Multi-drug resistance was common among non-carbapenem antimicrobials with only 3.7% of *E. coli* and 7.0% of *K. pneumoniae* susceptible to ciprofloxacin, gentamicin/tobramycin and TMP-SMX while 31.0% and 39.6% of isolates, respectively, were fully resistant to

all three alternative classes. Susceptibility rates for *E. coli* were stable over time, while *K. pneumoniae* susceptibility rates for ciprofloxacin decreased from 57.1% in 2004 to 9.4% in 2009. There was no difference in susceptibilities between academic and community hospitals for *E. coli*; however, overall susceptibility patterns for *K. pneumoniae* in the community were higher for gentamicin (46.8% vs. 35.7%) and tobramycin (45.1% vs. 24.7%) while lower for ciprofloxacin (17.6% vs. 36.2%) and TMP-SMX (17.7% vs. 21.9%). Carbapenem resistance was rare, with only four non-susceptible isolates (2008 – 1 imipenem resistant, 2009 – 2 imipenem and 1 meropenem resistant).

**Conclusions:** Over a 6 year period, the incidence of ESBL-E has increased in Toronto. Isolates were multi-drug resistant and the degree of fluoroquinolone resistance was higher than has been observed in ESBL-E. Treatment of serious ESBL-E infection in this region will require the use of carbapenems. This is concerning given the identification of carbapenem resistant isolates in this region beginning in 2008.

**P1696 Prevalence of ESBL and carbapenemase-producing phenotypes of Enterobacteriaceae identified in the 2010 AWARE surveillance programme**

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**Objective:** ESBL and carbapenemase-producing organisms are well recognized as a global problem with high prevalence rates being reported in Eastern Europe, Asia and South America. The aim of this study was to determine the frequency of these resistance phenotypes among clinical isolates of *Escherichia coli* (EC), *Klebsiella pneumoniae* (KP) and *Klebsiella oxytoca* (KO) collected from USA subjects during the 2010 Assessing Worldwide Antimicrobial Resistance Evaluation (AWARE) Surveillance program.

**Methods:** Clinical isolates (1 per patient) of EC (n = 657), KP (n = 653) and KO (n = 250) were collected from 65 medical centres geographically distributed across the USA. Specimen infection sources included; blood (53.6%), skin (SSSI; 10.0%), respiratory (RTI; 11.4%) and urine (UTI; 22.3%). All isolates were centrally tested for susceptibility to various antimicrobials by CLSI broth microdilution methods (M100-S21). ESBL phenotypes included isolates with MICs  $\geq 2$  mg/L for ceftriaxone, ceftazidime or aztreonam. Carbapenem-resistant isolates included those with MICs  $\geq 2$  mg/L for meropenem. Resistant phenotypes were evaluated among different specimen sources and by geography using the nine USA census regions.

**Results:** ESBL phenotypes were present for >12% of all collected EC, KP and KO from all specimen sources. ESBL phenotypes ranged from 10.6% for KP to 16.6% for EC from SSSI, from 10.4% for KP to 31.4% for EC among RTI isolates, and from 4.7% for KO to 7.3% for EC for UTI isolates. ESBL phenotypes of EC and *Klebsiella* spp. were most prevalent in the East South Central region of the USA at 22% and 23%, respectively and least prevalent in the Pacific region (8.3% and 6.7%, respectively). ESBL phenotypes were greater than two-fold higher among nosocomial organisms (18.5%) when compared with community-acquired organisms (8.1%). ESBL phenotypes were often resistant to fluoroquinolones ( $\geq 70\%$ ). Carbapenem resistance was detected among 27 (4.1%) isolates of KP with the highest prevalence rates being observed in the Mid-Atlantic (13.8%) and West South Central regions (8.0%).

**Conclusions:** ESBL and carbapenemase-producing Enterobacteriaceae are prevalent among USA clinical isolates and will continue to pose a threat to the existing armamentarium of beta-lactam antibiotics. Continued surveillance will be important to monitor resistant phenotypes and for evaluating new agents that circumvent these mobile resistance mechanisms.

**P1697** Characterisation of carbapenem non-susceptible *Pseudomonas aeruginosa* isolates in Danish hospitals: a nationwide study

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**Objectives:** In many European countries an increase in carbapenem non-susceptible *Pseudomonas aeruginosa* has been observed. Until 2011, no systematic data from Denmark had been registered, so a consecutive collection of carbapenem resistant *P. aeruginosa* was enacted, to investigate the carbapenem resistance mechanisms in Danish *P. aeruginosa* isolates.

**Methods:** From 1 January 2011 through 30 June 2011, 116 nonreplicate, non-cystic fibrosis related *P. aeruginosa* isolates with reduced carbapenem susceptibility were collected from 12 out of 13 Danish Departments of Clinical Microbiology. The presence of acquired beta-lactamases was assessed using a combination tablet method, and the isolates were antimicrobial susceptibility tested against relevant antipseudomonal agents. Beta-lactamase subgroup specific PCR assays, subsequent sequencing analysis as well as an efflux pump inhibitor assay were performed.

**Results:** Eight isolates produced the metallo-beta-lactamase VIM-2 and one isolate produced both OXA-10 and a VEB-group enzyme. Furthermore, 67 isolates displayed a derepressed AmpC phenotype, deduced from cloxacillin or boronic acid synergy with either ceftazidime or meropenem. Phenotypic indications of increased efflux pump activity were seen in 44 isolates. Efflux and AmpC positive results occurred more frequently in isolates resistant to both meropenem and imipenem than in isolates resistant only to imipenem. This suggests loss of porin as the main resistance mechanism in the imipenem resistance group of isolates. Activity of doripenem was less affected than that of meropenem in isolates with increased efflux activity. Resistance to ceftazidime and cefepime was primarily seen in the AmpC derepressed isolates. The rate of aminoglycoside resistance was relatively low against gentamicin (8%), amikacin (9%) and tobramycin (11%), while 56% were resistant to ciprofloxacin.

**Conclusion:** Although relatively low in number, the occurrence of eight VIM-2 producing isolates from six different hospitals stresses the necessity of a continued effort to detect and confirm isolates with a potential for spread of acquired beta-lactamases. Based on the phenotypic findings in this study, reduced permeability of the outer cell membrane and/or increased efflux pump activity, often in combination with overexpression of chromosomal AmpC, appeared to be the most likely main explanation for reduced carbapenem susceptibility in Danish *P. aeruginosa* isolates.

**P1698** Carbapenemase producers in the Czech Republic – current situation

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**Objectives:** Monitoring of the epidemiological situation in carbapenemase-producing Gram-negative bacteria is necessary to prevent the rapid spread of this type of carbapenem resistance. The objective of this work is to summarize the current incidence and spectrum of carbapenemase producers in the Czech Republic.

**Methods:** All blood isolates of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* have been sent to the National Reference Laboratory for Antibiotics since 2005 for the purpose of EARS-Net survey. Moreover, Enterobacteriaceae and *Acinetobacter baumannii* isolates resistant to meropenem or imipenem according to the CLSI or EUCAST criteria were sent to the NRL for Antibiotics and/or to the Pilsen laboratory for confirmation of resistance to carbapenems. The approximation disk test was used to detect MBLs, KPCs and AmpC enzymes. Carbapenemase activity was formerly confirmed by

imipenem spectrophotometric hydrolysis assay and recently by MALDI-TOF MS hydrolysis assay. Identification of carbapenemase was performed by PCR followed by amplicon sequencing. MLST was performed in *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolates.

**Results:** The most of the analysed isolates were resistant to the carbapenems due to the porin mechanism. Carbapenemase production has been confirmed in minority of the cases. The KPC-2 carbapenemase was firstly detected in *K. pneumoniae* (ST258) isolated from a patient repatriated from Greece, followed by KPC-3-producing *K. pneumoniae* (ST515) from a patient previously hospitalized in Italy. VIM-1 metallo-beta-lactamase was detected in two strains of *Serratia marcescens* isolated independently in two hospitals and also in *K. pneumoniae* isolates. VIM-1-producing *K. pneumoniae* has been responsible for local outbreaks in one hospital in Prague. In spring 2011, the first NDM-1 producer was observed in the Czech Republic. NDM-1 was detected in *Acinetobacter baumannii* (ST1) isolated from a patient repatriated from Hurghada, Egypt. Metallo-beta-lactamase-producing *P. aeruginosa* isolates (VIM-2, IMP-7) have been detected in at least seven hospitals causing local outbreaks.

**Conclusions:** Although the incidence has been mostly sporadic in the Czech Republic, the outbreak potential of these isolates has already been shown. In 2011, rapid increase of carbapenemase-producing enterobacteria was observed.

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**P1699** Rapid dissemination of OXA-163 carbapenemase, an emerging OXA-48 variant, in species of Enterobacteriaceae in multiple hospitals from Argentina: multiples clones and detection issues

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OXA-48-producing Enterobacteriaceae (Ent) isolates are emerging worldwide and represent a serious threat. Recently, OXA-163, a novel carbapenemase related to OXA-48 with increased activity against extended-spectrum beta-lactams, was detected in Argentina.

**Objective:** To describe the evolution of the epidemiology of OXA-163-producing Ent in Argentina.

**Methods:** We designed an algorithm to detect carbapenemases in Ent at the level of the clinical microbiology lab, which was implemented among the National Quality Control Program in Bacteriology (Argentinean Ministry of Health; 432 labs). By means of this algorithm, all Ent with decreased susceptibility to carbapenems and a negative synergy test result between the carbapenems disks and boronic acid or EDTA, were considered as suspicious of OXA production and referred to the National Reference Lab. The presence of several beta-lactamase genes, including blaOXA-48/163 was assessed by PCR. Sequencing of amplicons and PFGE using XbaI was performed on all OXA-producers. Antimicrobial susceptibility was confirmed by agar dilution (CLSI).

**Results:** A total of 13 strains were confirmed in the INEI as OXA-163 producers: nine *Klebsiella pneumoniae* (Kpn) and four *Enterobacter cloacae* (Ecl). Co-production of CTX-M was detected in one Ecl. Strains were recovered from April 2010 to February 2011 from 10 different Hospitals in Buenos Aires. Only one hospital had multiple isolations of Kpn OXA-163 (n = 4). All the strains were resistant to penicillins, extended-spectrum cephalosporins, aztreonam and cefoxitin. The range of carbapenem MICs (mg/L) (% of susceptible-S-) was: 0.12–8 imipenem (91%); 0.25–16 meropenem (14%); >16 ertapenem (0%). Tigecycline was the most active drug (77% of S), followed by colistin (61%) and fosfomycin i.v. (25%). Three strains displayed a pan-resistant phenotype. By PFGE, seven clones were observed among nine Kpn isolates and four clones among four Ecl isolates. A single clone of Kpn or Ecl was found in all except one hospital where two Kpn clones were detected.

**Conclusions:** This is the first report describing the rapid spread of OXA-163 worldwide. The emergence of multiple clones of Ent OXA-163+ was responsible of this dissemination. Ertapenem resistance was the only effective marker of OXA-163 production, but in countries with high prevalence of CTX-M plus impermeability producing strains, this indicator could be very limited. The silent spread of OXA-163 among multiples hospitals constitutes a public health concern.

**P1700 Occurrence and genetic analysis of OXA-48-producing strains in European countries, 2007–2010**

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**Objectives:** To evaluate the dissemination and genetically characterize OXA-48-producing strains in Europe. Increasing traveling and immigration among countries in the Mediterranean area seem to promote the spread of blaOXA-48. We used the SENTRY Program network to assess the occurrence of OXA-48-producers among 16 European nations.

**Methods:** Among 201 (1.3%; 15 520 strains) carbapenem-resistant (R) Enterobacteriaceae strains collected during 2007–2010 in 16 European countries, 42 OXA-48-producing strains were detected using Modified Hodge Test (MHT) and PCR for carbapenemase-encoding genes. Clonality was assessed by PFGE. Gene location was determined by S1 endonuclease restriction, followed by hybridization. Genetic environment was amplified using primers targeting IS1999 anchoring on the OXA-48 gene. Amplicons were digested with AluI, RsaI and Sau3IA and different types were sequenced.

**Results:** OXA-48-producers were collected in 2007 (three strains), 2008 (6), 2009 (28) and 2010 (6). Forty-one strains were detected in Turkey (all years) and one *K. pneumoniae* (KPN) in Italy. The latter was collected in April/2009 from a 79 y/o female patient hospitalized in Sicily. Isolates belonged to four bacterial species: KPN (23 strains; all years), *E. coli* (EC; 14 strains; 2008 and 2009), *E. cloacae* (ECL; four strains; 2010 only); *K. oxytoca* (KOX; three strains; 2009 only) and one *E. aerogenes* (2009). Imipenem (IMI) MIC values ranged from 1 to >8 mg/L and meropenem (MER) from 0.25 to >8 mg/L (mode, 4 and 1 mg/L, respectively). One KPN strain was MHT negative (IMI and MER MIC, 2 and 1 mg/L, respectively). KPN displayed great genetic diversity by PFGE (12 patterns). Clonality was observed only in 2009 (one cluster of five strains and three clusters of two). Among EC, eight patterns were noted and seven of 10 strains from 2009 belonged to the one cluster. ECL strains displayed two patterns and KOX were identical. All strains carried blaOXA-48 in plasmids and two different blaOXA-48 genetic elements were observed: IS1999 ( $\pm$ IS1 tnpA disruption)/blaOXA-48/IS1999.

**Conclusions:** OXA-48-producing strains were found to be disseminated in Turkey and one strain was detected in Italy. These strains were not observed on other European countries (five in the Mediterranean region) surveyed by the SENTRY Program. High rates of OXA-48-producers in 2009 seemed to be related to clonal spread. This gene seems to disseminate via plasmid or genetic element with no boundaries among Enterobacteriaceae species.

**P1701 OXA-48 carbapenemase in an isolate of the uropathogenic ST131 clone of *Escherichia coli* in the UK**

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**Introduction:** OXA-48 carbapenemase was initially identified in Turkey, but recent reports indicate its spread, mainly in *Klebsiella pneumoniae* isolates, in the Middle East and north Africa. There have been several hospital outbreaks in Europe. The blaOXA-48 gene is associated with transposon Tn1999, which is often carried on well-conserved, transferable plasmids. These have aided spread of the carbapenemase into multiple strains and genera of the Enterobacteriaceae. We report the production of OXA-48

carbapenemase by an *Escherichia coli* isolate belonging to the international ST131 clonal lineage, which is notorious for its role in the global dissemination of CTX-M-type ESBLs.

**Methods:** The *E. coli* was isolated from blood and had been referred to the HPA's Antibiotic Resistance Monitoring and Reference Laboratory from a UK hospital. MICs were determined by agar dilution and interpreted using BSAC/EUCAST criteria. Carbapenemase genes were sought by PCR. Multilocus sequence typing (MLST) was used to determine clonal type. Plasmid DNA was extracted and transformed into electrocompetent *E. coli* DH5 cells, with transformants selected from colonies growing around piperacillin/tazobactam discs. Plasmids were analysed with PCR mapping and sequencing used to identify the environment of the carbapenemase gene.

**Results:** The isolate was resistant to ertapenem (MIC, 8 mg/L), but MICs of imipenem (2 mg/L) and meropenem (1 mg/L) remained in the susceptible range. PCR identified blaOXA-48 whilst MLST showed that the isolate belonged to ST131. Plasmid analysis showed a ca. 60-kb plasmid carrying the blaOXA-48 gene, which was flanked upstream and downstream by IS1999 elements, thus forming a Tn1999 structure.

**Discussion:** The detection of OXA-48 carbapenemase in an ST131 *E. coli* isolate is highly worrying. This is a pandemic clone with demonstrated high virulence and ability to spread. To date, OXA-48 has been found mainly in *K. pneumoniae*, and outbreaks have only been reported in hospitals. Acquisition by *E. coli* ST131 may lead to community dissemination, which will make control extremely difficult.

**P1702 Carbapenem-resistant Enterobacteriaceae surveillance: 2 years cohort with successful control**

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**Objectives:** Few reports of active surveillance and cohort have been published about the control of Carbapenem-Resistant Enterobacteriaceae (CRE) in Latin America. This work aims to report the long-term and successful control of CRE in a 450 beds university hospital in Brazil using a surveillance program.

**Methods:** The first case of CRE was detected in the Medical Surgical Intensive Care Unit (ICU) in February 2009. A surveillance program was initiated. Patients with the following criteria were screened for CRE by specimen collected through rectal swab: (i) inpatients who had contact with CRE cases; (ii) presence of at least three of the subsequent criteria at the time of admission: hospitalization in the last 6 months, pressure ulcers, antimicrobial use, recent abdominal surgery, use of invasive devices. Patients with criteria (i) had three rectal swabs followed by one rectal swab per week till discharge and (ii) had three rectal swabs at admission. Detection of CRE was performed according to CLSI 2009. Geographical plus healthcare workers cohort at the ICU and clinical wards for the CRE suspected patients, and contact precautions plus privative room for the CRE positive patients were adopted.

**Results:** From February 2009 to May 2011, 614 patients (pts) were screened, with 2006 samples collected. There were 53 CRE positive pts (93% autochthonous), and 69,8% of those were revealed through surveillance (positivity of 1.5%). The majority of our cases were colonizations (77.4%). Almost all CRE pts had  $\geq 1$  surveillance culture positive, with their first culture positive, however two pts only had their second surveillance culture positive, and another two only had their third surveillance culture positive. Of the 53 ERC isolates, we had 44 *Klebsiella pneumoniae*, six *Enterobacter cloacae*, one *Enterobacter aerogenes*, one *Escherichia coli*, one *Providencia stuartii*, and one *Proteus mirabilis*. One patient had both *K. pneumoniae* and *E. coli*.

**Conclusion:** The 2 years geographical plus healthcare workers cohort combined with our active surveillance has prevented CRE from becoming endemic in our hospital, only appearing in small and controlled outbreaks, and this has lasted till the present report.

**P1703** Effectiveness of infection control measures and active surveillance to reduce the prevalence of carbapenem-resistant *Klebsiella pneumoniae* in an acute care Greek hospital

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**Objectives:** The dissemination of carbapenem-resistant *Klebsiella pneumoniae* (CRKP) represents a major public health problem in several regions worldwide. In our country KPC- or both KPC- and MBL-producing *K. pneumoniae* isolates have emerged during the last years. In this study we present the effectiveness of implementing infection control measures and active surveillance to reduce the incidence of CRKP in an acute care Greek hospital.

**Methods:** During January 2009–October 2011 all new cases of CRKP in the ICU and medical wards were recorded. Combined-disk tests employing meropenem disks without and with phenylboronic acid (PBA), EDTA or both EDTA and PBA were used to phenotypically detect production of KPC, MBL or KPC and MBL genes. A modified CLSI test with the addition of both PBA and EDTA was used to detect coproduction of ESBLs. KPC, MBL and ESBL production was genotypically confirmed by PCR and sequencing. Immediately after the end of 2009 the increase of CRKP isolates in ICU was led to the reinforcement of infection control measures that included continuous program of hand hygiene promotion, patients' isolation or cohorting, contact precautions, limiting patients' transfer, post discharge cleaning and decontamination of room and collection of environmental specimens. From the beginning of 2011 an active surveillance was implemented to new ICU admissions.

**Results:** Seventy CRKP isolates were prospectively recovered from separate patients hospitalized during the study period. KPC genes were detected in 50 cases, MBL genes in 11 cases, while both KPC and MBL genes in nine cases. ESBL genes were coproduced in 45 CRKP isolates. Additionally, active surveillance revealed 10 carriers of KPC genes among 78 patients admitted to the ICU during the third year of the study. The first year of the study a rate of 0.52 CRKP cases per 1000 patient-days was recorded. During the second year of the study, after the implementation of infection control measures, the incidence fell to 0.32 cases/1000 patient-days. During the third year of the study, when the active surveillance program was additionally implemented, a significant reduction in the incidence of CRKP cases was recorded (0.21 cases/1000 patient-days) compared to the postintervention period ( $p < 0.05$ ).

**Conclusions:** Infection control measures along with active surveillance reduced considerably the infection rate due to CRKP strains in our hospital. The accurate phenotypic detection of carbapenemase genes contributed to the early implementation of infection control measures.

## Epidemiology and genetics of clinical isolated carrying NDM

**P1704** Molecular characterisation of NDM-1-bearing *K. pneumoniae* isolates from Croatia

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**Objective:** Molecular genetic characterization of 4 blaNDM-1 positive *K. pneumoniae* strains isolated from Croatia.

**Methods:** Four carbapenem-resistant *Klebsiella pneumoniae* were isolated from clinical samples from four different hospitals. Three of them are situated in Zagreb and one in Bjelovar. Species identification was carried out by API. MICs were performed by microdilution method and interpreted by the EUCAST criteria. Investigation of genes encoding for beta-lactamases (ESBL, MBL, OXA-type and plasmidic AmpC), plasmid-mediated quinolone resistance determinants (qnrA, qnrB, qnrS, qnrC, qnrD, qepA, aac(6')-Ib-cr variant) and 16S rRNA methylase (armA, rmtB, rmtC) was performed by PCR. PCR-based replicon typing provided the plasmid scaffold of the strains. Multilocus

sequence typing (MLST) was performed. Conjugation experiment was also carried out.

**Results:** All the strains were blaNDM-1 positive. MLST yielded sequence type (ST) 15 (three strains) and ST16 (one strain). Three ST15 strains produced, also TEM-1, SHV-12, CTX-M-15, OXA-1- group beta-lactamases and harboured qnrB1 gene. All three possessed a common plasmid that, surprisingly, was IncR and not A/C as described in most previous reports. Beside IncR plasmid, one strain harboured colE and the third strain carried both L/M and colE plasmids. The *K. pneumoniae* strain belonging to ST16 possessed A/C and colE plasmids and harboured CIT-type acquired AmpC gene together with blaOXA-1, blaCTX-M-15, blaSHV-1, blaTEM-1. The transconjugants obtained from IncR type strain were positive for blaNDM-1 and IncR genes by PCR.

**Conclusion:** This study revealed diverse genetic features among several NDM-1 positive *K. pneumoniae* strains isolated in four Croatian hospitals. The results confirmed the presence of multiple beta-lactamase genes other than NDM-1 as well the presence of determinants of resistance to other antibiotic classes, as typical for these strains. The plasmid profiles revealed the presence of either one or two plasmids, with different patterns among the strains. The finding of IncR plasmids, so far not reported, is of particular relevance.

**P1705** The genetic context of blaNDM-1 in *Acinetobacter baumannii* from a burns unit outbreak in Swansea, Wales

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**Objectives:** Cultures from five patients on a burns unit in Swansea grew a multi-drug resistant *A. baumannii*. Isolates from two patients with no significant travel history and the environment were New Delhi metallo-beta-lactamase-1 (NDM-1) producers. An earlier isolate with the same sensitivity pattern, from a patient who had undergone medical intervention in Egypt, had not been saved for further analysis. We set out to define the genetic context of blaNDM-1 and determine whether it was on a mobile genetic element capable of dissemination to other bacteria.

**Methods:** Isolates were identified biochemically (Phoenix) and by PCR for blaOXA-51-like genes. blaNDM-1 and blaOXA-23 were detected by PCR. Genomic DNA was digested with *ApaI* and endonuclease S1 and fragments separated by pulsed field gel electrophoresis. *EcoRI* and *HindIII* digests were separated by standard electrophoresis. Gels were probed for blaNDM-1 and ISAbA125. PCR primers were designed to published sequences of the blaNDM-1 context in *A. baumannii* to perform primer walking and PCR products sequenced. Conjugation experiments were performed with recipients of various species including *Escherichia coli* and *Acinetobacter*.

**Results:** All blaNDM-1 positive isolates were of a single strain type by PFGE and all blaOXA-51-like (supporting *A. baumannii* ID) and blaOXA-23-like positive by PCR. S1 digests showed that this strain harboured several plasmids. Gene probing the S1 and *ApaI* digests showed blaNDM-1 was on the chromosome and on an *ApaI* fragment of <50 kb. Primer walking and sequencing revealed a complete ISAbA125 upstream of blaNDM-1, with ble and truncated trpF genes downstream. The genes groEL, groES and the ISCR16-like gene commonly found near blaNDM-1 were not detected. blaNDM-1 was on approx. 1 and 8 kb *EcoRI* fragments, indicating there is a second copy in a novel genetic context, since there are *EcoRI* sites either side of the blaNDM-1 sequence obtained so far. Multiple attempts at conjugation were unsuccessful.

**Conclusion:** Transfer of blaNDM-1 was not observed in vitro but it remains possible that a transposable element, like ISAbA125, could transfer blaNDM-1 on to broad host range plasmids capable of dissemination to other bacteria. The genetic context in this strain further supports the association of blaNDM-1 with ISAbA125 elements. ISAbA125 is predominantly found in *A. baumannii*, suggesting that NDM-1 established itself in *A. baumannii* prior to spreading into the Enterobacteriaceae.



**P1706** European dissemination of NDM-1-producing *Acinetobacter baumannii*

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**Background:** Carbapenem resistance in *Acinetobacter baumannii* is increasingly reported worldwide. That resistance is mostly related to carbapenem-hydrolyzing class D beta-lactamases and less frequently to class B carbapenemases. The blaNDM-1 gene has been mostly found as plasmid-located and mostly from *Klebsiella pneumoniae* and *Escherichia coli*. Reports of NDM-1 producing *A. baumannii* are rare.

**Methods:** NDM-1 producing *A. baumannii* isolates were identified by using the API20NE system (bioMérieux, Marcy l'Etoile, France) and identification was confirmed by 16S rDNA gene sequencing and culture at 44°C. A collection of five NDM-producing *A. baumannii* recovered from four European countries, namely France (n = 2), Germany, Switzerland and Slovenia. Sequence-typing, Multi Locus Sequence Typing, Diversilab and PFGE was used to evaluate the clonality of the isolates. Shotgun cloning and PCR mapping were used to determine genetic context of the blaNDM-1 gene. Its genetic support was determined by Southern hybridization. MICs were determined by E-test.

**Results:** The *A. baumannii* isolates were resistant to all beta-lactams, including carbapenems. Genomic analysis identified three clones among the five isolates. The blaNDM-1 gene was located in each case on their chromosome within the Tn125 composite transposon that is made of two copies of the ISAbal25-like insertion sequence bracketing a ca. 8 kb fragment encompassing the blaNDM-1 gene. Two out of the four isolates harboured an isoform of Tn125 with the insertion of ISAbal4. One isolate co-expressed the carbapenemase OXA-23.

**Conclusions:** This the first epidemiological study of NDM-1 producing *A. baumannii* with European isolates. This study showed that the dissemination of the blaNDM-1 gene in *A. baumannii* is multiclinal in Europe. The genetic structure associated with the blaNDM-1 gene was always related to Tn125 or an isoform.

**P1707** NDM-4-producing and carbapenem-resistant *Escherichia coli* from Cameroon to France

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**Objectives:** We investigated the mechanisms responsible for carbapenem resistance in an *Escherichia coli* isolate recovered from a Cameroonian patient.

**Methods:** The MICs were determined by Etest and PCR assays followed by sequencing were carried out for detection and identification of carbapenemase genes. The genetic environment of the blaNDM-4 gene was studied by PCR combination using specific primers of ISAbal25, bleMBL (bleomycin resistance gene) and blaNDM-1 followed by sequencing. Multi locus sequence typing (MLST) was performed to type the strain.

**Results:** *Escherichia coli* FEK was recovered from rectal swab of a patient transferred from Cameroon to France. He had been hospitalized for 1 month in Douala for an inflammatory syndrome associated to a kidney failure prior to his transfer to France. Isolate FEK was resistant to all beta-lactams including carbapenems, with MICs values of IMP, MER, ERT > 32 mg/L. It produced a new variant of the class B carbapenemase NDM, NDM-4, that shares a single amino-acid substitution (Met to Leu) at position 154 compared to NDM-1. Together with NDM-4, isolate FEK produced the extended-spectrum beta-lactamase CTX-M-15, the narrow-spectrum beta-lactamase OXA-1, and the cephalosporinase CMY-6. The blaNDM-1 gene was located on a conjugative plasmid (~150-kb) and bracketed upstream by the insertion sequence ISAbal25 and downstream by the bleMBL gene. MLST analysis revealed that this isolate belonged to ST405 type. Interestingly, although the patient did not receive any carbapenem-based treatment, his history revealed that he had received

chemotherapy based on bleomycin to treat an Hodgkin lymphoma since 2008.

**Discussion:** This study indicates that (i) NDM-producing enterobacterial isolates are present in West Africa (ii) NDM-4 may be spread as well as NDM-1 (iii) screening at the admission of any patient transferred from abroad is justified.

**P1708** Emergence of NDM-1 carbapenemase producing Enterobacteriaceae in Abu Dhabi Emirate, United Arab Emirates

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**Objectives:** The aim of the study was to assess the prevalence and molecular background of blaNDM in carbapenem resistant Enterobacteriaceae isolated in four major hospitals of Abu Dhabi Emirate.

**Methods:** Thirty-two Enterobacteriaceae strains non-susceptible to at least one carbapenem and isolated between 2009 and 2011 in Tawam (22), Mafraq (4), Al Ain (1) Hospitals and Sheikh Khalifa Medical Center (5) were collected. Species identification and antibiotic susceptibility testing were performed using VITEK 2. MBL production was confirmed by E-test. The presence of blaNDM, ESBL and aminoglycoside resistance coding genes was detected by PCR. The blaNDM type was determined by direct sequencing of the amplicon. Plasmid analysis was conducted using the alkaline lysis method. Conjugative transfer of beta-lactam resistance genes was carried out using an azide-resistant derivative of *E. coli* J53 as recipient. Plasmid replicon types were determined by multiplex PCR. Molecular fingerprints of the isolates were obtained by ERIC PCR and PFGE analysis. Multi locus sequence types of *K. pneumoniae* and *E. coli* were determined. Patient data on nationality and travel history was also collected.

**Results:** Seven strains (21.9% of the collection) were NDM producer. They were isolated in Tawam (6) and Mafraq (1) Hospitals and belonged to four different species: *K. pneumoniae* (3), *E. coli* (2), *Citrobacter freundii* (1) and *Enterobacter cloacae* (1). They all carried blaNDM-1 on conjugative plasmids of varying size. Antibiotic sensitivity, sequence type of isolates, size and type of plasmid carrying the blaNDM-1 with respective patient data are shown in Table 1. Only two *K. pneumoniae* isolates exhibited similar PFGE patterns and sequence types and epidemiological link could be established between them. The remaining five strains did not share any common trait, except that three of them carried NDM-1 on a plasmid of similar size coding for beta-lactam resistance, only. Four patients had connection to the Indian subcontinent either by travel or by nationality. However, three patients: an Omani, an Iraqi and an Emirati, carried NDM-1 producer strains without any travel history outside of the Gulf region.

Strain	Hospital	Species	Specimen	Patient's nationality	Travel history	Sequence type	Antibiotic susceptibility	Size of blaNDM-1 plasmid	Plasmid replicon type	Other resistance genes on the blaNDM-1 plasmid
ABC40	Tawam	Enterobacter cloacae	Wound swab	Emirati	None	Not determined	Amikacin, Cotrimoxazole, Colistin	approx. 50 Kb	Non typable	blaNDM-1
ABC32	Tawam	Klebsiella pneumoniae	Tracheal aspirate	Emirati	India	ST11	Tetracycline, Colistin	approx. 50 Kb	Non typable	blaNDM-1
ABC33	Tawam	Klebsiella pneumoniae	Tracheal aspirate	Iranian	None	ST11	Tetracycline, Colistin	approx. 50 Kb	Non typable	blaNDM-1
ABC34	Tawam	Escherichia coli	Urine	Pakistani	None	ST206	Nalidixic acid, Ciprofloxacin, Mirocycillin, Amikacin, Gentamicin, Colistin	approx. 50 Kb	Non typable	blaNDM-1
ABC40	Tawam	Citrobacter freundii	Urine	Bangladeshi	None	Not determined	Tetracycline, Mirocycillin, Amikacin, Gentamicin, Colistin	approx. 50 Kb	Non typable	blaNDM-1
ABC33	Mafraq	Klebsiella pneumoniae	Nasal screen	Iraqi	Iraq	Single locus variant of ST14	Colistin	approx. 170 Kb	Non typable	armA, blaNDM-1
ABC35	Tawam	Escherichia coli	Tracheal aspirate	Omani	None	ST253	Chloramphenicol, Colistin	approx. 150 Kb	A/C	intC, blaNDM-1

**Conclusion:** Our data demonstrate that NDM-producing Enterobacteriaceae are spreading within Abu Dhabi Emirate. This supports previous suggestions that these bacteria have already disseminated in the Middle East and threaten successful antibiotic treatment in this region as well.

**P1709** OXA-48, OXA-23 and NDM-1 carbapenemases in gram-negative bacteria from patients from Libya

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**Objectives:** Multidrug-resistant gramnegative bacteria pose an important challenge for treatment and infection control. Resistance to carbapenems is of special clinical importance and therefore it is necessary to monitor respective resistance mechanisms like carbapenemase production. No data regarding the presence of carbapenemases, extended-spectrum beta-lactamases or acquired AmpC-beta-lactamases have been published for Libya so far.

**Methods:** Health-care authorities in Germany strongly recommended to screen patients from Libya for multidrug-resistant gramnegative pathogens and to refer those strains to the German reference laboratory for multidrug-resistant gramnegative bacteria. Isolates were tested for the presence of carbapenemases by combined disk-tests with boronic acid or EDTA, modified Hodge-Test, a microbiological bioassay based on cell-free extracts and PCR and subsequent sequencing for KPC, VIM, IMP, NDM, OXA-48, OXA-23, OXA-40 and OXA-58.

**Results:** Several young patients injured during the recent conflict in Libya were transferred to hospitals in Germany for treatment. From 21 October till 7 November, 2011 isolates of 17 male patients with an age between 14 and 41 years were referred.

*Klebsiella pneumoniae* harbouring OXA-48 as well as CTX-M-15 was found in 13 patients. *Acinetobacter baumannii* with OXA-23 was found in four patients, two of which also carried a OXA-48 producing *K. pneumoniae*. Two patients were colonized with a NDM-1 producing *A. baumannii*. In one of those patients also a *K. pneumoniae* with OXA-48 was found. One patient was colonized with a *K. pneumoniae* strain harbouring both a CTX-M-15 ESBL and a DHA-1 AmpC-beta-lactamase.

**Conclusion:** A considerable number of patients previously hospitalized in Libya carry multidrug-resistant *K. pneumoniae* strains with OXA-48 as well as *A. baumannii* strains with OXA-23 or NDM-1. Sometimes co-colonization with both species occurs. Because those patients were screened for multidrug-resistant bacteria immediately after transfer to German hospitals and cared for in single rooms it is highly likely that they acquired those multidrug-resistant strains while hospitalized in Libya. OXA-48, OXA-23 and NDM-1 carbapenemases likely show a high prevalence in Libyan hospitals.

**P1710** First detection of New Delhi metallo-beta-lactamase in a multiple resistant strain of *Klebsiella pneumoniae* in Iran

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**Objectives:** New Delhi Metallo-beta-lactamase (NDM-1) is a novel metallo-beta-lactamase. Sporadic cases of NDM-1 positive strains have been reported from different countries suggesting a widespread dissemination. The aim of this study was to detection of Metallo-beta-lactamases in gram-negative bacilli isolated from patients in Tehran hospitals.

**Methods:** After specific identification tests, the susceptibility to the antibiotics was done by Kirby Bauer method and broth microdilution. Carbapenem resistant isolates were tested for carbapenemase production using modified Hodge test. Carbapenem resistant strains screened for blaKPC gene and genes encoding Metallo-beta-lactamases by using specific primers targeting blaNDM-1, blaVIM-1, blaVIM-2, blaIMP-1, blaIMP-2, and blaSPM. PCR products were sequenced in both directions.

**Results:** Among 360 collected gram-negative isolates, 23 isolates (6.3%) were resistant to meropenem, 11 isolates (3%) were resistant to ertapenem, and four isolates (1.1%) were resistant to imipenem. Majority of carbapenem resistant isolate were *Klebsiella* spp. (52.1%) and *Escherichia coli* (39.1%). Modified Hodge test was positive in 11 (47.8%) of carbapenem resistant isolates showing carbapenemase production. Resistance to third-generation cephalosporins in this study was 65.7% averagely. Of the cephalosporins, cefepime showed the highest activity against all species. In March 2011, we detected a multiple drug resistant *Klebsiella pneumoniae* isolate that it was resistant to all tested antibiotics except colistin. PCR confirmed that this isolate contained blaNDM-1, blaTEM, blaSHV, and blaCTX-M. Modified Hodge test showed weakly positive result. MBL screening by E-test strips was positive. Class 1 Integron detected in this isolate after PCR using by specific primers. The nucleotide sequence of NDM-1 gene was submitted to the GenBank databases under accession number JN664262.

**Conclusion:** In this study, detection of blaTEM, blaSHV, blaNDM-1, and blaCTX-M combined with the mobility of class 1 integron in *K. pneumoniae* suggests that resistance to cephalosporins and carbapenems will continue and these resistances can easily transfer to many different gram negative bacteria. This is the first report of detection of metallo-beta-lactamase NDM-1 in Iran. The rapid spread of NDM-1 positive bacteria showed a major challenge for treatment and control of infectious diseases and all clinicians should be aware the NDM-1 as a potential health threat.

**P1711** New Delhi metallo-beta-lactamase-1 in *Acinetobacter baumannii*: a report from a tertiary care centre in South India

M. Shanthi\*, U. Sekar, A. Kamalanathan, S. Balaraman (Chennai, IN)

**Objectives:** New Delhi Metallo beta lactamase-1 (NDM-1) is a growing threat worldwide. Though it has been increasingly associated with Enterobacteriaceae, reports of its occurrence in *Acinetobacter baumannii* has been sporadic. This study was done to detect the presence of NDM-1 in clinical isolates of carbapenem resistant *A. baumannii*.

**Methods:** The study was conducted in a 1600 bedded university teaching hospital between April and October 2010. One hundred and sixteen consecutive, clinically significant carbapenem resistant (by disc diffusion test as per CLSI guidelines) *A. baumannii* obtained from respiratory secretions (62), blood (25), exudative specimens (18), body fluids (7) and urine (4) were included in the study. Minimum Inhibitory concentrations (MIC) to imipenem and meropenem were determined by broth microdilution method with suitable controls. Screening for production of carbapenemases and metallo-beta-lactamases (MBL) was done by the Modified Hodge test (MHT) and inhibitor potentiated disk diffusion test using ethylene diamine tetraacetic acid (EDTA) respectively. Presence of blaNDM-1 was detected by Polymerase chain reaction (PCR). Coexistence of other carbapenemases like blaOXA, blaIMP and blaVIM were looked for in NDM-1 producers. Gene sequencing was performed for representative isolates.

**Results:** Thirteen of 116 carbapenem resistant *A. baumannii* harboured the blaNDM-1 gene. Amongst them, six also had the blaVIM, blaOXA-23 like and blaOXA-51 like genes, while six others had blaOXA23 and blaOXA51 without blaVIM along with the blaNDM-1. One isolate harboured blaNDM-1 alone. All the 13 isolates exhibited a positive result with MHT and inhibitor based disc test for the presence of carbapenemases and MBL. Their MIC90 to imipenem and meropenem were 32 and 64 mg/L respectively. These NDM-1 producers were obtained from respiratory secretions (9), blood (3) and cerebrospinal fluid (1). The majority of the isolates were from one multidisciplinary intensive care unit (ICU) of the hospital.

**Conclusion:** With limited therapeutic options, NDM-1 in *A. baumannii* is a cause for concern in critically ill patients with life threatening infections. They are most often present along with other carbapenemases like blaOXA and blaVIM. Since *A. baumannii* has the

potential to disseminate in the environment, molecular surveillance is required to detect their presence and distribution. This will help in source control and early intervention to prevent outbreaks in ICU.

**P1712** **Increasing prevalence of New Delhi metallo-beta-lactamase -1 in Enterobacteriaceae: the challenge**

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**Objectives:** Acquired carbapenemases confer extensive antibiotic resistance in Enterobacteriaceae. The emergence and spread of New Delhi metallo-beta-lactamase-1 (NDM-1) is a cause for concern. This study was undertaken to detect the production of NDM-1 metallo-beta-lactamase (MBL) in Enterobacteriaceae at a tertiary care centre in India.

**Methods:** One hundred and eleven clinically significant Enterobacteriaceae resistant to one of the cephalosporins subclass III were collected during the period April–October 2010. They included *Klebsiella pneumoniae* -52, *Escherichia coli* -25, *Citrobacter freundii* -16, *Enterobacter cloacae* -16 and *Providencia rettgeri* -2, isolated from blood (23), respiratory secretions (24), exudative specimens (19) and urine (45) of patients in Intensive care units (ICU) and non-ICU settings. Susceptibility to imipenem and meropenem was performed by disc diffusion and Minimum inhibitory concentration (MIC) determined by broth microdilution with suitable controls. Results were interpreted according to CLSI January 2011 guidelines. Carbapenemase production was screened by Modified Hodge test (MHT) and MBL production by inhibitor potentiated disk diffusion tests with ethylene diamine tetraacetic acid (EDTA). The isolates were subjected to Polymerase chain reaction (PCR) for detection of blaNDM-1. Gene sequencing was performed.

**Results:** blaNDM-1 was detected in 64 isolates. While the MHT was positive in 57 of them, the MBL screening test was positive in 43. The sensitivity of these tests were 89.1% and 79.6%. MIC<sub>90</sub> values for imipenem and meropenem were 4 and 2 mg/L respectively. blaNDM-1 positive isolates were from blood (17), exudative specimens (13), respiratory secretions (9) and urine (25). The majority of these isolates were *K. pneumoniae* (31), followed by *E. coli* (13), *E. cloacae* (10), *C. freundii* (9) and *P. rettgeri* (1). The NDM-1 producers were distributed equally between ICU and non-ICU patients. Twenty-seven isolates were susceptible to carbapenems as per CLSI 2011 interpretation.

**Conclusion:** NDM-1 producing Enterobacteriaceae cause a multitude of infections both in ICU and non ICU settings. Screening tests have poor sensitivity for NDM-1 producers. Interpretation based on CLSI 2011 guidelines can lead to erroneous susceptibility report. Since the significance of presence of NDM-1 in isolates susceptible to carbapenem is unclear, the need for screening all Enterobacteriaceae with resistance to cephalosporins subclass III is to be considered.

**P1713** **The genetic context of blaNDM-1 in *Acinetobacter baumannii* from clinical isolates dating back to 2005 from Chennai, India**

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**Objectives:** We set out to define the genetic context of blaNDM-1 in six isolates dating back to 2005 from Chennai, India. To our knowledge these are the earliest known bacterial isolates producing NDM-1. *A. baumannii* has been proposed to be an intermediate source for the dissemination of blaNDM-1 into Enterobacteriaceae so the genetic context in these isolates is of significant interest.

**Methods:** Isolates were identified biochemically (Phoenix) and by PCR for blaOXA-51-like genes. blaNDM-1 and blaOXA-23 were detected by PCR. Genomic DNA was digested with *ApaI* and endonuclease S1 and fragments separated by pulsed field gel electrophoresis. Further digests with *EcoRI* and *HindIII* were separated by standard electrophoresis. Gels were probed for blaNDM-

1 and ISAbA125. PCR primers were designed to published sequences of the blaNDM context in *A. baumannii* to perform primer walking and PCR products sequenced.

**Results:** Six blaNDM-1 positive isolates exhibited three *ApaI* digest patterns by PFGE. blaOXA-51-like (supporting *A. baumannii* ID) and blaOXA-23-like PCRs were positive for all isolates tested. S1 digests and probing showed that blaNDM-1 appeared to be on multiple plasmids, but these are probably plasmid multimers. blaNDM-1 was on an *ApaI* fragment of <50 kb. In isolate 45-1 primer walking and sequencing revealed a genetic context which is similar but not identical to that in *Acinetobacter* described so far. ISAbA125 is upstream of blaNDM-1, with ble downstream. Further downstream *trpF*, *groEL*, *groES* and the ISCR16-like gene appear to be present but full sequencing of these PCR products is ongoing. blaNDM-1 was on ~1 and 5 kb *EcoRI* fragments, suggesting there is a second copy of blaNDM-1 in a novel genetic context, since there are *EcoRI* sites either side of blaNDM-1 in all *A. baumannii* sequences available so far, including 45-1.

**Conclusion:** The genetic context in these earliest known NDM-1 producing isolates is similar to that found in *A. baumannii* previously. The consistent association with *A. baumannii* IS element ISAbA125 supports the suggestion that blaNDM-1 may have established itself in *A. baumannii* prior to spreading into the Enterobacteriaceae. This is only the second study of which we are aware showing NDM-1 on plasmids in *A. baumannii* and it will be interesting to see whether transfer into other bacteria occurs in vitro.

**Commercially available systems for detection of ESBLs including KPC and AMPC**

**P1714** **Carbapenemase identification by matrix assisted laser desorption/ionisation time-of-flight mass spectrometry**

R. Walkova\*, V. Studentova, E. Chudackova, T. Bergerova, J. Hrabak (Plzen, CZ)

**Objectives:** Resistance of Gram-negative rods to carbapenems has been an increasing problem all over the world. This resistance is caused by an alteration in the outer membrane of the cell wall, by an overexpression of the efflux pumps or by carbapenemase production. Carbapenemases can be detected by methods based on the ability of some compounds to inhibit these enzymes, molecular genetic techniques and by direct visualization of carbapenem hydrolysis. We describe here the use of MALDI-TOF mass spectrometry to detect a carbapenem antibiotic and its degradation by carbapenemases.

**Methods:** Buffered meropenem solution (0.1 mM Tris-HCl, pH 6.8) was mixed with an overnight culture of bacteria. After 3 hours incubation, the reaction mixture was centrifuged and supernatant was analysed by MALDI-TOF MS using 2,5-dihydroxybenzoic acid as a matrix. The presence or absence of peaks representing meropenem and its sodium salts was crucial. Inhibitor-based methods have also been tested for the identification of carbapenemase type. The samples containing inhibitors (e.g. EDTA, dipicolinic acid, phenylboronic acid) cannot be directly measured by MALDI-TOF MS due to the interference of inhibitors with matrix. Therefore, some procedures for a partial purification of the sample were tested.

**Results:** The sensitivity of this method, validated on 145 strains, including 41 carbapenemase-producing strains, is higher than 97%, with a specificity of a similar value. The strains used for the study produced different KPC enzymes (KPC-2 and KPC-3), VIM, IMP and NDM-1 metallo-beta-lactamases. Positive results were obtained also in NDM-1-producing *Acinetobacter baumannii*. The inhibitors can be optimally removed from the sample using ZipTip Pipette Tips (Millipore). Validation of the inhibitor-based method for identification of carbapenemase type is under the evaluation.

**Conclusion:** MALDI-TOF MS assay for carbapenemase detection was introduced to the routine laboratory praxis in the author's laboratories. The results are comparable with the reference spectrophotometric

imipenem hydrolysis assay using crude bacterial extract. We believe that this method can become a standard technique for a quick carbapenemase identification in routine diagnostic laboratories.

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**P1715 Evaluation of Brilliance™ CRE agar for the detection of carbapenem-resistant gram-negative bacteria**

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**Objectives:** Infections caused by carbapenem-resistant enterobacteria are an emerging problem worldwide. Various selective agar media have been developed for preliminary screening allowing different carbapenem-resistant enterobacterial species to be recognised easily. The aim of this work was to evaluate the performance of the new chromogenic medium, Oxoid Brilliance™ CRE Agar (Thermo Fisher Scientific) for determining the limit of detection of carbapenem-resistant bacteria.

**Methods:** A total of 70 clinical isolates were studied. Of these, 30 were well-characterized carbapenem-resistant enterobacterial strains, including *Klebsiella pneumoniae* producing KPC-type (n = 15) or VIM-type (n = 3) enzymes, VIM-positive *Enterobacter cloacae* (n = 3), and *Escherichia coli* (n = 1), and isolates characterized by porin loss associated with ESBL production (*K. pneumoniae*, n = 3), or AmpC hyperproduction (*E. coli*, n = 2; *Serratia marcescens*, n = 2; *E. cloacae*, n = 1). Ten additional carbapenemase-producing non-fermentative isolates (*Pseudomonas aeruginosa*, n = 7; and *Acinetobacter baumannii*, n = 3) were also included in the study as well as 30 carbapenem-susceptible Gram-negative isolates. Carbapenem-resistant strains were inoculated at three different concentrations onto Brilliance CRE Agar starting from  $1.5 \times 10^4$  CFU/mL up to  $1.5 \times 10^4$  CFU/mL whereas carbapenem-susceptible isolates were inoculated at the fixed concentration of  $1.5 \times 10^2$  CFU/mL. After 18–24 hour incubation at 36°C, the plates were evaluated in order to verify number, size and colour of colonies.

**Results:** The Brilliance CRE Agar was consistently able to sustain the growth of carbapenem-resistant isolates, showing a detection limit of  $1.5 \times 10^4$  CFU/mL in 31/40 cases whereas the remaining grew at  $1.5 \times 10^2$  CFU/mL. No growth was observed with carbapenem-sensitive control strains. Concerning enterobacteria, blue colonies were obtained in the case of *K. pneumoniae*, *E. cloacae*, and *S. marcescens* whereas a pale pink colour was observed for *E. coli*. Finally, *P. aeruginosa* showed a light brown colour and *A. baumannii* grew with small colourless colonies.

**Conclusions:** Our data demonstrate that the new Brilliance CRE Agar allows the growth of carbapenem-resistant isolates with low detection limits thus representing a useful screening medium for carbapenem-resistant enterobacteria. In our experience, carbapenem-resistant *P. aeruginosa* and *A. baumannii* were clearly distinguishable from enterobacterial strains based on different size and colour.

**P1716 Evaluation of the Oxoid Brilliance™ CRE agar for detection of carbapenemase producing Enterobacteriaceae**

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**Objectives:** Rapid detection of carbapenemase producing Enterobacteriaceae is pivotal for adequate antibiotic therapy and infection control. Selective agars play an essential role in the detection of such isolates in clinical specimens. The aim of this study was to determine the test characteristics of the Oxoid Brilliance™ CRE Agar (Thermo Fisher Scientific, UK) for detection of carbapenemase producing Enterobacteriaceae.

**Methods:** The test characteristics and specificity of the colony colours were determined, using an international panel of 234 non-repeat

Enterobacteriaceae including 74 isolates (56 *Klebsiella pneumoniae*, eight *E. coli*, six *Enterobacter* spp., two *P. mirabilis*, two *Serratia marcescens*) producing the following carbapenemases: 36 KPC-2/3, four KPC plus VIM, four NDM-1, six GIM, 20 VIM, four OXA-48. Of the 160 carbapenemase negative control isolates, 97 produced an ESBL (63 CTX-M, 14 TEM, 12 SHV, eight other ESBL genes or combinations), 37 isolates an AmpC (13 plasmid mediated, 24 chromosomal), seven co-produced AmpC and ESBL, eight isolates were K1 hyperproducing *K. oxytoca*, and 11 isolates were beta-lactamase negative. The plates were inoculated with 10 µL of a 0.5 McFarland suspension. Plates were read after 24 and 48 hours.

**Results:** The sensitivity for detection of carbapenemase producers after both 24 and 48 hours was 72/74 (97%). The growth was inhibited of two VIM-1 positive isolates (one *E. coli*, and one *P. mirabilis* with ertapenem MICs of 0.25 and <0.25 mg/L, respectively, with meropenem MICs of 0.5 and 2 mg/L, respectively, and both with an imipenem MIC of 6 mg/L. Of the control strains 46/160 (29%) were recovered at 24 hours, and 59/160 (37%) at 48 hours, corresponding to a specificity of 71% and 63%, respectively. The carbapenemase negative isolates growing at 24 hours were predominantly producers of ESBL or derepressed AmpC, with an increased ertapenem MIC (median: 1 mg/L range ≤0.0625 to >8 mg/L; MIC 50 1 mg/L; MIC 90 > 8 mg/L). All colonies of *K. pneumoniae*, *Enterobacter* spp., *P. mirabilis* and *S. marcescens* were blue, whereas *E. coli* colonies were brownish.

**Conclusion:** The Brilliance™ CRE Agar is a highly sensitive and rapid method for detection of carbapenemase producing Enterobacteriaceae, but confirmation of carbapenemase production and species is required.

**P1717 Evaluation of a novel chromogenic medium for detecting carbapenemase-producing Enterobacteriaceae in surveillance rectal swabs**

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**Objectives:** Carbapenem-producing Enterobacteriaceae (CPE) is an increasing problem worldwide and rectal swab surveillance is recommended as a component of infection control programs.

**Methods:** The performance of a prototype chromogenic medium (chromID CARBA, bioMérieux, Marcy l'Étoile, France) was evaluated and compared with four other screening methods: (i) overnight selective enrichment in 9 mL tryptic soy broth with a 10-µg ertapenem disk followed by plating onto MacConkey agar (CDC-TS), (ii) short selective enrichment in 5 mL brain heart broth with a 10-µg ertapenem disk followed by plating onto chromID ESBL medium (ESBL-BH) (bioMérieux), (iii) direct plating onto chromID ESBL medium and (iv) direct plating onto MacConkey agar supplemented with meropenem (1 mg/L) (MCM) for the detection of CPE in 200 rectal swabs from ICU hospitalized patients. Identification and antimicrobial susceptibility testing of isolates was performed by the VITEK2 system (bioMérieux). Carbapenem MICs were verified by Etest (bioMérieux) using CLSI 2011 criteria. Carbapenemase enzymes were confirmed using combined disk tests and PCR assays for KPC, VIM, IMP, and OXA-48 genes.

**Results:** A total of 73 rectal swabs (36.5%) were found to contain Enterobacteriaceae that produced a carbapenemase for at least one of the screening media and 92 CPE isolates were recovered from these samples. The predominant species was *K. pneumoniae* (n = 85, 56 KPC-positive, 29 VIM-positive), followed by *Enterobacter aerogenes* (n = 7, all KPC-positive).

ChromID CARBA, ESBL-BH and chromID ESBL exhibited the highest sensitivity (92.39%), followed by CDC-TS and MCM (89.13%). The specificity was greater for ESBL-BH (93.2%) and chromID CARBA (96.9%) than for CDC-TS (86.4%), MCM (85.2%) and chromID ESBL (84.7%). ESBL-BH and chromID CARBA showed higher overall accuracy (93.0% and 95.1%) than CDC-TS (87.4%), MCM (86.7%) and chromID ESBL (85.1%).

**Conclusion:** ChromID CARBA and ESBL-BH were found the most efficient screening methods for CPE detection in rectal swabs 24 hour upon reception.

**P1718 Evaluation of a new chromogenic medium, chromID CARBA, for the detection of carbapenemase-producing Enterobacteriaceae**

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**Objective:** Carbapenemase-producing Enterobacteriaceae (CPE) are multi-resistant emerging bacteria which can be responsible for hospital acquired infections and outbreaks. Detection of CPE carriers particularly is of importance for prevention and epidemiological monitoring of these infections. In this context, chromogenic media for CPE should make the screening easier due to their selectivity and the use of different colours to discriminate targeted species. The aim of this study was to evaluate the performance of two chromogenic media for CPE detection, namely chromID CARBA (a prototype from bioMérieux) and CHROMagar KPC (CHROMagar). One selective home-brewed medium commonly used so far, MacConkey supplemented with 1 mg/L imipenem (McC + I), was also included in the study.

**Method:** A total of 194 isolates was tested, including 127 CPE with different types of carbapenemases (53 KPC, 44 NDM, 13 VIM, 11 IMP, six OXA-48) and 67 isolates that do not produce a carbapenemase. All microorganisms were inoculated directly onto each medium with 10 µL of a 0.5 McFarland calibrated suspension before 24 hour incubation at 34–38°C. Two batches of chromID CARBA were used: one freshly prepared (B1) and one close to the expiry date (B2).

**Results:** For chromID CARBA, the sensitivity for CPE detection varied from 89.8% (B1) to 96.1% (B2). By comparison, the sensitivity was 89.0% for CHROMagar KPC and only 68.5% for McC+I. Variation in sensitivity was dependent on the types of carbapenemases. Detection of NDM (97.7%) and KPC (96.2–100%) by chromID CARBA was the easiest, followed by VIM (84.6–92.3%), OXA-48 (66.7–100%) and IMP (45.5–72.7%).

Specificity was almost identical between the different media: chromID CARBA, 97% (B1) and 94% (B2); CHROMagar KPC, 94%; McC+I, 96%.

**Conclusion:** This study highlights the superiority in sensitivity of both chromogenic media over the imipenem supplemented MacConkey. In comparison to CHROMagar KPC, chromID CARBA presents three advantages: (i) trend to higher sensitivity, (ii) ready to use plates and (iii) extended shelf life. As such, it has the potential of being a very useful tool for the screening of patients who carry the widespread KPC- and NDM-producing Enterobacteriaceae. These results should be confirmed with clinical samples such as rectal swabs.

**P1719 Comparison and development of faecal screening methods for detection of carbapenemase-producing Gram-negative bacteria**

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**Objectives:** Carbapenems are the ultimate drug choice for treatment of serious Gram negative infections in many hospitals. Increasing reports of bacteria producing carbapenemases, such as NDM, especially in outbreak scenarios, are of concern. Faecal screening policies for at risk patients are now commonplace. However the method for performing faecal screening accurately and for detecting carbapenemase producing bacteria is difficult. Commercial screening agars are available and official guidance has been issued but the issue remains problematic, especially in carbapenemase producing bacteria which exhibit low MICs to carbapenems. Here we compare the ability of commercial agars and official guidance to detect a variety of carbapenemase producing bacteria (CPB) at various concentrations within a faecal bacterial mix.

**Methods:** Commercial agars CRE (Oxoid) & ChromagarKPC (E&O) plus in-house agars containing MacConkey (MAC) + 8 mg/L vancomycin (V) + 1 mg/L meropenem (M) and UTI agar + 8 mg/L V + 1 mg/L ertapenem (ERT) were compared with a standard UTI agar (Oxoid) and HPA recommended MAC+ERT disc. CPBs at 109, 107, 105 & 103 CFU/mL were added to mixes of 109 *Pseudomonas aeruginosa* (PSA) or *E. coli* (EC) plus 107 *Enterococcus* sp. (E). CPB used were: *Klebsiella pneumoniae* (KPN) containing NDM (low MIC), *E. cloacae* (ECL) + NDM, *Acinetobacter baumannii* (AcB) + NDM (high MIC), PSA + VIM (high MIC), PSA + VIM (low MIC), KPN + IMP, KPN + KPC, AcB + GES, KPN + oxacillinase (OXA) Table 1.

**Results:** For agars CRE & UTI + V + ERT CPB were detected with no loss of quantity and ease of distinguishing against background mix was good for all mixes. For KPC & MAC + V + MER, CPB were detected with only slight loss of quantity in 105 and 103 quantities in mixes 15–18. For MAC+ERT disc, 11 background isolates grew making ease of distinguishing CPB reasonable for mixes 1, 3, 4 (109/107), 5 (109–105), 6, 13 (109/107), 15, 16, 17 (109), and 18 (109). At lower concentrations (105/103) ease of detection in mixes 2, 4, 5, 7 to 13, 17, 18 was poor and in mix 4 at 103 not detected.

Mix No.	Background mix	CPB			
		10 <sup>9</sup>	10 <sup>7</sup>	10 <sup>5</sup>	10 <sup>3</sup>
1	10 <sup>9</sup> PS A + 10 <sup>7</sup> E	KPN NDM (L)			
2	10 <sup>9</sup> EC + 10 <sup>7</sup> E	KPN NDM (L)			
3	10 <sup>9</sup> PS A + 10 <sup>7</sup> E	ECL NDM			
4	10 <sup>9</sup> EC + 10 <sup>7</sup> E	ECL NDM			
5	10 <sup>9</sup> PS A + 10 <sup>7</sup> E	AcB NDM (H)			
6	10 <sup>9</sup> EC + 10 <sup>7</sup> E	AcB NDM (H)			
7	10 <sup>9</sup> PS A + 10 <sup>7</sup> E	PS A VIM (H)			
8	10 <sup>9</sup> EC + 10 <sup>7</sup> E	PS A VIM (H)			
9	10 <sup>9</sup> PS A + 10 <sup>7</sup> E	PS A VIM (L)			
10	10 <sup>9</sup> EC + 10 <sup>7</sup> E	PS A VIM (L)			
11	10 <sup>9</sup> PS A + 10 <sup>7</sup> E	KPN IMP			
12	10 <sup>9</sup> EC + 10 <sup>7</sup> E	KPN IMP			
13	10 <sup>9</sup> PS A + 10 <sup>7</sup> E	KPN KPC			
14	10 <sup>9</sup> EC + 10 <sup>7</sup> E	KPN KPC			
15	10 <sup>9</sup> PS A + 10 <sup>7</sup> E	AcB GES			
16	10 <sup>9</sup> EC + 10 <sup>7</sup> E	AcB GES			
17	10 <sup>9</sup> PS A + 10 <sup>7</sup> E	KPN OXA			
18	10 <sup>9</sup> EC + 10 <sup>7</sup> E	KPN OXA			

**Conclusions:** CRE commercial plate and UTI + VAN + ERT performed best at detecting CPB and for ease of distinguishing from background mix. MAC + ERT disc performed variably depending on the quantity of CPB present.

**P1720 Evaluation of a new Etest® strip for Klebsiella carbapenemase detection on a large collection of genotypically characterised strains**

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**Objective:** Carbapenemase producing Enterobacteriaceae (CPE) pose a serious problem in the management of healthcare-associated infections and accurate detection is key for infection control and prevention. Non-expensive, reliable confirmatory tests for CPE are needed and should be accessible to any laboratory. Specific synergy-based tests often are preferred to the Modified Hodge test which may lack sensitivity and specificity. To complement the recently available Etest® MBL (MP/MPI), a new Etest® is being developed for KPC identification. The objective of this study is to evaluate this strip using a panel of genetically characterized isolates for resistance genes.

**Methods:** Etest® KPC MP/MPB is based on the reduction of the meropenem inhibitory concentration (MP side of the strip) in

presence of a boronic acid derivative (MPB side). This strip is tested with the procedure for standard Etest<sup>®</sup> MIC testing of Gram negative aerobes (inoculum 0.5 McF – Mueller Hinton agar and 16–20 hour incubation at 35°C). A total of 128 Gram negative isolates (81 positive for KPC and 47 producing various broad spectrum beta-lactamases other than KPC) were tested. After incubation, the Inhibitory Concentrations (IC) were read on each side of the strip. A three-fold-or-greater decrease (>3 log<sub>2</sub> dilutions) of the MP IC in the presence of inhibitor or presence of an ellipse deformation (“key hole” image) on the MP side is interpreted as a positive test for KPC. All other results are considered either as negative or non-determinable (ND).

**Results:** In comparison to the presence of the KPC gene, Etest<sup>®</sup> KPC had a sensitivity of 97.4% and a specificity of 97.8% with a PPV and NPV of 98.7% and 95.7% respectively. Moreover, the results are deemed ND for only 4.7% of the isolates.

**Conclusion:** In this study, the Etest<sup>®</sup> KPC strip shows very promising results by combining good sensitivity and specificity. While this study demonstrates feasibility of Etest<sup>®</sup> KPC MP/MPB, additional studies are needed for validation of this test.

**P1721 Evaluation of the modified KPC + MBL confirm ID kit for the phenotypic detection of class A and B carbapenemases in *Klebsiella pneumoniae* isolates**

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**Objectives:** The KPC + MBL Confirm ID kit (ROSCO Diagnostica) is a reliable method for the phenotypic detection of MBL- or KPC-producing enterobacteria. However, spread of isolates producing multiple carbapenemases, mainly in Greece, compromised the performance of all inhibitor based phenotypic methods. To address this issue, we evaluated a new version of the kit that was modified to bypass the problem imposed by the multiple carbapenemase producers.

**Methods:** The KPC + MBL confirm ID kit included four discs: MEM, MEM + APBA, MEM + DPA and MEM + Cloxacillin. In the modified kit, a fifth disc containing MEM + APBA + DPA was added. Performance was tested using 225 previously characterized *K. pneumoniae* isolates obtained from Greek hospitals in 2010. The collection included 102 KPC-positive, 22 MBL-positive and 39 isolates co-producing MBL and KPC carbapenemases. The remaining isolates were either ESBL and/or AmpC producers (n = 27) or susceptible to newer b-lactams (n = 35). The novel method was compared with a previously described procedure utilizing four discs: MEM, MEM + EDTA, MEM + PBA and MEM + EDTA + PBA.

**Results:** An increase of ≥5 mm in zone diameter around discs containing DPA as compared to the MEM disc, was considered as indicative of MBL production; a ≥4mm increase with the APBA-containing disc indicated KPC production. An increase of ≥4mm in the zone of the triple combination as compared to the MEM + DPA and MEM + APBA was considered as indicating production of both MBL and KPC enzymes. Cloxacillin test was negative for all isolates. All MBL producers and 101 of the 102 KPC producers were positive with the DPA or APBA test, respectively. With the triple combinations, 20 out of the 39 MBL + KPC-producers were correctly classified as double-carbapenemase producing isolates. The remaining 19 were characterized as single producers. None of the sensitive isolates was misclassified, whereas only one of the isolates with other resistant mechanisms was falsely classified as MBL-positive. Sensitivity, specificity, positive and negative predictive values of the modified KPC + MBL confirm ID kit were 91.8%, 99.4%, 98.2%, and 97% for MBLs and 87.9%, 100.0%, 100.0%, 83.2% for KPCs, respectively. The overall results were comparable to those of EDTA and PBA tests.

**Conclusion:** The modified KPC + MBL confirm ID kit can reliably identify most carbapenemase producers among *K. pneumoniae*. Yet, there remains room for improving detection of MBL + KPC-producers.

**P1722 Phenotypic detection of carbapenemase associated with extended-spectrum beta-lactamase in *Klebsiella pneumoniae***

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**Objective:** Although the presence of carbapenemase in Enterobacteriaceae in France is relatively rare, reports from national agency in charge of human health (INVS) show a disturbing increase. Microbiologists should aware of the resistance to carbapenems is not always easy to detect especially for imipenem. During an outbreak, we proposed an alternative phenotypic detection in our routine.

**Methods and Results:** Isolates of multiresistant *K. pneumoniae* were detected in seven patients hospitalized at St. Joseph Hospital Group in different wards. Antibiotic susceptibility was tested by the technique of agar diffusion. Strains of *K. pneumoniae* were resistant to all beta-lactam except carbapenems (MICs ertapenem: 0.38 mg/L, imipenem: 0.19 mg/L, meropenem: 0.125 mg/L, doripenem: 0.125 mg/L). The double disk synergy test between cefotaxime, ceftazidime, cefepime and clavulanate performed with and without cloxacillin was negative. The cloxacillin test eliminated an acquired cephalosporinase. Genetic analysis of resistance to beta-lactams has identified genes blaVIM-1 and blaTEM. Hodge test suggested the presence of a carbapenemase. Imipenem-EDTA disc indicated the presence of a metallo-beta-lactamase. Molecular typing by RAPD showed that seven patients had a single clone. In this epidemic context, rectal swabs were discharged on ESBL chromogenic agar. Colonies were tested on Mueller Hinton agar with a central disk of amoxicillin + clavulanate surrounded by a disc of aztreonam, cefotaxime, cefotaxime + EDTA and cefepime. This allowed the detection of carbapenemase-producing strains of type VIM-1 associated with ESBLs.

**Conclusion:** An isolated synergy between aztreonam and amoxicillin + clavulanic acid associated with multidrug resistance to beta-lactams suggests the presence of a metallo-beta-lactamase. Indeed, this type of enzyme can affect the activity of carbapenems moderately complicating their detection. Combined with an ESBL, a metallo-beta-lactamase may mask the synergy 3rd generation cephalosporin/clavulanic acid forming a trap. The detection of carbapenemase-producing strains is essential to limit dissemination.

**P1723 Comparative epidemiology of viral infections causing influenza-like illness. A survey in France during winter 2010–2011**

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**Introduction:** Influenza like illness (ILI) can be caused by many respiratory viruses. Apart influenza or respiratory syncytial viruses (RSV), little is known about the comparative epidemiology of these viral infections.

**Objective:** To compare the demographic and clinical characteristics of patients with ILI according to viral aetiology.

**Methods:** Molecular detection of 18 respiratory viruses was performed using microarray by three virological centres in patients seeking for medical advice for ILI definition. Nasal pharyngeal swabs, as well as demographical and clinical data were collected from each patient.

**Results:** The study was carried out over 14 weeks, from January to April 2011. We obtained complete demographical clinical and virological data in 587 included patients (93.5%). Four hundred and thirty-five (74%) were positive for at least one virus and co-infections were detected in 90 (21%) of positive patients. Influenza viruses were the most common viruses (82%) with influenza B and A/H1N1 2009 detected in equal proportions. Overall, positivity to any tested respiratory virus decreased with age (p = 0.004) except for human metapneumovirus which increased from 4% to 6% in children and adults under 45 years of age to 13–15% in older adults and the elderly (p = 0.0008). Among clinical symptoms, conjunctival hyperemia was

associated with a positive influenza B diagnosis, headaches with a negative rhinovirus diagnosis and rhinorrhea with a positive diagnosis to at least one virus.

**Conclusions:** These results contribute to a better understanding of the dynamic of respiratory viruses during an ILI epidemic. It also gives insights on the associations between a virological diagnosis and clinical or demographical characteristics of patients.

**P1724** **Detection of KPC carbapenemase with the EasyQ Kpc system, using NASBA technology**

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**Objectives:** Fifty-five *Klebsiella pneumoniae* from a collection of clinical isolates, with different level of resistance to carbapenems and well characterized mechanism, were analysed with the new EasyQ<sup>®</sup> KPC system (BioMerieux) and compared with Modified Hodge Test (MHT) and PCR, to determine the strains producers of the carbapenemase KPC.

The study was conducted with 17 KPC producers, five MBL producers (VIM and NDM), 17 ESBL producers and 16 strains with porin defect.

**Materials and methods:** MICs were performed by microdilution and interpreted according to EUCAST documents. Carbapenemase was investigated by hydrolysis of imipenem. The presence of bla(KPC), blaIMP, blaVIM, blaNDM were investigated by PCR and MHT for detection of class A carbapenemase.

The EasyQ<sup>®</sup> KPC test was performed following the instruction of manufacture and time of analysis is about 2 hours.

**Results:** The phenotype of all strains was confirmed for the presence of carbapenemase by PCR and hydrolysis of carbapenems.

Twenty strains have a positive result with the Modified Hodge Test, other than: the 17 KPC producers, also two MBL producers and one strain with a porin defect. These three strains continued to be positive any time the test was repeated.

All the strains were also tested with the new system EasyQ<sup>®</sup> KPC. All the KPC producers were positive at first analysis like as the MBL producers were negative. Five strains with porin defect and two ESBL producers resulted as KPC producers at the first analysis, but the amplification curve analysis of these discordant strains showed to be clearly different from the amplification curve of positive strains.

In comparison with PCR both methods, MHT and EasyQ<sup>®</sup> KPC showed a 100% of sensitivity. The specificity was respectively of 92% and 81.6%, but specificity of EasyQ<sup>®</sup> KPC improved to 100% with a modified interpretation of the cut-off value.

**Conclusions:** The EasyQ<sup>®</sup> KPC system presents a very high sensitivity (100%) and is able to detect all the KPC producers strains without false negative results. It present a specificity of 81.2% detecting false positive between strains producing ESBL and/or with porin defects. A better interpretation of the amplification curve, choosing a more sensitive cut-off will able to increase the specificity of test to 100%.

Three hours it will be sufficient to screen directly from the clinical sample all the patients infected or colonized by KPC producer strains, reducing noticeable the time of analysis.

**P1725** **Comparison of different molecular methods for the detection of the blaKPC gene**

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**Objectives:** The spread of carbapenem resistant *Klebsiella pneumoniae* (CR-KP) asks for a correct and prompt detection of blaKPC gene producers to guide for the best treatment and to implement infection control measures. The aim of this study is the comparison of three commercial tests for the identification of blaKPC with phenotypic methods and an home made molecular test.

**Methods:** From September 2010 to June 2011, 25 CR-KP strains isolated at Ospedali Riuniti di Bergamo were evaluated by:

1 phenotypic tests: Modified Hodge Test (MHT), Double Disk Synergy Test (DDST) and Combination Disk Test (CDT), by the use of boronic acid, EDTA and dipicolinic acid.

2 genotyping tests:

3 home made test by the use of blaKPC primers as described in the literature (Bradford, CID 2004).

4 Easy Q KPC (BioMerieux SA) for the detection of RNAKPC by a NASBA Real Time method

5 Hy-KPC Detection Real Time Kit and Hy-KPC Detection PCR Kit (Neomed, srl), both detecting blaKPC from bacterial DNA.

We used ATCC1706 as KPC negative control and ATCC1705 as KPC positive control.

The phenotypic tests and the home made PCR were performed directly on strains before their storage at -80°C. DNA extract was frozen at -20°C.

The Easy Q KPC was performed directly on seven strains before their storage and on 20 frozen strains.

Hy-KPC Detection Real Time was performed only on frozen DNA extracts, Hy-KPC Detection PCR was performed on 25 frozen DNA extracts and on two frozen strains.

**Results:** Phenotypic tests, home made PCR and Hy-KPC Detection Real Time Kit classified 23 strains plus the ATCC1705 as KPC producers and two strains plus the ATCC1706 negative for KPC. Easy Q KPC resulted negative in three KPC-positive strains. Hy-KPC Detection PCR Kit was negative in two KPC-positive strains.

**Conclusions:** Phenotypic tests, home made PCR and Hy-KPC Detection Real Time Kit were in agreement in the classification of the studied strains. Easy Q KPC and Hy-KPC Detection PCR Kit were negative respectively in three and two frozen strains. The frozen false negative strains were reevaluated by phenotypic tests and home made PCR and they also resulted negative. Probably the storage at -80°C caused the loss of blaKPC. Phenotypic tests need at least overnight incubation, while molecular methods allow faster results in about 2 hours. Home made PCR has more affordable costs.

**P1726** **New microarray check-point for the detection of ESBL, AmpC betalactamases and Carbapenemases**

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**Objectives:** The aim of this study was to use the new array Check-MDR CT103 in the molecular detection of ESBLs (Extended Spectrum Betalactamases), AmpC betalactamases and Carbapenemases as confirmation of the results obtained by conventional methodology.

**Methods:** Sixteen multidrug resistant enterobacterias clinical isolates obtained at the Hospital de la Princesa in Madrid were selected for the study. Its resistant detection was primary based on phenotypic testing: microdilution method (Microscan WalkAway Siemens), double-disk synergy test (cephalosporin and cephalosporin with boronic acid) or IP/IP EDTA E-test in the cases that were required. Clinical isolates were defined by phenotypic method as ESBL, AmpC, carbapenemase producer or multiresistant phenotype. DNA extraction was performed with the Nuclisens Easymag system (bioMérieux). Check-MDR CT103 (Hain Lifescience Spain) was used to detect the genes involves in the resistant mechanisms. Microarray uses a multiplex ligation detection reaction followed by PCR and hybridization. One array detects the presence of many of the tree enzymes types: ESBL, AmpC and Carbapenemase.

**Results:** The results are shown in the table. It should be interesting to point out the detection of a double AmpC: CMY II + DHA, a double ESBL: CTX M-9 + TEM 164C and the new NDM-1 metalobetalactamase. Moreover, all the AmpC betalactamases detected were plasmidic.

**Conclusion:** All isolates had concordant phenotypic and genotypic detection. The microarray was more accurate than classical methods to detect ESBL, AmpC and Carbapenemases. Check-MDR CT103 detected successfully the different genes and also the combination mechanisms none detected by phenotypic analysis: ESBL with AmpC, ESBL with Carbapenemase and AmpC with Carbapenemase.

N° isolates	Phenotypic detection	Genotypic detection
5	<i>E.coli</i> AmpC	CMY II + TEM 164C
		CMY II
		CMY II
		CMY II
		CMY II
5	<i>E.coli/K.pneumoniae</i> multiresistant	CMY II
		CMY II
		CMY II + CTX M-15like
		VIM + CMY II
		VIM + CTX M-15like
4	<i>E.coli/K.pneumoniae</i> ESBL	CMY II + CTX M-9 + TEM 164C
		CMY II+DHA + CTX M-15 like
		CMY II + CTX M-1
		CMY II + CTX M-15 like
2	<i>K.pneumoniae</i> metalobetaactamase	VIM + CMY II
		NDM-1 + CTX M-15

Maldi/ identification	FEP-CT result	CLOX-CT result	Check MDR CT 102 result
<i>S. marcescens</i>	-	+	-
<i>S. marcescens</i>	-	+	-
<i>S. marcescens</i>	-	+	-
<i>E. cloacae</i> complex	+	-	-
<i>E. cloacae</i> complex	+	-	-
<i>E. cloacae</i> complex	+	+	-
<i>C. freundii</i>	+	-	-
<i>E. cloacae</i> complex	+	+	CTX-M 1
<i>E. cloacae</i> complex	+	+	CTX-M 1
<i>E. cloacae</i> complex	-	+	CTX-M 1 & SHV 2385 + 240K
<i>C. freundii</i>	+	+	CTX-M 9
<i>E. aerogenes</i>	+	-	CTX-M 9
<i>E. cloacae</i> complex	+	+	CTX-M 9
<i>E. cloacae</i> complex	+	+	CTX-M 9 & SHV 2385 + 240K
<i>E. cloacae</i> complex	+	+	CTX-M 9 & SHV 2385 + 240K
<i>E. cloacae</i> complex	+	+	SHV 2385
<i>E. cloacae</i> complex	+	+	SHV 2385
<i>C. braakii</i>	+	+	SHV 2385 + 240K
<i>C. freundii</i>	+	+	SHV 2385 + 240K
<i>C. freundii</i>	+	+	SHV 2385 + 240K
<i>C. freundii</i>	+	+	SHV 2385 + 240K
<i>C. freundii</i>	+	+	SHV 2385 + 240K
<i>E. cloacae</i> complex	-	+	SHV 2385 + 240K
<i>E. cloacae</i> complex	+	+	SHV 2385 + 240K
<i>E. cloacae</i> complex	+	+	SHV 2385 + 240K
<i>E. cloacae</i> complex	+	+	SHV 2385 + 240K
<i>E. cloacae</i> complex	+	+	SHV 2385 + 240K
<i>E. cloacae</i> complex	+	+	SHV 2385 + 240K
<i>E. cloacae</i> complex	+	+	SHV 2385 + 240K
<i>S. marcescens</i>	+	+	SHV 2385 + 240K
<i>E. cloacae</i> complex	+	+	SHV 2385
<i>E. cloacae</i> complex	+	+	SHV 240K
<i>C. braakii</i>	+	+	TEM 104K
<i>C. freundii</i>	+	+	TEM 104K

**Conclusion:** The estimated prevalence of ESBL in chromosomal AmpC producers is 3,5%. The specificity of the BD Phoenix for ESBL detection in chromosomal AmpC producers is low (<85%). ESBL confirmation is necessary to prevent inappropriate infection control measures. The confirmation method needs to be easy and cheap to implement in the routine clinical laboratory as many isolates need to be tested. Both phenotypic tests performed equally but three isolates with microarray confirmed ESBL were only detected by 1 test. These three isolates as well as the seven isolates with a negative microarray result will be characterized by PCR and sequencing.

**P1727 Phenotypic ESBL detection in Enterobacteriaceae producing chromosomal AmpC beta-lactamases**

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**Objectives:** Phenotypic ESBL confirmation by the combination disk test demonstrating synergy between cefotaxime and/or ceftazidime and clavulanate (CTX-CAZ-CT) is unreliable in Enterobacteriaceae with inducible or derepressed chromosomal AmpC resulting in false negative outcomes. Two phenotypic detection methods to overcome the masking effect of the AmpC were compared.

**Methods:** During a 14 month period, all consecutive non-duplicate clinical isolates of chromosomal AmpC producers identified by the Phoenix as (suspected) ESBL producers were prospectively collected. Additionally, a maximum of 10 (suspected) ESBLs of less frequently encountered species stored prior to the study period were included. The combination disk test with clavulanate and cefepime which is a poor substrate for AmpC (FEP-CT), and the CTX-CAZ-CT with cloxacillin as AmpC inhibitor added to all disks (CLOX-CT) were performed on all isolates. For molecular confirmation, the Check MDR CT 102 microarray was performed on isolates with at least 1 positive phenotypic ESBL test.

**Results:** In the study period, 190 of 796 non-duplicate isolates producing chromosomal AmpC were identified as (suspected) ESBL by the Phoenix. Of these, 30 were not stored and therefore not available for testing. Of the 160 study isolates (*Enterobacter* spp. [n = 87], *Citrobacter* spp. [n = 59], *M. morgani* [n = 6], *H. alvei* [n = 1] and *Serratia* spp. [n = 7]), 28 were positive in one or both ESBL confirmation tests (18%). Of the additional five *E. aerogenes*, 10 *S. marcescens* and four *C. braakii*, eight tested positive. Three isolates with genetically confirmed ESBL were not detected by both tests: two isolates tested positive only in the CLOX-CT and one only in the FEP-CT. On the other hand, seven isolates with a positive phenotypic test were not confirmed by the microarray. Species identification by Phoenix and MALDI-TOF were identical.

**P1728 Rapid identification and confirmation of extended-spectrum beta-lactamase utilising Brilliance ESBL agar and Sensititre ESBL antimicrobial susceptibility panels**

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**Objectives:** Rapid identification and confirmation of extended-spectrum beta-lactamase (ESBLs) in the clinical setting is imperative in improving patient outcomes and preventing spread. This study was undertaken to determine if Sensititre® ESBL antimicrobial susceptibility panels could be used directly with Brilliance™ ESBL agar for the rapid identification, confirmation and MIC determination of suspect organisms.

**Methods:** A collection of 65 clinical isolates (31 *Klebsiella pneumoniae*, 21 *Escherichia coli* and 13 Enterobacteriaceae) including ESBL and non-ESBL producing strains, along with challenging organisms containing other resistance mechanisms known to give false positives on ESBL screening media, were inoculated onto Brilliance ESBL agar in accordance with the manufacturer's instructions and onto Columbia Blood Agar (CBA) plates as a control. Plates were incubated at 35–37°C for 24 hours; negative Brilliance ESBL agar plates were incubated for an additional 24 hours. The Sensititre ESBL panel inocula was prepared from colonies grown on both the Brilliance ESBL agar and the CBA plates. QC testing was performed daily following the manufacturer's instructions and CLSI M100.

**Results:** The Sensititre panel correctly differentiated the ESBL positive isolates from the organisms containing other resistance mechanisms that were presumptive positive on the Brilliance ESBL agar. The MIC was unaffected by inoculation directly from the selective Brilliance ESBL



agar compared to the non selective Columbia blood agar producing a 97% essential agreement.

**Conclusions:** The Sensititre panel can be used as a direct confirmatory test for suspect organisms isolated on the Brilliance ESBL agar. The prior use of the Brilliance ESBL media does not influence the MIC result. The combination of Brilliance ESBL agar with the Sensititre ESBL panel offers a rapid, simple solution for identification, confirmation and susceptibility of ESBL producing organisms.

**P1729 Evaluation of a new chromogenic test (betaLACTA™ test) for rapid detection of third-generation cephalosporins nonsusceptible Enterobacteriaceae**

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**Objectives:** A new chromogenic test (betaLACTA™; Bio-Rad) was developed for rapid detection (2–15 minute) of third-generation cephalosporins nonsusceptible Enterobacteriaceae (3GCns-E). Performances were determined through retrospective and prospective studies.

**Methods:** Retrospective analysis was performed on 72 3GCns-E producing well-characterized beta-lactamases (ESBL, n = 36; AmpC, n = 10; carbapenemases, n = 12; and multiple beta-lactamases, n = 14). Prospective study (3 months period) was performed on primary cultures isolated from various clinical samples inoculated on different agar media. BetaLACTA™ results were compared to disc diffusion method (ceftazidime and cefotaxime, 3GC) according to CA-SFM 2009 guidelines, then 3GCns-E were characterized by molecular techniques. BetaLACTA™ was performed by suspending 1–3 colonies in reagents and waiting for 2–15 minute to interpret results as follow: no color change: negative; shift to red or purple: positive (any other color change was considered as non-interpretable).

**Results:** Retrospective study: Of the 72 3GCns-E, 66 were positive, four were negative (two CMY-2, one DHA-1, one TEM-29) and two were non-interpretable (one CMY-13, one OXA-48).

Prospective study: 571 isolates (80% from urines) including strains of *Escherichia coli* (n = 405), *Klebsiella pneumoniae* (n = 75), *Enterobacter* spp. (n = 29) and other Enterobacteriaceae (n = 62) were tested. 6/571 (1%) were non-interpretable with the test, other isolates gave the following results (see Table 1):

Of the 77 3GCns-E, 64 produced an ESBL (83% belonged to CTX-M family), 13 produced an AmpC (nine chromosomally derepressed and six plasmid-mediated). BetaLACTA™ detected 100% of ESBL strains. The five false negatives were AmpC-producing strains and the two false positives were *K. oxytoca* hyperproducing chromosomal K1 beta-lactamase. In comparison with disc diffusion method, the test showed a sensitivity of 93.5%, a specificity of 99.6%, a positive predictive value of 97.3% and a negative predictive value of 98.9%.

betaLACTA™	Disc diffusion method		
	3GC susceptible	3GC nonsusceptible	Total
Negative	486	5	491
Positive	2	72	74
Total	488	77	565

**Conclusion:** The betaLACTA™ test is a rapid and reliable test for the detection of third-generation cephalosporins nonsusceptible Enterobacteriaceae. With excellent specificity and negative predictive value, we conclude that it should be used for therapeutic guidance and should help to monitor beta-lactamase resistance in clinical settings.

**P1730 Evaluation of chromID ESBL and CCDA media for detection of extended-spectrum beta-lactamase (ESBL) producing Enterobacteriaceae in stool samples**

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**Background and objectives:** ESBL are plasmid-mediated enzymes, which are reported in increasing numbers worldwide. The aim of this study was to evaluate two selective media for cephalosporin resistance (CCDA and chromID ESBL) with and without selective enrichment broth with cefpodoxime in stool samples.

**Methods:** Setting: The study was performed at Aarhus University hospital (Denmark) from November 2010 to December 2010. Stool samples submitted to the Department of Clinical Microbiology were included in the study.

Inoculation of media and incubation: Stool samples were plated both with and without prior overnight enrichment in selective enrichment broth (SSI) with 10 µg cefpodoxime, onto, chromID ESBL (bio-Mérieux) and CCDA (SSI, a cefoperazone-containing media for the isolation of *Campylobacter* species). The chromID ESBL plates were incubated aerobically at 36°C and the CCDA plates were incubated microaerophilic at 36°C. Both plates were examined after 18–24 hour of incubation.

Identification of ESBL producers: The plates were read according to the color chart provided by the manufacturer. All isolates of Enterobacteriaceae growing on CCDA and other than *E. coli* on chromID ESBL were identified by MALDI-TOF MS (Bruker Daltonics). Confirmation of ESBL producers was performed by the AMPC + ESBL detection set (MAST group).

**Results:** From 506 patients, a total of 612 stool samples were obtained, of which 290 (47.3%) samples were obtained in a hospital setting. Of the 62 detected ESBL-producers, 93.5% were *E. coli* and 6.5% were *K. pneumoniae*. Plating on chromID ESBL was significantly more efficient than the CCDA media for detecting ESBL (see Table 1). The enrichment step increased the detection rate by 12.9% using the CCDA media and by 3.2% using chromID ESBL.

	CCDA		chromID ESBL	
	Without enrichment	With enrichment	Without enrichment	With enrichment
Sensitivity (%)	80,8	93,5	96,8	100
Specificity (%)	47,6	64,8	82,1	86,2
PPV (%)	14,4	25,7	49,6	49,6
NPV (%)	99,2	92,7	92,3	97,8

**Conclusion:** ChromID ESBL is a significantly better culture medium for detection of ESBL-producing Enterobacteriaceae in stool samples than CCDA. The enrichment step only increased detection rate, using chromID ESBL media, with 3.2% but postponed the result with another 24 hour. Therefore, we recommend the use of chromID ESBL without prior enrichment for detection of ESBL in stool samples.

**P1731 A multi-center evaluation of three selective screening agars for the detection of extended-spectrum beta-lactamases**

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**Introduction:** Enterobacteriaceae producing Extended-Spectrum beta-Lactamases (ESBLs) are an emerging problem. This sustains the need for rapid and accurate screening methods, directly on stool samples or rectal swabs.

**Materials and methods:** Over a 4-month period (November 2010–February 2011), admission screening was performed at two Belgian hospitals: AZ St Lucas Brugge and AZ Gezondheidszorg Oostkust

Knokke-Heist. All patients admitted at the intensive care unit or at the geriatric ward were screened using a rectal swab (Eswab<sup>®</sup>, Copan). One hundred microlitre of the transport medium was inoculated onto three commercial screening agars: ChromID<sup>™</sup> (BioMérieux), Brilliance<sup>™</sup> agar (Oxoid) and BLSE agar (Chemunex). All agars were overnight incubated. If there was growth on at least 1 agar, identification and antimicrobial susceptibility testing was performed on the Microscan WalkAway<sup>®</sup> plus system (Microscan) (Siemens). Further confirmation of ESBL was performed using the double disk method and the ESBL plus<sup>®</sup> panel on the Microscan.

**Results:** In total 504 samples were collected: 310 samples yielded no growth and 194 samples yielded growth on at least 1 agar. Overall 66 ESBL producing strains were found. One strain was missed with the ChromID<sup>™</sup> agar and eight strains were missed using the Brilliance<sup>™</sup> agar. The study with the BLSE agar was prematurely stopped due to low sensitivity and specificity. Results are shown in Table 1. The prevalence of ESBL's was 14.7% at the intensive care unit in Brugge and was respectively 9.6% and 16.4% at the intensive care unit and at the geriatric ward in Knokke. The sensitivity of the screening agars was respectively: 98.5% (ChromID<sup>™</sup>), 88.0% (Brilliance<sup>™</sup> agar) and 83.7% (BLSE agar). The specificity was equal for ChromID<sup>™</sup> and Brilliance<sup>™</sup> agar: 87% and was only 70% for the BLSE agar. The ESBL plus<sup>®</sup> panel contains a higher range of dilutions and is therefore much more sensitive to detect ESBL's compared to the conventional gram negative panel on the Microscan.

Table 1

	BM	OX	AES
Total number of samples	n=504	n=504	n=425*
Growth	n=120	n=114	n=153
ESBL positive samples	n= 65	n=58	n=41
<i>E. coli</i>	54	50	36
<i>E. aerogenes</i>	4	3	1
<i>K. oxytoca</i>	3	2	1
<i>K. pneumoniae</i>	3	3	3
<i>P. vulgaris</i>	1	-	-

\* The study with the BLSE agar was prematurely stopped due to low sensitivity and specificity.

**Conclusion:** There are no clear guidelines in literature about the size of inoculum for these screening agars. These agars are easy to use and give a first result after 24 hour incubation. The ChromID<sup>™</sup> and the Brilliance<sup>™</sup> agar show similar performance in specificity, whereas ChromID<sup>™</sup> shows slightly higher sensitivity compared to Brilliance<sup>™</sup> agar. Further confirmation of ESBL remains necessary with both agars.

#### P1732 Multicentre evaluation of the AST-piperacillin/tazobactam card for use on the Vitek 2 and Vitek 2 compact systems (bioMérieux Inc.)

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**Objectives:** BioMérieux, Inc. has developed an antimicrobial susceptibility test (AST) card with piperacillin-tazobactam (TZP) for use with the Vitek2 (V2) and Vitek2 Compact (V2C) systems. The AST-TZP card was evaluated for FDA submission by three sites in the USA including the Cleveland Clinic, CMI Inc., and Wishard Health Services. The purpose was to demonstrate the safety and effectiveness of the AST-TZP card.

**Methods:** One thousand four hundred and fifty-six (72.1% fresh and 27.9% frozen stock) clinical isolates were tested with the AST-TZP card using the V2 automatic dilution (auto) mode and CLSI broth microdilution as the reference method. Seven isolates did not grow in the card (0.5%). Ninety-five challenge isolates were tested with the V2

auto and manual modes, V2C manual mode, and the reference method. The card results from one site were compared to consensus reference results from all three sites. Ten reproducibility isolates were tested in triplicate on each of 3 days using the V2 auto and manual modes and V2C. Three Quality Control strains were tested a minimum of 20 times by all sites in the same manner as the challenge isolates. Data was analyzed using CLSI and EUCAST breakpoints as appropriate.

**Results:** Clinical isolates: Using CLSI breakpoints, the overall essential agreement (EA) was 94.8% (1373/1449) and the overall category agreement (CA) was 94.3% (1367/1449) with 0 very major errors. Using EUCAST breakpoints, the EA for Enterobacteriaceae was 96.6% (1231/1274) and the CA was 95.3% (1214/1274) with 1 very major error. For *Ps. aeruginosa*, the EA was 92.9% (156/168) and the CA was 93.5% (157/168) with 1 very major error. Challenge isolates: For V2 auto, V2 manual, and V2C, using CLSI breakpoints, the EA was  $\geq 92.6\%$  and the CA was  $\geq 93.7\%$ ; using EUCAST breakpoints, the EA was  $\geq 92.1\%$  and the CA was  $\geq 89.5\%$ . Reproducibility: Best case calculation assuming the off-scale result is within one well of the mode was  $\geq 95.9\%$ . Quality Control: Results were within the expected results range  $>95\%$  of the time.

**Conclusion:** Piperacillin-tazobactam results were obtained for 1544 clinical and challenge Gram-negative bacilli isolates with a newly formulated Vitek2 card (AST-TZP, pending FDA clearance). Good essential and categorical agreement was obtained as compared to a standard broth microdilution method using CLSI or EUCAST breakpoints. There were no very major errors using CLSI breakpoints and only 2 very major errors obtained using EUCAST breakpoints.

#### P1733 Evaluation of a new multiplex PCR assay system for rapid detection of multidrug-resistant gram-negative bacteria

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**Objectives:** Multidrug-resistant gram-negative bacteria (MDR) became an emerging problem throughout Europe within the last years. To prevent transmission of these bacteria especially in the hospital setting and to start earlier with an appropriate antibiotic therapy new and rapid molecular-based diagnostic tools are needed. Here we extended our previous preliminary study and have evaluated a newly developed multiplex PCR assay system to address this issue.

**Methods:** The complete assay allows differentiation of 17 pathogens based on 23S rRNA sequences and simultaneously the detection of 22 resistance markers. For detection of MDR the multiplex PCR targets three classical Ambler class A beta-lactamases (tem, shv, ctx-M) and two families of plasmid encoded ampC genes (Ambler class C). Moreover, an integrase gene as surrogate marker for multidrug resistance was included. Evaluation was performed on 239 clinical isolates (196 Enterobacteriaceae, 43 non-fermenter). One hundred and ninety-four of these isolates exhibited multidrug-resistance based on phenotypic methods. MDR was defined as resistance to at least three antibiotic classes or an ESBL phenotype.

**Results:** Ninety-nine percent of the pathogens were identified correctly and no false positive results of non-MDR phenotypes were observed. In 89% of the Enterobacteriaceae and 87% of the non-fermenter included in this study the MDR phenotype was confirmed. As expected, detection of genes coding for beta-lactamases in Enterobacteriaceae as well as occurrence of more than one gene coding for the same phenotype was very common and species specific. For example in 82% and 91% of the *Klebsiella pneumoniae* isolates ctx-M and shv could be detected, respectively. Whereas *Escherichia coli* isolates prefer ctx-M and tem (76% and 57%, respectively). Interestingly, detection of the MDR surrogate marker exhibited strong correlation with an ESBL phenotype in Enterobacteriaceae as well as multidrug-resistance in *Pseudomonas aeruginosa*. Furthermore, in *Acinetobacter baumannii* isolates included in this study it was suitable to predict resistance to aminoglycosides, whereas the correlation in Enterobacteriaceae was poor.

**Conclusion:** Especially, the new approach to use the integrase gene as surrogate marker for multidrug resistance was shown to be a valuable

target. The new multiplex PCR assay system was proven to be suitable to detect >80% of clinical relevant MDR and therefore should help the clinician to select an appropriate antibiotic therapy in time.

## News on fluoroquinolone resistance

### **P1734** Complete sequence of pJIE203, a plasmid from *Klebsiella pneumoniae* carrying blaDHA-1 and qnrB4

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**Objectives:** blaDHA-1 is one of the dominant plasmid-borne ampC genes worldwide, but only one plasmid carrying the entire blaDHA-1 gene, pKP048 from China, had been completely sequenced. Here we sequenced and assembled pJIE203, a plasmid carrying blaDHA-1 and qnrB4 from a clinical *Klebsiella pneumoniae* isolate from Sydney, Australia.

**Methods:** *Klebsiella pneumoniae* JIE203, isolated at Westmead Hospital, Sydney, Australia, in 2006, had a cefoxitin MIC of >16. A plasmid carrying blaDHA-1 that gave no amplicons by standard PCR-based replicon typing was obtained by filter mating with *Escherichia coli*. DNA extracted from a transconjugant was amplified using Genomiphi (GE Healthcare), quantified, sequenced (GS-FLX; Roche 454) and assembled (Newbler, Roche). Contigs were linked by PCR and additional sequencing. Genes were annotated with RAST (<http://rast.nmpdr.org/rast.cgi>), translated proteins were analysed using BLASTp (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) and insertion sequences were identified with IS Finder (<http://www-is.biotoul.fr/is.html>).

**Results:** Newbler assembly gave 15 contigs (0.2–21 kb, 13-585x coverage). Searches of GenBank identified related plasmid sequences and suggested that three contigs included fragments of the plasmid backbone. Other contigs corresponded to long repeats and intervening segments making up the resistance region. These findings were used to direct PCR to enable assembly of a complete 136 790 kb plasmid. The blaDHA-1, qnrB4, aac(3)-IIId, tetA(A), strAB, sul1 and cata2 resistance genes, a class 1 integron with the [dfrA12|gcuF|aadA2] cassette array, three copies of IS26 and other mobile elements are clustered in a 47 kb region that shares some components with pKP048. The pJIE203 backbone is related to those of other resistance plasmids from *K. pneumoniae*, including pKP048, but neither of the two replicon regions present corresponds to a standard IncFIIK-type replicon. pJIE203 carries a complete conjugation region plus genes associated with plasmid maintenance and stability, including toxin-antitoxin systems.

**Conclusions:** Many components of the pJIE203 resistance region are found in pKP048 or multiresistance regions on other plasmids. pJIE203 also shares some backbone regions with pKP048, but other regions are more closely related to different plasmids from *K. pneumoniae*. This illustrates the mosaicism of both multiresistance regions and the backbones of certain plasmid types.

### **P1735** Characterisation of qnrA6 genetic environment in *Proteus mirabilis* PS16 confirms mobilisation from Shewanellaceae

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**Objectives :** The aim of this study was to determine genetic location and environment of qnrA6 in *Proteus mirabilis* PS16, the first published strain carrying this gene, and to determine quinolone susceptibility conferred by this gene.

**Methods :** Transfer experiments and Southern-blotting on plasmid and pulse-field gel electrophoresis of genomic DNA of *P. mirabilis* PS16 were performed to determine qnrA6 location. Combinatorial PCR with qnrA6 primers and specific primers for genes usually surrounding qnrA were used for determining the genetic environment. The qnrA6 encoding region was cloned into the constitutively expressed tetA gene of vector pBR322 and in pDrive cloning vector. The ligations products were electroporated into competent *E. coli* TOP10 and *E. coli* DH10B.

**Results :** The qnrA6 quinolone resistance determinant was found as chromosomally encoded in *P. mirabilis* PS16 and its genetic environment was very similar to that of qnrA2 in *Shewanella* algae (Genbank HQ449669.1). We described a 5138-bp region up- and downstream from qnrA6 that contains an IS10 element disrupting the putative qnrA6 promoter. This 5.1-kb region was surrounded by two ISCR1 recombinase. Cloning of qnrA6 in pBR322 and pDrive showed that the gene did not confer any raise in MIC to quinolones contrary to the others qnr genes.

**Conclusions:** This work describes the first chromosomally located qnrA in Enterobacteriaceae. It also confirms that water-borne Shewanellaceae is a reservoir for Qnr-like quinolone determinant. qnrA6 environment was probably excised from chromosomal DNA of *S. algae* or similar organisms and was integrated in *P. mirabilis* PS16. This mobilization of resistance genetic environment probably occurred through the ISCR1 recombinase as it was described for blaCTXM-2 in *Kluyvera ascorbata*. Furthermore, the fact that this allele doesn't confer any resistance might explain the rarity in the literature of the reported clinical isolates carrying qnrA6.

### **P1736** High prevalence of plasmid-mediated quinolone resistance determinants in Enterobacteriaceae producing plasmid-AmpC-type-beta-lactamases, metallo-beta-lactamases or both isolated in 34 Spanish hospitals

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**Objective:** To investigate the occurrence of plasmid-mediated quinolone resistance determinants (PMQR) in clinical isolates of Enterobacteriaceae producing plasmid-AmpC-type-beta-lactamases (pAmpC), metallo-beta-lactamases (MBL) or both isolated in 34 Spanish hospitals.

**Methods:** Two hundred eighty-three genetically unrelated clinical isolates of Enterobacteriaceae producing pAmpC (n = 257; CMY-type: 73%, DHA-type: 23%, ACC-1: 3%, FOX-type: 1%), MBL (n = 23; VIM-1: 91%, IMP-type: 9%), or both types of enzymes (n = 3; VIM-1 + DHA-type: 75%; VIM-1 + CMY-type: 25%), collected between February and June 2009 in 34 different Spanish hospitals, were included in this study. Genetic relatedness was analyzed by PFGE. Susceptibility to ciprofloxacin (CIP) was studied using the MicroScan WalkAway system applying EUCAST interpretative criteria. The presence of PMQR qnrA, qnrB, qnrC, qnrD, qepA and aac(6')Ib-cr (AAC) was studied by PCR and sequencing.

**Results:** Seventy-eight percent of the isolates were non susceptible to CIP. PMQR were present in 78 of the 257 pAmpC producers (30%), of which 55, 16, 9, 6 and 3 carried qnrB4, AAC, qnrD, qnrS1 and qnrS2, respectively. qnrC and qepA were absent. Among MBL producers, PMQR were detected in 10 of the 21 VIM-1 producers (48%), of which 6, 4, 2, 1 and 1 carried qnrS2, AAC, qnrA1, qnrS1 and qnrB19, respectively. PMQR were also present in the three isolates producing both types of enzymes, of which 2, 1 and 1 carried qnrB4, AAC and qnrD, respectively. PMQR carriage rate was significantly higher among isolates producing DHA-type-pAmpC (91%) than in CMY-type-pAmpC producers (11%) (p < 0.001). Fifty-six out of the 57 qnrB4 detected (98%) were present in DHA-type producers [*Klebsiella pneumoniae* (n = 24), *Escherichia coli* (n = 21), *Proteus mirabilis* (n = 3), *K. oxytoca* (n = 3), *Enterobacter cloacae* (n = 2), *Citrobacter koseri* (n = 2), and *Salmonella enterica* (n = 1)]. All the qnrD were detected in *P. mirabilis* producing CMY-2. Eleven pAmpC-producing isolates (4%) harboured two PMQR, nine of them were DHA-1 producers. The most frequent combination found was qnrB4 + AAC (46%), followed by qnrB4 + qnrS1 (27%), qnrS2 + AAC (18%), and qnrB4 + qnrS2 (9%). Among VIM-1-producers, four (19%) harboured two PMQR, qnrS2 + AAC. No significant differences in terms of non-susceptibility to CIP were observed between isolates PMQR-negative and those harbouring one or two PMQR.

**Conclusion:** A high prevalence of PMQR among clinical isolates of Enterobacteriaceae producing DHA-type-pAmpC and those producing VIM-1 was observed. A correlation between qnrB4 and DHA-type-production was also observed as well as between qnrD and *P. mirabilis* producing CMY-2.

**P1737** **Beta-lactams and trimethoprim induce the expression of qnrB and smaqr genes by SOS depending regulation**

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**Objectives:** Direct SOS-depending regulation, mediated by LexA, of qnrB genes by fluoroquinolones (FQ) has been recently reported. smaqr gene, from the chromosomal of *Serratia marcescens*, contains a putative LexA box. The aim of this study was to evaluate whether smaqr gene is induced via a SOS depending mechanism, and to investigate whether others antimicrobial agents induce the expression of qnrB and smaqr leading to low-level of FQ resistance.

**Methods:** *Serratia marcescens* 257 reference strain (Institute Pasteur) and *Escherichia coli* J53 carrying a natural plasmid that harboured qnrB1 were used for RT-PCR assays. Expression of smaqr and qnrB genes were studied by real time RT-PCR and quantified compared to rpoB gene for *S. marcescens* and mdh gene for *E. coli*. Cefazidime (CAZ) at MIC concentration was tested as possible inducer. Strains were grown to exponential phase at 37°C and the inducer was added during 45 minute, leaving one culture as control. Additionally, promoter regions of qnrB and smaqr were cloned in pMS201, fused to GFP protein and used for gene report assays. Three *E. coli* wild-type strains (MG1655, ATCC25922 and DR1) and *E. coli* HB101 (recA deficient and isogenic with DR1) were used for these assays. Controls using the promoter region of recA and the empty vector were included. Disk diffusion and E-test were used to evidence the induction with ciprofloxacin (CIP), betalactams, trimethoprim (TMP), imipenem (IP) and colistin (CS). Mytomycin C was used as positive control for induction.

**Results:** RT-PCR assays showed that both qnrB and smaqr were induced at MIC of CAZ increasing transcription 2- and 3.5-fold compared to the basal expression, in *E. coli* and *S. marcescens*, respectively. Gene report assays showed that qnrB and smaqr genes were induced by CIP, as expected, but also by (CAZ), cefepime (FEP), ampicillin or TMP in the *E. coli* wild-type strains, but not in the recA-deficient *E. coli* HB101. Induction was not evident for IP or CS in any case. Fluorescent quantification showed that CIP, CAZ and FEP increased the level of expression 55%, 65% and 74% for qnrB and 44%, 60% and 68% for smaqr, compared to recA expression.

**Conclusions:** Beta-lactams and trimethoprim induce the transcription of qnrB and smaqr genes by a SOS depending regulation. These results show a direct SOS-dependent regulation of a low-level FQ-resistance mechanism in response to others antimicrobials. Its consequences in terms of cross resistance are currently unknown.

**P1738** **Identification of the new variant QepA3, a plasmid-mediated quinolone resistance determinant, collected in a CMY-2-producing *Escherichia coli***

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**Objectives:** The efflux pump QepA confers decreased susceptibility to hydrophilic fluoroquinolones (e.g., norfloxacin, ciprofloxacin, and enrofloxacin). In this study, we characterized the third variant, named qepA3, collected from an *Escherichia coli* isolate in Portugal.

**Methods:** INSRA6015 was isolated in 2005 from the urine of a 77-year-old female patient hospitalized at the Hospital Fernando Fonseca, Portugal. Susceptibility testing was performed by disk diffusion and MIC methods, (SFM and EUCAST guidelines, respectively). PCR and sequencing were used to screen and identify bla (blaTEM, blaSHV, blaOXA, blaCTX-M and plasmid-mediated ampC) genes, as well as

plasmid-mediated quinolone resistance (qnrA, qnrB, qnrC, qnrD, qnrS, qepA and aac(6')Ib-cr), and the quinolone resistance-determining regions (QRDR: gyrA, gyrB, parC, and parE) genes. PCR-mapping was used to characterize the genetic environment of the new qepA3 gene. Transfer of resistance of the QepA3 determinant, was performed through electroporation, using the *E. coli* TOP10 as recipient. Plasmid content was characterized by PCR-based replicon typing.

**Results:** Molecular characterization of INSRA6015 showed the presence of blaTEM-1, blaCMY-2 and a new variant of qepA possessing two nucleotide substitutions, leading to Phe85Leu and Val134Ile changes. This variant, named QepA3, conferred a similar phenotype to that of the QepA1 and QepA2 determinants. Sequencing of the QDRD detected substitutions Ser83Leu and Asp87Asn in the GyrA subunit and Glu84Lys in the ParC subunit, which are consistent with the high resistance to ciprofloxacin observed in the MICs. Sequence analysis of qepA3 genetic environment revealed that the gene was located inside a genetic structure identical to that of previously described for qepA1 and qepA2. It is noteworthy that qepA3 gene, as qepA2, was not associated with the rmtB gene encoding an aminoglycoside ribosomal methylase, contrarily to qepA1. PCR-based replicon typing indicated the presence of the IncF plasmid.

**Conclusion:** We have identified and characterized a new variant of the plasmid-mediated efflux pump QepA, which is responsible for the increased levels of resistance to several clinically important quinolones, such as ciprofloxacin, and norfloxacin. This is, at our knowledge, the first description of the co-production of QepA and CMY-2. The study highlights the need of surveillance of this resistance mechanism and reinforces a more careful use of quinolones.

**P1739** **High prevalence of fluoroquinolone efflux pump OqxAB in ESBL-producing *Klebsiella pneumoniae* in Spain**

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**Objectives:** Plasmid-mediated quinolone resistance is an emergent phenomenon, especially in Enterobacteriaceae, and includes mechanisms of target protection (qnr), antimicrobial inactivation (aac(6')Ib-cr) or active efflux systems (qepA and oqxAB). The efflux pump OqxAB-TolC was originally described in *E. coli* and later in *K. pneumoniae*. The aims of this study were (i) to analyze the presence of oqxA and oqxB genes in a collection of ESBL-producing *K. pneumoniae* strains, (ii) to determine its chromosomal and/or plasmidic location and (iii) to analyze their expression levels in relation to susceptibility or resistance to quinolones.

**Methods:** A collection of 114 non-repetitive isolates of ESBL-producing *K. pneumoniae* from a multicenter study in Spain was used. 37.8% of isolates were susceptible to ciprofloxacin (CIP), while 62.2% were resistant or intermediate. Detection of oqxA and oqxB genes was performed by PCR. Chromosomal and/or plasmidic location was performed using plasmid DNA (Kieser technique) and subsequent hybridization. oqxA gene expression was analyzed by real time RT-PCR. Normalized expression levels of the target gene transcripts were calculated in comparison to the expression of rpoB using the 2<sup>-</sup>DDCT method. *K. pneumoniae* ATCC 27799 and *K. pneumoniae* ATCC 700603 were included in the study.

**Results:** Both oqxA and oqxB were detected in *K. pneumoniae* at high prevalence, 77% and 75% respectively. Ten amplicons were sequenced and showed 100% homology with previously described genes showing a high degree of conservation. Hybridization assays showed the simultaneous presence of oqxA (16%) and oqxB (13%) in both chromosome and large size plasmids locations. These plasmids were not transferable by transformation into *E. coli*. RT-PCR assays showed a higher expression (four-folds) in strains with reduced susceptibility to quinolones compared to that of susceptible strains. Interestingly, *K. pneumoniae* ATCC 700603 showed an 18-folds higher expression than *K. pneumoniae* ATCC 27799. These differences were in accordance to the MICs of CIP (0.5 and 0.125 mg/L, respectively).

**Conclusions:** The prevalence of the OqxAB efflux pump (both chromosomal and plasmidic) is high in ESBL-producing *K. pneumoniae* in Spain, representing a potential reservoir for the spread of such genes. High expression of this pump seems to contribute to reduced susceptibility to quinolones in clinical isolates of *K. pneumoniae*.

**P1740** First report of a quinolone resistance mutation in the *gyrA* gene of a clinical *Chlamydia trachomatis* isolate

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**Objectives:** Genital *Chlamydia trachomatis* (CT) infection is the most prevalent STI worldwide. So far, mutational resistance to fluoroquinolones (FQs), which are used, along with macrolides and tetracyclines, for treatment of CT infection, has not been described in clinical CT isolates, although selection of FQ resistance mutations in primary target (GyrA) has been shown in vitro. In Russia, FQs are used widely and available over-the-counter thus potentially exerting significant selective pressure for resistance development in STI pathogens. This study aimed to assess the presence of FQ resistance mutations in quinolone resistance determining regions (QRDRs) of the CT *gyrA* and *parC* genes in clinical samples.

**Methods:** Two sets of urogenital samples (cervical swabs from females and urethral swabs from males) were screened for mutations in the CT *gyrA* and *parC* genes using a newly designed real-time PCR assays. One set comprised 33 samples collected in St. Petersburg in 2006–2008 from 16 patients with posttreatment recurrence of CT infection. The other consisted of 557 consecutive CT positive samples from gynaecological and urological patients collected in Smolensk in 2009–2011 and St. Petersburg in 2010–2011 during routine testing by PCR. The presence of mutations was inferred by postamplification melting temperature ( $T_m$ ) analysis of fluorescent probes complementary to the sequences encoding amino-acids 80–87 of GyrA and 80–85 of ParC (*Escherichia coli* numbering). Samples showing altered probe  $T_m$  as compared to a wild type control (CT L2) were further characterized by sequencing of QRDR containing fragments.

**Results:** Of the 590 CT positive samples, 551 and 543 were positive in the PCR assays targeting the *gyrA* and *parC* genes, respectively. No samples were found to contain mutations in *parC*, but five samples revealed the presence of mutations in *gyrA*. Three of them had only silent substitution at *gyrA* codon for His80. Two samples, both obtained from the same patient in the group of patients with posttreatment CT recurrence, had Ser83-Gly substitution, which is known to be associated with FQ resistance in various Gram-negative bacteria, and two additional mutations, Val61-Ala and His129-Gln, outside the *gyrA* QRDR.

**Conclusion:** The results of our study indicate that, although extremely rare, *gyrA* mutations associated with FQ resistance may occur in clinical CT isolates. Clinical relevance of these findings is to be assessed.

**P1741** Correlation between fluoroquinolone resistance and mutations in the *gyrA/gyrB* genes observed in *M. tuberculosis* clinical isolates collected in France from 2004 to 2010

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As a consequence of the use of fluoroquinolones (FQ) to treat multidrug-resistant tuberculosis (MDR-TB; i. e. resistant to isoniazid and rifampin), resistance to FQ has emerged, leading to nearly untreatable extensively drug resistant TB (XDR-TB; i.e. MDR strains resistant to FQ and amikacin or kanamycin or capreomycin). Mutations in DNA gyrase (GyrA2GyrB2), the sole target of FQ in *M. tuberculosis*, represents the main mechanism of FQ resistance in this species. A full

understanding of the pattern of mutations found in FQ resistant clinical strains, and of their proportions, is crucial to improve the molecular methods intended to detect FQ resistance in *M. tuberculosis*.

**Objective:** To evaluate ofloxacin resistance in *M. tuberculosis* strains of clinical origin, in parallel with the patterns of associations of mutations in the *gyrA/gyrB* genes.

**Methods:** The *gyrA* and *gyrB* sequences performed routinely on suspected MDR clinical isolates received from 2004 to 2010 at the French NRC laboratory were compared to the susceptibility profiles of the corresponding bacteria to FQs.

**Results:** Eight hundred and twenty-two *gyrA* and 524 *gyrB* sequences were determined for 822 strains, of which 37% were MDR and 1.8% were XDR (n = 15). Among the 67 strains carrying mutation in *gyrA* and the 23 strains carrying a mutation in *gyrB*, for which susceptibility to FQ was available, 28 were susceptible (mainly harbouring silent polymorphisms) and 45 were resistant to FQ. More than 70% of the strains displaying resistance to FQ harboured a GyrA substitution at position 90 and 94, while 15% had a single mutation in *gyrB*. The presence of a *gyrA/B* mutation known from biochemical studies to be involved in FQ resistance correlated well with resistance to FQ, a mutation in *gyrB* being significantly associated with a low level of resistance.

**Conclusions:** These data highlight that the sequencing of both *gyrA* and *gyrB* is required to investigate FQ susceptibility in *M. tuberculosis*. Moreover, the molecular approach is complementary to but not yet able to substitute for the standard susceptibility testing carried out on solid media. Finally, a possible efficacy of moxifloxacin in the treatment of the patients infected with strains harbouring *gyrB* mutations conferring low-level of resistance is still a matter of debate.

**P1742** Detection of fluoroquinolones resistance and efflux pumps activity by flow cytometry

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**Objectives:** Fluoroquinolones are bactericidal drugs which have been widely used due to its high activity against Gram-negative bacteria like Enterobacteriaceae. Multidrug resistance is an increasing health concern worldwide. Increased efflux pumps expression has been documented in association with resistance to fluoroquinolones. An assay based upon flow cytometry was developed to quickly detect resistance to fluoroquinolones and, in that case, whether it results from active efflux pumps.

**Methods:** Clinical isolates of Enterobacteriaceae resistant (n = 35) and susceptible (n = 25) to ciprofloxacin and levofloxacin previously evaluated by VITEK 2 and Etest (BioMérieux, Paris) were selected. Genetically modified *E. coli* AG100A with all the efflux pumps systems inactivated and *E. coli* AG100TET with overexpressed efflux pumps (a kind gift by Dr Miguel Viveiros) were used as controls. Bacterial cells were grown in Luria Bertani (LB) broth, and then sub-cultured in LB supplemented with serial concentrations of ciprofloxacin or levofloxacin (Sigma-Aldrich) for 1 hour at 37°C. For susceptibility profile, 1 mL aliquots were pelleted, washed twice with PBS and stained with Syber-Green I (SB) before flow cytometric analysis. For efflux pumps activity studies subinhibitory concentrations of the drugs were diluted in PBS supplemented with glucose and stained with Ethidium Bromide (Sigma-Aldrich) (EB) at 1 µg/mL, a concentration described to be an excellent probe for efflux. In parallel the same strains were incubated with 20 µg/mL of Chlorpromazine (Sigma-Aldrich), a pump inhibitor and the EB protocol repeated. Flow cytometric analysis (FL2 –585 nm) was performed and the intensity of fluorescence was registered.

**Results:** Susceptible strains showed a decrease in the intensity of fluorescence compared to control (without drug); conversely, resistant strains did not show that decrease even after incubation with high concentrations of the drugs. Resistant strains, including the control strain with efflux overexpression, exhibited a low intensity of fluorescence when stained with EB which increased in the presence of chlorpromazine. On the other hand, susceptible strains, as well as

the control strain without efflux pumps, exhibited higher intensity of fluorescence after EB staining.

**Conclusions:** Flow cytometry demonstrated to be an excellent approach to evaluate the resistance to fluoroquinolones and the responsibility of efflux pumps on such resistance.

#### **P1743** The presence of virulence factors in quinolone-resistant uropathogenic *Escherichia coli* in outpatients with UTI

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**Objectives:** Urinary tract infections are among the most common human infections and *Escherichia coli* is their main cause. Uncontrolled use of quinolones leads to resistance to this group of antibiotic.

Aim of this study was to get an insight of characteristics of quinolone-resistant *E. coli*, taking into consideration presence of virulence factors (hemolysin, P fimbriae, type 1 fimbriae, cytotoxic necrotizing factor 1), as well as to determine difference in characteristics of strains that are quinolone-susceptible.

**Methods:** During 1 year period all quinolone-resistant *E. coli* strains (n = 87) were collected from urine samples obtained from outpatients with symptoms of urinary tract infections in the Split and Dalmatia County, Croatia. Control group (n = 87) was formed by taking the next isolated quinolone-susceptible strain of *E. coli* for each quinolone-resistant strain. Virulence factors are determined by the molecular or phenotypic methods. Production of hemolysin was determined by phenotypic method, by observing the presence of hemolysis near the colony of the tested strain on blood agar. The presence of P fimbriae, type 1 fimbriae, and cytotoxic necrotizing factor 1 was proven by polymerase chain reaction, determining the presence of genes pap, fim and cnf1. The statistical significance of the difference between quinolone-resistant *E. coli* strains and quinolone-susceptible strain of *E. coli* was determined by Chi-square test.

**Results:** Virulence factors were found statistically significantly less frequently (all p < 0.002) in quinolone-resistant strains (fim gene in 21.84%, pap gene in 20.69%, cnf1 gene in 1.15% and hemolysis in 2.30% strains) than in quinolone-sensitive strains (fim gene in 90.80%, pap gene in 50.57%, cnf1 gene in 12.64% and hemolysis in 52.87% strains).

**Conclusions:** There are numerous clinical observations that quinolone – resistant strains of *E. coli* are less virulent and more frequently cause cystitis and asymptomatic bacteriuria than invasive urinary tract infections like pyelonephritis and febrile urinary tract infections. Studies in the available literature that differentiate the presence of virulence factors in strains resistant to quinolones and their presence in sensitive strains are rare.

Results of this study confirm that the virulence factors are significantly less present in quinolone – resistant strains than in quinolone – sensitive strains.

#### **P1744** Dynamics of the emergence of *Escherichia coli* resistance to fluoroquinolones in the faecal flora from healthy volunteers

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**Background:** *Escherichia coli* resistance to fluoroquinolones (Fq) is an increasing clinical problem. Selection of quinolone-resistance (Q-R) in *E. coli* primarily emerges in commensal bacteria under selective pressure. Whether resistance is due to the selection of resistant strains already present in the microbiota, or acquired exogenously during therapy is unknown. We describe the dynamics of Q-R *E. coli* in the stools of healthy volunteers treated by ciprofloxacin (Cipro).

**Methods:** Forty-eight healthy volunteers received different dosing regimens of Cipro for 14 days. Stools were collected on days (D) 0, 8, 14 and 42. Susceptibility to Fq was determined in the dominant *E. coli* population. Q-R subpopulations were detected by plating faecal

samples on Drigalski agar containing 16 mg/L nalidixic acid (Nal) or 1 mg/L Cipro. Resistant strains were characterized phenotypically and genetically. Profiles of Q-R *E. coli* strains were compared to *E. coli* strains collected on different days by RAPD-PCR and pulse-field gel electrophoresis.

**Results:** Thirty-three subjects had no Q-R strains detected at any time. For 15 subjects (31%), Q-R *E. coli* were evidenced at least once:

**1** nine subjects with no Q-R at D0 had low (n = 8) or high-level (n = 1) resistant *E. coli* strains detected at D42. In all cases, Q-R strains phenotypically and genetically differed from the susceptible *E. coli* of the dominant flora on D0.

**2** four subjects had low-level resistant *E. coli* strains (single gyrA mutation) detected on D0: one cleared the resistant strains; one acquired Cipro-resistant strains (gyrA + parC mutations) on D42, genetically different from D0 strains; and two had the same strains detected again after the end of treatment at D42.

**3** two subjects had Cipro-R *E. coli* (gyrA + parC mutations) on D0 and throughout the study (D8-D42). Genetically identical resistant strains colonized the two subjects from D0 to D42.

No plasmid-mediated Q-R mechanisms were evidenced in any of the strains.

**Conclusions:** Q-R emerges frequently in commensal *E. coli* under Cipro pressure. When Q-R strains are already present at D0, same resistant strains may persist or not after treatment, depending on the level of resistance. When Q-R strains emerged during or after Fq treatment, exogenous acquisition of resistant strains seems to prevail rather than selection from the original dominant flora. Dynamics of the emergence of resistance to Fq in the gut microbiota is a more complex phenomenon than expected.

## External quality assessment of resistance detection

#### **P1745** External quality assessment of culture-based detection of vancomycin-resistant enterococci by a network of European laboratories

M. Gazin\*, C. Lammens, L. Derde, M. Bonten, C. Brun-Buisson, H. Goossens, S. Malhotra-Kumar on behalf of the MOSAR WP2 Study Team

**Objectives:** An external quality assessment (EQA) was carried out to assess the culture-based methods utilized by 10 hospital laboratories in six European countries and their proficiency in detecting vancomycin-resistant Enterococcus (VRE).

**Methods:** The panel included *E. faecium* (n = 2), *E. faecalis* (n = 1) and *E. casseliflavus* (n = 1) harbouring vanA, vanB, vanC2 and vancomycin MICs of 256 (n = 2), 8 (n = 1) and 4 (n = 1) mg/L respectively, and one strain each of *Acinetobacter* spp., vancomycin-sensitive *E. faecium*, and *Lactobacillus* spp. as pure strains or in mixtures at varying concentrations (Table 1). All 10 participants analyzed the panel utilizing their in-house culture protocol (IHP) for VRE detection, and a standardized protocol (SP) implemented as a diagnostic intervention in clinical trials as part of the EU-FP6 project, MOSAR. SP included sample inoculation on a selective medium (Enterococcosel agar, ECCV with 8 µg/mL vancomycin, BD Diagnostics) and VRE confirmation by catalase and L-pyrilodiny-beta-naphthylamide (PYR) tests.

**Results:** Of the 10 participants, eight (80%) could detect up to 10 colony forming units (CFU) of absolute VRE loads using both protocols (Table 1). Overall, vanA-harboring VRE were detected by a larger no. of participants than vanB-harboring VRE. SP and IHP results showed 92.9% concordance (kappa: 0.70, 95% CI: 0.55–0.86). SP showed higher specificity than IHP; two participants reported the vancomycin-sensitive strain as VRE using IHP (blood agar, confirmatory tests: catalase, Phoenix, PYR, and Vitek). However, vanA- and vanB-harboring VRE at 1 CFU loads were also more frequently missed with SP. One participant reported 10 VRE samples (pure strains: 1000-1 CFU, and mixtures) as negative with both SP and

Sample characteristics	Absolute bacterial loads inoculated on media* (CFU)	VRE confirmed by in-house protocol (IHP) N (%)	VRE confirmed by standardized MOSAR protocol (SP) N (%)
<b>Pure strains</b>			
VRE-1 ( <i>E. faecium</i> , vanA)	1000	9 (90%)	9 (90%)
VRE-1 ( <i>E. faecium</i> , vanA)	100	10 (100%)	10 (100%)
VRE-1 ( <i>E. faecium</i> , vanA)	100	9 (90%)	9 (90%)
VRE-1 ( <i>E. faecium</i> , vanA)	10	9 (90%)	9 (90%)
VRE-1 ( <i>E. faecium</i> , vanA)	1	8 (80%)	6 (60%)
VRE-2 ( <i>E. faecium</i> , vanB)	1000	9 (90%)	9 (90%)
VRE-2 ( <i>E. faecium</i> , vanB)	100	8 (80%)	8 (80%)
VRE-2 ( <i>E. faecium</i> , vanB)	100	8 (80%)	8 (80%)
VRE-2 ( <i>E. faecium</i> , vanB)	10	8 (80%)	8 (80%)
VRE-2 ( <i>E. faecium</i> , vanB)	1	8 (80%)	5 (50%)
VRE-3 ( <i>E. faecalis</i> , vanB)	100	9 (90%)	9 (90%)
VRE-4 ( <i>E. casseliflavus</i> , vanC2)	100	5 (50%)	8 (80%)
<b>Mixtures</b>			
VRE-1 + <i>Acinetobacter</i> spp. ( <i>E. faecium</i> , vanA)	10+100	10 (100%)	10 (100%)
VRE-2 + <i>Acinetobacter</i> spp. ( <i>E. faecium</i> , vanB)	10+100	9 (90%)	9 (90%)
<b>Negative controls</b>			
VSE ( <i>E. faecium</i> )	100	2 (20%)	0
<i>Lactobacillus</i> spp.	100	0	0
Double-distilled H <sub>2</sub> O	0	0	0

\*Bacterial load in 100 µl of sample inoculated on culture plates as part of IHP and SP.

VRE: vancomycin-resistant *Enterococcus* spp.; VSE: vancomycin-sensitive *Enterococcus* spp.; CFU: colony forming units

IHP (ECCV with 8 µg/mL vancomycin; confirmatory tests: E-test, Phoenix). As IHP, most participants (n = 6) utilized selective (ECCV, Bile Esculin agar) and chromogenic (Brilliance VRE, Oxoid; ChromID VRE, bioMérieux) media either alone (n = 5) or combined with conventional medium (n = 1). Catalase and PYR confirmatory tests were most commonly performed (n = 7).

**Conclusions:** This is the first EQA programme assessing culture-based detection of VRE. Performance of the participating laboratories was generally high with the majority implementing ‘‘rapid’’ selective or chromogenic media as IHP that allow a presumptive detection of VRE within 18 hours.

#### P1746 External quality assessment of culture-based detection of extended spectrum beta-lactamase producing gram-negative bacteria by a network of European laboratories

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**Objectives:** An external quality assessment (EQA) was carried out to assess the culture-based methods utilized by eight hospital laboratories in seven European countries and their proficiency in detecting ESBL-GNB.

**Methods:** The panel included five extended spectrum beta-lactamase producing Gram-negative bacteria (ESBL-GNB) and three non-ESBL-producing GNB as pure strains or in mixtures at varying concentrations (Table 1). All eight participants analyzed the panel utilizing their in-house culture protocol (IHP) for ESBL detection, and a standardized protocol (SP) implemented as a diagnostic intervention in clinical trials as part of MOSAR (EU-FP6 project). SP included sample inoculation on a chromogenic medium, Brilliance ESBL (Oxoid) and ESBL confirmation by double disk synergy test (DDST).

**Results:** Of the eight participants, seven (88%) successfully detected the ESBL-harboring *E. coli*, and *K. pneumoniae* at 100 colony forming units (CFU) absolute loads using both protocols (Table 1). However, at 1 CFU absolute loads, no. of participants able to confirm ESBL-GNB was drastically reduced. The ESBL-producing *E. cloacae* was detected by a limited number of participants while none were able to detect *P. aeruginosa* expressing the ESBL PER-1. SP and IHP results

Sample characteristics	Absolute bacterial loads inoculated on media* (CFU)	ESBL confirmed by in-house protocol (IHP) N (%)	ESBL confirmed by standardized MOSAR protocol (SP) N (%)
<b>Pure strains</b>			
ESBL 1 ( <i>E. coli</i> , bla <sub>CTX-M10</sub> )	100	7 (88%)	7 (88%)
ESBL 1 ( <i>E. coli</i> , bla <sub>CTX-M10</sub> )	100	7 (88%)	7 (88%)
ESBL 1 ( <i>E. coli</i> , bla <sub>CTX-M10</sub> )	1	5 (63%)	2 (25%)
ESBL 2 ( <i>E. cloacae</i> , bla <sub>CTX-M3</sub> )	100	5 (63%)	5 (63%)
ESBL 2 ( <i>E. cloacae</i> , bla <sub>CTX-M3</sub> )	100	5 (63%)	5 (63%)
ESBL 2 ( <i>E. cloacae</i> , bla <sub>CTX-M3</sub> )	1	2 (25%)	3 (38%)
ESBL 3 ( <i>K. pneumoniae</i> , bla <sub>CTX-M3</sub> + bla <sub>SHV12</sub> )	100	7 (88%)	7 (88%)
ESBL 3 ( <i>K. pneumoniae</i> , bla <sub>CTX-M3</sub> + bla <sub>SHV12</sub> )	100	8 (100%)	8 (100%)
ESBL 3 ( <i>K. pneumoniae</i> , bla <sub>CTX-M3</sub> + bla <sub>SHV12</sub> )	1	3 (38%)	1 (13%)
ESBL 4 ( <i>P. aeruginosa</i> , bla <sub>PER-1</sub> )	100	0	0
ESBL 4 ( <i>P. aeruginosa</i> , bla <sub>PER-1</sub> )	100	0	0
ESBL 4 ( <i>P. aeruginosa</i> , bla <sub>PER-1</sub> )	1	0	0
ESBL 5 ( <i>K. pneumoniae</i> , bla <sub>TEM4</sub> )	100	7 (88%)	7 (88%)
ESBL 5 ( <i>K. pneumoniae</i> , bla <sub>TEM4</sub> )	100	7 (88%)	7 (88%)
ESBL 5 ( <i>K. pneumoniae</i> , bla <sub>TEM4</sub> )	1	2 (25%)	2 (25%)
<b>Mixtures</b>			
ESBL 1 + 3 (bla <sub>CTX-M3</sub> + bla <sub>CTX-M3</sub> + bla <sub>SHV12</sub> )	10+10	8 (100%)	8 (100%)
ESBL 4 + 5 (bla <sub>PER-1</sub> + bla <sub>TEM4</sub> )	10+10	7 (88%)	7 (88%)
ESBL 1 + <i>P. mirabilis</i> (bla <sub>CTX-M10</sub> )	10+100	6 (75%)	5 (63%)
ESBL 3 + <i>E. coli</i> (bla <sub>CTX-M3</sub> + bla <sub>SHV12</sub> )	10+100	8 (100%)	8 (100%)
ESBL 4 + <i>C. freundii</i> (bla <sub>PER-1</sub> + bla <sub>AmpC</sub> )	100+100	0	0
<b>Negative controls</b>			
<i>C. freundii</i> (bla <sub>AmpC</sub> )	10000	1 (13%)	1 (13%)
<i>E. coli</i>	10000	1 (13%)	1 (13%)
<i>P. mirabilis</i>	10000	0	0
Double-distilled H <sub>2</sub> O	0	0	0

\*Bacterial load in 100 µl of sample inoculated on culture plates as part of SP and IHP.

ESBL: extended-spectrum beta-lactamase

showed 95.4% concordance (kappa: 0.90, 95% CI: 0.84–0.96), thus no difference in detection rates. One participant reported 16 ESBL-GNB samples (pure strains: 1–100 CFU, and mixtures) as negative and a non-ESBL producing *E. coli* as ESBL-positive with both SP and IHP (McConkey and blood agar, confirmatory tests: E-test and BD Phoenix). The AmpC-producing *C. freundii* was reported as ESBL-positive by one participant with both SP and IHP (blood agar, confirmatory tests: DDST and CLSI disc diffusion procedure using cephalosporin alone and with inhibitor). As IHP, majority of the participants (n = 5) utilized conventional media, while three used chromogenic media (ChromID ESBL, bioMérieux). Among confirmatory tests, DDST was most commonly performed (n = 5) and was combined with CLSI procedure (n = 2), Phoenix (n = 1), Phoenix and E-test (n = 1), and CLSI procedure and Vitek (bioMérieux) (n = 1). No two participants performed an identical IHP.

**Conclusions:** This is the first EQA programme assessing culture-based detection of ESBL-GNB and highlights the need for standardized detection algorithms. Detection of ESBL harbouring GNB other than *E. coli* and *K. pneumoniae* was more challenging for the participating laboratories, majority of which utilized conventional media as IHP for ESBL detection.

#### P1747 The 2011 antimicrobial susceptibility testing external quality assessment exercise organised for EARS-Net participants

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**Objective:** The United Kingdom National External Quality Assessment (EQA) Service for Microbiology (NEQAS) provides external quality assessment (EQA) for antimicrobial susceptibility testing to the European Centre for Disease Prevention and Control (ECDC) EARS-Net. In 2011 the annual EQA exercise was the second

in collaboration with ECDC but the tenth in succession organised for EARS-Net (formerly EARSS) laboratories. The objective of this EQA exercise was to highlight and improve susceptibility testing across Europe.

**Methods:** Participation was invited from 908 laboratories in 31 countries, results were returned by 818 laboratories. The organisms distributed were of *K. pneumoniae*, *E. coli*, *S. pneumoniae*, *E. faecium*, *P. aeruginosa*, and *S. aureus*. Participants' results for identification and antimicrobial susceptibility testing were assessed.

**Results:** The level of performance was generally high, with a few exceptions for specific organism-antimicrobial agent combinations. Specimen 0270, an *E. coli* with slightly raised MICs of amikacin (4–8 mg/L) proved problematic, with variable results (61.8% S, 31.6 I, 6.6% R). A high discrepancy rate was also seen for piperacillin-tazobactam with this isolate. Specimen 0271 was a *K. pneumoniae* producing both an ESBL and a carbapenemase. Only 54.2% reported the presence of an ESBL and carbapenem susceptibility reports were variable. Specimen 0272 was a *S. pneumoniae* with reduced susceptibility to penicillin (MIC 0.5 mg/L). Reporting of penicillin susceptibility varied among participants depending on the reported site of infection and the guidelines followed. Specimen 0273 was an *E. faecium* with VanB glycopeptide resistance. Reduced susceptibility to vancomycin was detected by 92% participants (8.7% I, 83.3% R). Specimen 0274 was a *P. aeruginosa* and no significant problems were seen with this isolate. Specimen 0275 was a methicillin resistant *S. aureus* with dissociated resistance to clindamycin. There were significant discrepancies with reporting of clindamycin (74.2% R, 1.8% I, 24.0% S) and 5% laboratories failed to detect methicillin resistance.

**Conclusion:** EQA is a valuable tool in the quality assurance of antimicrobial susceptibility testing performed in laboratories reporting to EARS-Net and helps to validate data between laboratories in Europe. In this exercise overall concordance between participating laboratories was generally high except in cases where susceptibility was borderline or different guidelines were used.

#### **P1748** Standardised methods and harmonised guidelines for antimicrobial susceptibility testing – an international external quality assessment provider's perspective

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**Objective:** Participants of the United Kingdom National External Quality Assessment Service (UK NEQAS) international scheme for antimicrobial susceptibility testing (AST) report susceptibility results interpreted primarily according to either EUCAST or CLSI guidelines. The drive to standardise AST methods and harmonise guidelines is making good progress but EQA highlights some outstanding issues.

**Methods:** Performance of participants was analysed according to methods and guidelines used for recently distributed EQA specimens. The organisms included were isolates of Enterobacteriaceae, *Enterococcus* spp., *H. influenzae*, *P. aeruginosa*, *S. aureus* and *S. pneumoniae*.

**Results:** Concordance with the intended results was generally good with an overall concordance with reference results of >95% but some problems were highlighted. For ESBL-producing Enterobacteriaceae reporting of susceptibility was very variable for beta-lactamase inhibitor combinations, partly related to different approaches to reporting ESBL-producers. *Enterococcus* spp. without high-level resistance to gentamicin were often reported resistant, and one high-level resistant strain was not reported resistant by 9.5% of participants. Problems detecting glycopeptide resistance in *Enterococcus* spp. are most common with disc diffusion methods. Problems in testing susceptibility of *H. influenzae* to co-amoxiclav were particularly common in isolates with resistance mediated by PBP changes. Borderline susceptibility to piperacillin-tazobactam in *P. aeruginosa* was reflected in variable results, largely related to differences in breakpoints between EUCAST and CLSI. *S. pneumoniae* intermediate to penicillin were reported variably depending on different

interpretation of guidelines for reporting isolates from cases of pneumonia or meningitis. With *S. aureus* there was variable reporting of mupirocin and fusidic acid susceptibility dependant on guidelines followed and there was variation in reporting dissociated resistance to clindamycin.

**Conclusion:** EQA is a valuable tool in the quality assurance of AST in the diagnostic laboratory. Discrepancies were identified mainly where there were differences in interpretation of breakpoints between guidelines and where adherence to the stated guideline was not observed.

#### **P1749** Proficiency of Spanish laboratories for accurate susceptibility testing, detection, and interpretation of beta-lactam resistance phenotypes in *Pseudomonas aeruginosa*

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**Objective:** To evaluate the proficiency of Spanish laboratories for accurate susceptibility testing, detection, and interpretation of *P. aeruginosa* betalactam resistance phenotypes.

**Methods:** A panel of 13 characterized strains was sent to 54 centers. The panel included reference strains PAO1 and ATCC27853, clinical strains producing diverse horizontally-acquired betalactamases (PSE-1 [along with MeXY-OprM overexpression], Extended-spectrum betalactamases [ESBLs PER-1 or OXA-161], and class A [GES-5] and B [VIM-2] carbapenemases) as well as mutants showing different combinations of chromosomal resistance mechanisms (AmpC hyperproduction, OprD loss and overexpression of MexAB-OprM or MexCD-OprJ efflux pumps). The centers were requested to use their routine approach to evaluate the susceptibility to six betalactam (and five non-betalactam) antipseudomonal agents, provide raw/interpreted clinical categories (RCC/ICC respectively), and infer the resistance mechanisms, reporting the additional tests used in each case. Consensus results from two reference centers were used to assign minor, major or very major errors (mE, ME or VME).

**Results:** Most centers (94.4%) used automated devices, mainly Vitek 2, MicroScan WalkAway, and Wider, each accounting for 25–30%. CLSI/EUCASST breakpoints were used in 86/14% of the determinations. RCC discrepancies exclusively due to the differential application of breakpoints were higher (up to 15%) for aztreonam, followed by piperacillin-tazobactam (PTZ) and meropenem. The lowest % of VME was documented for Vitek 2 users, followed by Wider and MicroScan. By contrast, MicroScan system showed the lowest % of ME, followed by Vitek2. The strains yielding the overall highest % of VME (average for all antibiotics close to 6%), were CC-4 (AmpC hyperproduction + OprD loss), and CC-9 (GES-5 producer), while among antibiotics, the overall highest % of VME (22%) was documented for PTZ. Appropriate inference of resistance mechanisms was high for the VIM-2 producing strain (83%) and the OprD mutant (74%), but low (<40%) for clinical strains producing PER-1, OXA-161 or GES-5.

**Conclusions:** The use of different breakpoints and devices, the complexity of mutation-driven resistance mechanisms and the lack of standard tests to detect ESBLs and carbapenemases in *P. aeruginosa* leads to an extraordinary variability and lack of accuracy in susceptibility testing reports which may have important consequences in the treatment and control of infections.

#### **P1750** Detection of MRSA encoding mecALGA251: problems and solutions?

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**Objectives:** The recent identification of MRSA encoding a newly described mecA homologue (mecALGA251) highlighted the fact that



accurate detection and identification of these organisms could pose a challenge for clinical diagnostic laboratories using standard protocols. To improve awareness and increase case ascertainment, we sought to (i) include a representative isolate in the UK NEQAS MRSA screening scheme and (ii) assess the performance of phenotypic methods for their detection.

**Methods:** A mecALGA251-positive MRSA (oxacillin and ceftoxitin MIC = 16 mg/L) was distributed to 301 UK NEQAS participants to screen for MRSA using their standard culture and/or molecular methods. The second arm of the study examined 40 isolates representing three different lineages of *S. aureus* from bovine mastitis cases in England (n = 13) and human sources in England and Scotland (n = 15 and 12 respectively). Isolates were cultured onto bioMérieux chromID MRSA selective agar and a non-selective medium (nutrient agar). In addition, isolates were screened for PBP2a using two commercially available kits (MASTALEX™ and Clearview® Exact).

**Results:** 1 Analysis of the UK NEQAS EQA results showed that of the 68 participants that reported on the detection of MRSA using molecular methods, 16 (24%) reported positive molecular results (three were true positives from an in-house PCR), 49 (72%) reported a negative result, and three (4%) reported an invalid result. Of the participants that reported culture results, 75% (225/299) detected MRSA.

2 None of the mecALGA251-positive MRSA gave a positive result with MASTALEX whether cultured on selective or non-selective media. In contrast, all isolates gave a positive result with the Clearview® Exact test but only when tested from bioMérieux chromID MRSA selective agar.

**Conclusion:** Results from laboratories participating in the EQA distribution have highlighted problems in identifying mecALGA251-positive MRSA with the commercial molecular kits used. Where phenotypic and genotypic data for methicillin resistance in *S. aureus* are discrepant, the possibility of mecALGA251-positive MRSA should be considered. MRSA harbouring either mecA or mecALGA251 can be detected successfully using Clearview® Exact following ceftoxitin induction, but differentiation between these two gene homologues requires the use of specific PCR-based methods. This combined study alerts the routine diagnostic microbiology laboratory to the challenges of accurately detecting emerging MRSA variants.

## Diagnostic microbiology methods - non molecular

**P1751** Comparative evaluation of processing- untreated, with heat and acid- water samples within the ISO 11731 for qualitative and numerical isolation of *Legionella* spp. and serotypes

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**Objective:** Comparative evaluation of three treatment procedures (Cold-untreated, Hot-heat treatment, Acid-acid treatment, (as proposed in ISO 11731), are applied for the isolation of *Legionella* spp. from environmental samples.

**Methods:** One thousand nine hundred and eighty-three recent water samples were simultaneously processed using the above treatments in the Central Public Health Laboratory. Overall, 460 samples were found positive for the bacterium using any of the three treatments. The results were analyzed by X2, Wilcoxon, Friedman and Kruskal-Wallis tests.

**Results:** Statistically significant correlation between the method of sample treatment and the number of isolated colonies was found (Kruskal-Wallis test,  $p < 0.001$ ). In this context, the superiority of heat treatment process in the numerical recovery of bacteria was observed (Friedman test,  $p < 0.001$ ). Specifically, using the Cold treatment, more *L. pneumophila* serogroup 2-15 colonies were isolated (Friedman,  $p < 0.002$ ), while using the acid treatment less *L. pneumophila* serotype 1 and *L. pneumophila* serogroup 2-15 colonies were isolated (Wilcoxon,  $p < 0.001$ ). It should also be noted that, statistically significant correlation was revealed between the method of sample processing and the qualitative isolation of the bacterium ( $\chi^2$ ,

$p < 0.002$ ). When a positive sample was detected with only one of the three sample treatments (the rest two would not detect the microorganism), isolation of *Legionella* spp. was possible with Cold in 18.3%, with Hot in 17% and only in 4.8% with Acid treatment.

**Conclusions:** The heat treatment is superior in numerical recovery of bacteria than both Cold and Acid. Treatment with Acid is performing worse to the Cold and Hot ones, both qualitatively and numerically. The study continues, but appears that important benefit in financial and human resources would result from the omission or limitation of the acid treatment process in selective cases of samples.

**P1752** Evaluation of TRU Legionella®, a new rapid test for detection of *Legionella pneumophila* serogroup 1 antigen in urine samples

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**Introduction:** We evaluated the ability of a new antigen test (TRU Legionella assay, Meridian Bioscience, Cincinnati, USA) to diagnose Legionnaires' Disease (LD) using frozen urine samples from a well-described sample of patients with and without LD. The results were compared with those obtained with the Binax NOW urinary antigen test.

**Materials and methods:** Urine samples were collected between 2000 and 2011 and were stored at  $-70^{\circ}\text{C}$  until processing was performed. We included 139 urine samples from 139 patients with LD (cases). A case of confirmed *Legionella pneumonia* was defined according to the European Working Group on Legionella Infections (EWGLI) criteria (www.ewgli.org). The specificity of the test was determined by using urine samples collected from 73 patients with respiratory tract infections other than Legionella infections. The results were compared with those obtained by the BinaxNOW urinary antigen test. The urinary antigen tests were performed simultaneously and the results were interpreted according to the manufacturers' instructions.

**Results:** The sensitivities and specificities were 73.4% and 100%, respectively, for TRU Legionella test; and 77.0% and 100%, respectively, for the Binax NOW urinary antigen test. The sensitivity of the TRU Legionella test increased to 80.6% (112/139) if tests were reexamined after 60 minute of incubation. Prolonged incubation did not effect the specificity in both tests.

**Table 1:** Results of IC tests after 15 min and 1 hour of incubation. LD: Legionnaires' disease, RTI: respiratory tract infection.

LD (cases)	No. of patients with a positive test result/total number of patients (%)	
	20/15 min	1 hour
TRU Legionella®	102/139 (73%)	112/139 (81%)
NOW®	107/139 (77%)	113/139 (81%)
<b>RTI other than LD (controls)</b>		
TRU Legionella®	0/73 (0%)	0/73 (0%)
NOW®	0/73 (0%)	0/73 (0%)

**Conclusion:** The study provided data showing that the TRU Legionella test has a high degree of sensitivity and specificity, with a sensitivity that increased with incubation time. The two assays evaluated have similar performance characteristics and are suitable for the rapid diagnosis of LD.

**P1753** Evaluation of ESwab (COPAN) for the detection of *Ureaplasma urealyticum* and *Mycoplasma hominis* from genital specimens with Mycoplasma Duo kit (BIO-RAD)

J.M. Rousee\*, C. Rieder-Monsch, T. Gueudet (Strasbourg, FR)

**Objectives:** To validate ESwab as an alternate collection and transport medium for identification and titration of genital mycoplasma by using the Mycoplasma Duo kit.

**Methods:** Mycoplasma culture, identification and titration were compared according to three different methods: collection by a Rayon swab, transfer into the suspension medium of the Mycoplasma Duo kit and microplate seeding according to manufacturer's instructions (method 1); collection by ESwab and transfer of 200 µL of Eswab medium into the suspension medium of the Mycoplasma Duo kit and microplate seeding (method 2), collection by Eswab and direct seeding of the microplate by 100 µL of Eswab medium (method 3). In each case, an A7 agar was also inoculated as gold standard. Twenty-five patient specimens and various dilutions of *U. parvum* ATCC 27815 (Up) and *M. hominis* ATCC 27618 (Mh) strains were tested. Moreover, statistical data of patient's results were analyzed during one year before and after introduction of Eswab for mycoplasma diagnosis in our lab.

**Results:** With ATCC strains, there was no difference between the method 1 and 2. The method 3 didn't allow the detection of Mh at every concentration and Up at the lowest.

With 25 patients, 18 cultures were negative with every method and two samples were contaminated by yeast. Three specimen cultures were positive with *U. urealyticum* (Uu)  $\geq 10^4$  colour changing unit/mL for the three methods. One culture was positive with Uu  $\leq 10^3$  for methods 1 and 2 and negative for method 3. One culture was positive with Uu  $\geq 10^4$  for the three methods and Mh  $\geq 10^4$  for methods 1 and 3 and Mh  $\leq 10^3$  with method 2. In addition, no difference between the two study periods was found: 269 specimens (27%) were positive in 2009 vs. 320 (26%) in 2010 with about a thousand patient specimens in each cohort.

**Conclusion:** Transfer of 200 µL ESwab into the Bio-Rad suspension medium provides equivalent results in comparison with the method recommended by Bio-Rad. Use of ESwab for collection and transport of mycoplasma with Mycoplasma Duo kit simplifies the detection of genital mycoplasma by allowing multiple testing from the same original specimen.

#### P1754 Real-time evaluation of Uriswab vs. a dip-slide method for enumeration of urinary pathogens in outpatient settings

R. Rennie\*, J. Fuller, G. Tyrrell, T. Bennett, D. Dyrland (Edmonton, Red Deer, CA)

**Objective:** Time, temperature and transport from remote outpatient facilities to testing laboratories may compromise urine culture results. This study compared urine collected by Uriswab (Copan Innovation, Brescia, Italy) with a urine dip-slide medium paddle method commonly used in rural environments.

**Methods:** Three out-patient clinics were included; two ~100 km from the testing laboratory and one nearby the testing lab. From each facility, 100 consecutive urines, collected by physician request for culture, were inoculated according to manufacturers' instructions either to Uriswab, or Dip-N Count. Both samples were sent by the usual courier systems to the testing lab. On receipt the Uriswab sample was centrifuged for 5 minute at 3000 rpm and the urine was plated with a 0.001 mL loop on blood and MacConkey agars. The dip-slide and the plates from the Uriswab sample were incubated overnight at 35°C. Colony counts were performed, morphotypes were compared and identifications were performed. Time from collection to plating in the laboratory was noted.

**Results:** Of the 300 urine samples collected, 111 (37%) did not grow from the Uriswab sample; 72 (24%) from the dip-slide. There were four rejected from the dip-slide (dried out or unreadable), and one from the Uriswab (no urine in container). In all, 66 pathogens were recovered from the Uriswab (35% of the 189 samples that grew). For the dip-slide, 49 pathogens were isolated (21.5% of the 228 samples that grew). There was no difference between the species isolated from each sample, except that five beta-haemolytic streptococci were recovered from the Uriswab that were not seen in the dip-slide cultures. Transport times from collection to processing in the testing lab varied from 15 minute to 21 hour. There was no discernible difference in results based on delays in transport. Of the 39 (13%) samples that grew only on the dip-slide all were low colony count cultures with a single or multiple non-pathogens.

**Conclusions:** Significantly more pathogens were recovered from Uriswab samples than the dip slide. The Uriswab also failed to grow a large number of low colony count samples that did not contain a pathogen. This would reduce technologist time required for interpretation of organisms that are unimportant as urinary pathogens. The Uriswab offers a simple straightforward way of transporting urine for culture easily, at room temperature, and without loss of the sample even from more remote locations.

#### P1755 Evaluation of Vitek 2 for identification of *Acinetobacter* species

A. Schulte\*, H. Seifert (Cologne, DE)

**Objectives:** Phenotypic identification of acinetobacters to species level requires the use of more than 20 physiological and biochemical tests and is therefore not suited for routine diagnostic microbiology laboratories. Molecular identification methods such as tDNA spacer fingerprinting, AFLP, amplified ribosomal DNA restriction analysis (ARDRA), restriction analysis of the 16S-23S rRNA intergenic-spacer sequences, rpoB sequencing, and gyrB multiplex do not meet the demands of a diagnostic laboratory. Currently, most laboratories rely on commercially available semi-automated systems for identification of *Acinetobacter* spp.

**Methods:** We evaluated the performance of VITEK 2 (bioMérieux), the most frequently used commercial microbial identification system, using a set of 212 *Acinetobacter* isolates that had been identified to species level by reference identification methods including *A. baumannii*, n = 25; *A. bereziniae*, n = 11; *A. calcoaceticus*, n = 26; *A. gouillouiae*, n = 11; *A. haemolyticus*, n = 11; *A. johnsonii*, n = 11; *A. junii*, n = 12; *A. lwoffii*, n = 11; *A. nosocomialis*, n = 26; *A. pittii*, n = 26; *A. radioresistens*, n = 12; *A. schindleri*, n = 10; *A. ursingii*, n = 10; and *Acinetobacter* DNA group 14TU, n = 10. Tests were run in duplicate if VITEK2 identification did not match the reference method.

**Results:** Overall, 187/212 *Acinetobacter* isolates (88.2%) were correctly identified at the genus level while only 39 isolates (18.4%) were correctly identified to species level. Most importantly, members of the *A. baumannii* group, i.e. *A. baumannii*, *A. nosocomialis* and *A. pittii*, the clinically most important *Acinetobacter* species, while "correctly" identified as "Acinetobacter baumannii complex," could not be identified to species level. Among the species that were identified with a high reliability (83–100%) were *A. haemolyticus*, *A. radioresistens* and *A. ursingii*. Poor identification was achieved with *A. junii* and *A. lwoffii* isolates, while the remaining species were not represented in the database.

Microorganism	n	No. (%) of isolates correctly identified at	
		Genus level	Species level
<i>A. baumannii</i> *	25	25 (100)	0
<i>A. bereziniae</i>	11	9 (81.8)	0
<i>A. calcoaceticus</i> *	26	26 (100)	0
<i>A. gouillouiae</i>	11	7 (63.6)	0
<i>A. haemolyticus</i>	11	11 (100)	11 (100)
<i>A. johnsonii</i>	11	4 (36.4)	0
<i>A. junii</i>	12	12 (100)	7 (58.3)
<i>A. lwoffii</i>	11	4 (36.4)	1 (9.1)
<i>A. nosocomialis</i> *	26	26 (100)	0
<i>A. pittii</i> *	26	26 (100)	0
<i>A. radioresistens</i>	12	12 (100)	10 (83.3)
<i>A. schindleri</i>	10	5 (50)	0
<i>A. ursingii</i>	10	10 (100)	10 (100)
<i>Acinetobacter</i> DNA group 14TU	10	10 (100)	0

\* all isolates were identified as „*Acinetobacter baumannii* complex“

**Conclusion:** Apart from *A. haemolyticus*, *A. radioresistens* and *A. ursingii*, VITEK2 does not permit to reliably identify *Acinetobacter* isolates to species level. Members of the *A. baumannii* group, i.e. *A. baumannii*, *A. nosocomialis* and *A. pittii* are identified as "Acinetobacter baumannii complex," but correct species identification is not possible.

**P1756** Evaluation of Vitek 2 system for identification of most clinically important *Candida* species

M.E. Ochiuzzi, I. Maldonado, L. Guelfand, A. Arechavala, R. Soloaga\* (Buenos Aires, AR)

**Objective:** The species of *Candida* are the major cause of invasive fungal infections in hospitalized patients. Identification of yeasts to the species level has become a priority, not only for the increasing number of non-*Candida albicans* isolates but also for the differential susceptibilities to antifungal agents, being important at this point for the therapeutic management. VITEK 2 (BioMérieux, Inc, Hazelwood, MO, USA) is a fully automated microbiology instrument that allows the identification of microorganisms, including yeasts. This study utilized ID-YST card using a colorimetric system which include 47 biochemical tests (a variety of carbohydrate and organic acids assimilation tests, oxidase and arilamydase enzyme detection). The aim of our study was to evaluate the YST card of VITEK 2 System for the identification of the most frequent yeasts in clinical microbiology laboratory.

**Methods:** A total of 168 strains of *Candida* were studied: *C. albicans* (n = 26), *C. tropicalis* (n = 29), *C. glabrata* (n = 28), *C. parapsilosis* (n = 32), *C. krusei* (n = 26), *C. guilliermondii* (n = 20), *C. dubliniensis* (n = 7); 164 isolates came from clinical samples of hospitalized and ambulatory patients received in hospitals included in the Mycology Net of Buenos Aires, Argentina. Four pattern strains: *C. parapsilosis* ATCC 22019, *C. krusei* ATCC 6258, *C. glabrata* ATCC 90030 and *C. albicans* ATCC 64548 were included. The strains were transferred from storage at -70°C onto Sabouraud agar and subcultured after 24 hour on Sabouraud agar. All strains were tested with the ID-YST card and API 20C or API 32C system (BioMérieux, Marcy L'Etoile, France), according the manufacturer's instructions and the results were compared. The API was considered the reference standard for accuracy of identification.

**Results:** The percentage of concordance between API and VITEK 2 Yeast card was 98.3%. There were three misidentifications: one *C. parapsilosis*, one *C. krusei* and one *C. tropicalis*. The average time of identification was 18.2 hour (range 18.0-18.5 hour).

**Conclusions:** According to the results of our study VITEK 2 System is a reliable, simple and effective method for the identification of the most frequent *Candida* spp and besides, reduced the times for identification from 48 to 18 hour.

**P1757** *Neisseria gonorrhoeae* identification, usefulness of the Vitek 2 NH card

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**Objetivo:** Symptoms of infection with *N. gonorrhoeae* (NG) differ depending on the site of infection. It causes urethritis, cervicitis, prostatitis, orchitis, pharyngitis, conjunctivitis, pelvic inflammatory disease and disseminated gonococcal disease. The main way of transmission is the sexual activity. Infections in children is a sexual abuse indicator. For these reasons, wrong identification could have serious legal and social consequences.

In medicolegal cases some reference centers suggest three test: biochemical, immunological and molecular techniques. Rapid and reliable identification is critical.

The Vitek 2 NH card, based on colorimetric technology, contains 30 biochemical test in the following categories: 11 glycosidase and peptidase tests, 10 acidification tests, five alkalization tests, four miscellaneous tests.

The aim of this study was to determine the usefulness and reliability of the Vitek 2C NH card in the identification of NG.

**Method:** A total of 115 unique clinical isolates of *N. gonorrhoeae* corresponding to different phenotypic clones from the Gonococcal Antimicrobial Surveillance Argentinean Programm were tested by Vitek 2 C (bioMérieux, Marcy l'Etoile, France) using the NH card.

The isolates were previously identified to the species level by colony morphology, Gram stain, oxidase, superoxol, utilisation of carbohy-

drates testing and by latex agglutination with monoclonal antibodies using Phadebact Monoclonal GC Test (Bactus AB, Sweden).

Strains were isolated in pure culture on chocolate agar. A bacterial suspension made in 0.45% aqueous CINA was adjusted to a McFarland of 3 with Vitek 2 Densicheck instrument (bioMérieux). The time period from preparation of the inoculum to loading of the card was <10 minute. The inoculated card was loaded into the Vitek 2C automated identification system according to the manufacturer's instruction. A computer-assisted algorithm was used to interpret the results.

**Results:** The Vitek 2C NH card correctly identified 111 (96.5%) strains of 115 isolates; one strain was identified with low discrimination (0.86%), another one (0.86%) was misidentified (*N. meningitidis*) and another two (1.7%) remained unidentified. The results were available within 6 hours.

**Conclusion:** NH card could be considered a reliable and useful tool for routine use in the rapid NG identification.

**P1758** Optimisation of Phoenix automated identification and susceptibility testing of mucoid *Pseudomonas* species

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**Objectives:** *Pseudomonas aeruginosa* (PSA) is a clinically important pathogen causing serious infections such as pneumonia in immunosuppressed patients. They are a major cause of morbidity and mortality in cystic fibrosis (CF) patients. Often in these patients, organisms adopt a mucoid phenotype, making species identification and susceptibility testing difficult. Species identification and in particular susceptibilities are of the utmost importance in CF patients and many labs now use automated instruments to perform this task. Automated instruments fail to identify/issue susceptibility results for mucoid isolates, possibly due to the additional glycocalyx present. This study investigated various techniques to optimise the performance of an automated instrument to identify (ID) and susceptibility test (ST) these isolates.

**Methods:** Two mucoid PSA, which previously failed tests (ID + ST) on Phoenix (PHX) were used. Both isolates were pre-treated with 12 methods prior to re-testing on PHX and compared with the standard method (addition of 25 µL of McFarland (McF) 0.5 inoculum to AST broth). Pre-treatments include; (i) wash bacteria in sterile water, (ii) 30 minute incubation in saline + 20 µ/mL alginate lyase (AL), (iii) 4 hours growth in tryptone soya broth (TSB) + 20 µ/mL AL, (iv) growth on agar + 20 µ/mL AL, (v) heat to 60°C for 30 minutes, (vi) add 50 µL of McF 0.5 to AST broth, (vii) growth on DNase plate, (viii) addition of 20 µ/mL AL to AST broth, (ix) add 100 µL on McF 0.5 to AST broth, (x) add 50 µL of McF 1 to AST broth, (xi) add 50 µL of McF 2 to AST broth, (xii) add 50 µL to McF 4 to AST broth. The ability to produce an ID + ST was compiled on five occasions. Growth curves for the best pre-treatments were performed in AST broth.

**Results:** Results are shown in Table 1. Positive ID results were seen in all pre-treatments except 5 where ID results failed for both PSA. Positive ST results were seen in PSA (A) and (B) in pre-treatments 10

Pre-treatment	PHX ID present		PHX AST present	
	PSA (A)	PSA (B)	PSA (A)	PSA (B)
1	5/5	5/5	0/5	0/5
2	5/5	5/5	0/5	2/5
3	5/5	5/5	0/5	0/5
4	5/5	4/5	0/5	0/5
5	2/5	1/5	0/5	0/5
6	5/5	3/5	0/5	3/5
7	5/5	4/5	0/5	2/5
8	5/5	5/5	0/5	0/5
9	5/5	4/5	0/5	5/5
10	5/5	5/5	1/5	5/5
11	5/5	5/5	0/5	5/5
12	5/5	5/5	1/5	5/5

and 12 and 2, 6, 7, 9, 10, 11 and 12 respectively. Growth of PSA (A)/(B) for standard, pre-treatment 9, 10, 11 and 12 methods after 4 hours were:  $5.2 \times 106/3 \times 106$ ,  $7.9 \times 106/1.5 \times 107$ ,  $6.9 \times 106/1.2 \times 107$ ,  $1.3 \times 107/1.3 \times 107$  and  $1.7 \times 107/3.4 \times 107$  respectively.

**Conclusions:** The most effective pre-treatment for optimising ID and ST from the PHX was addition of higher density inoculums (pre-treatments 9–12). Susceptibility testing in PHX is reliant on growth of isolate in AST broths, increasing starting inoculums increases density of growth in AST broths and therefore ability to produce ST results.

**P1759** Performance comparison of chromID™ Strepto B and CHROMagar™ StrepB to isolate *Streptococcus agalactiae* in pregnant women

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**Objectives:** *Streptococcus agalactiae* (SGB) is a significant worldwide cause of morbidity and mortality in peripartum women and their newborn infants. SGB colonize the vaginal and gastrointestinal tracts in healthy women, with carriage rates ranging from 15% to 45%. Neonates can acquire the organism vertically in utero or during delivery from the maternal genital tract.

The current approach to the prevention of SGB infection in pregnancy requires intrapartum antimicrobial prophylaxis in women with culture evidence of recent vaginal or rectal SGB colonization.

The objective was to compare the usefulness of chromID Strepto B (CR) (bioMerieux, France) to CHROMagar™ StrepB (MD) (Mediatec, France) medium to detect *Streptococcus agalactiae* in pregnant women from the Todd Hewitt broth (THB) relating to the methods proposed by the CDC.

**Methods:** One thousand two hundred seventy-six swabs were analyzed, 638 from vaginal introitus (VI) and 638 rectal (RS) belonging to 638 women within 35–37 weeks of pregnancy. Samples were referred to the laboratory in Stuart medium. Both vaginal and rectal swabs were placed in one THB with 15 µg/mL supplement of nalidixic acid and 10 µg/mL of colistin. After 24 hours incubation, subcultures in CR medium, MD medium and agar with 5% sheep blood (ASO) were performed from THB. The reading time was 24 hours for MD and 48 hours for CR and ASO mediums. Suspicious colonies, red (CR and MD mediums) and grey ± beta hemolysis in ASO were identified by conventional test such as Gram stain, catalase, bile-aesculin, CAMP, hippurate hydrolysis and group B serology.

**Results:** *Streptococcus agalactiae* was isolated in 106 patients, with a prevalence of 16.6%. Sensitivity, specificity, positive and negative predictive value of THB subcultures to CR medium were 100%, 100%, 100% and 100% respectively. The corresponding values for MD medium were 83%, 96%, 82% and 97% respectively. Sensitivity of THB in ASO was 80%.

**Conclusions:** Todd Hewitt broth subculture performance in CR was outstanding as regards the method proposed by the CDC. The specificity of both chromogenic medium was comparable (p>0.76) but the sensitivity was significantly better (p < 0.05) with CR medium.

**P1760** Facilitating diagnostics of diarrhoeagenic *Escherichia coli* using a new chromogenic medium

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**Objectives:** Diarrhoeagenic *Escherichia coli* (DEC) are a common cause of diarrhoea in humans but remain underdiagnosed in the routine laboratory due to the difficulty of differentiating them from *E. coli* of the resident flora. We examined a collection of shiga-toxin producing *E. coli* (STEC) and other DEC of different serotypes for growth on a new selective chromogenic medium developed primarily for STEC screening.

**Methods:** Seventy-nine serotyped *E. coli* strains originating from stool cultures, including 17 STEC, 25 enteroaggregative *E. coli* (EAEC),

seven enteropathogenic *E. coli* (EPEC) and 30 *E. coli* harbouring no known virulence factors of DEC pathovars, were cultivated on CHROMagar STEC (CS) (Mast Diagnostica, Reinfeld) and McConkey (MC) agar as by manufacturer's instructions. Growth and colour of colonies were recorded after 24 and 48 hour of incubation.

**Results:** Thirteen of 17 STEC strains belonging to serogroups O26 (n = 4), O104 (n = 6), O145 (n = 1) and O157 (n = 2) grew on CS showing the typical mauve colour described by the manufacturer. Growth on CS was suppressed in four STEC strains (serogroups O91 and O103) although they were cultivable on MC. Nine of 25 EAEC strains and one of seven EPEC strains also grew as mauve colonies (serogroups O55, O86, O126, O127 and O128) in contrast to only one of 30 *E. coli* not belonging to any DEC pathovar (serogroup O127). All STEC strains of serogroups O26, O145 and O157 grew on CS, while EAEC and EPEC strains of the same serogroups did not.

**Conclusion:** The new Chromagar STEC is a useful selective medium for the most common serotypes of STEC. Few STEC (serogroups O91 and O103) were suppressed in growth and would have been missed. Several EAEC strains of different serogroups were also able to grow on CS. This data indicates that CS is a helpful tool for STEC diagnosis in the routine laboratory. However, it cannot fully replace the current diagnostic procedures for STEC detection.

**P1761** Comparison of CHROMagar StrepB, Columbia CNA agar and Lim broth for the isolation of Group B *Streptococcus* from vaginal and rectal swabs from South Africa

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**Objective:** Infections by Group B *Streptococcus* (GBS) is the significant cause of perinatal and neonatal infections. The implementation of maternal screening for GBS colonization followed by intrapartum antibiotic prophylaxis is effective in reducing GBS disease. A high incidence of antimicrobial resistance found in industrializing countries can compromise the recovery of GBS from selective media. We evaluated CHROMagar StrepB, Columbia CNA agar (CNA agar) and Lim broth for the isolation of GBS from swabs from pregnant women from South Africa.

**Methods:** Vaginal and rectal swabs were collected from 130 pregnant women at 20–30 weeks of gestation and transported to the laboratory in Amies transport medium, without charcoal. Samples were randomized to be plated first on either CNA agar or CHROMagar StrepB, following which, the swab tip was inoculated into Lim broth (Todd Hewitt broth with Gentamicin 8 µg/mL and Nalidixic acid 15 µg/mL) and incubated for 24 hours before being plated on sheep blood agar. Up to four GBS like colonies were isolated and confirmed as GBS by testing for CAMP factor, inability to hydrolyze esculin, catalase test and Group B antigen. Sensitivities were calculated by comparing the proportion of positive samples for each medium in relation to a composite positives for all test media.

**Results:** Out of 130 vaginal swabs 49 (37.7%) yielded a GBS on at least one medium. There were no statistically significant (Chi-square test) differences in recovery of GBS from vaginal swabs between the media, and relative sensitivities of recovery from the individual media were: CNA agar 34 (69.4%), CHROMagar StrepB 41 (83.7%) and LIM broth 39 (79.6%). Out of 130 rectal swabs 43 (33.1%) yielded a GBS on at least one medium and relative sensitivities of recovery from the individual media were: CNA agar 31 (72.1%), CHROMagar StrepB 38 (88.4%) and LIM broth 12 (27.9%). The sensitivity of recovery of GBS from rectal swabs following Lim Broth enrichment was significantly lower compared to CHROMagar StrepB (p > 0.0001) and CNA agar (p = 0.002).

**Conclusion:** CHROMagar StrepB performed better than CNA agar and Lim broth for the recovery of GBS from both vaginal and rectal swabs. Lim broth is not suitable for the recovery of GBS from rectal swabs in countries with a high prevalence of gentamicin and nalidixic acid resistant flora.

**P1762** The comparison between the cultures of endotracheal aspiration and mini-BAL used in the diagnosis of ventilator-associated pneumonia

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**Objectives:** This study was to compare the results of cultures obtained via mini-bronchoalveolar lavage (BAL) and endotracheal aspiration techniques, used for quick and accurate determination of pathogens causing ventilator-associated pneumonia in intensive care units.

**Result:** Of the 92 patients on mechanical ventilation followed at emergency intensive care unit of Gülhane Military Medical School between June 2010 and June 2011, 30 patients (32.2%) diagnosed of having ventilator-associated pneumonia were included in this study. After performing multivariate logistic regression analysis age of patient and duration of mechanical ventilation were found as independent risk factors for ventilator-associated pneumonia development. There was a statistically significant difference in clinical pulmonary infection score (CPIS) between patients developed ventilator-associated pneumonia and not ( $p < 0.001$ ). The use of CPIS for ventilator-associated pneumonia diagnosis was thought as beneficial in patients on mechanical ventilation. There was no positive correlation between culture techniques of mini-BAL and endotracheal aspiration ( $p = 0.464$ ). In endotracheal aspiration samples eight were contaminated and six were with no isolation, but there was no contamination in mini-BAL samples. Of the eight samples in endotracheal aspiration group, 7 (87.5%) demonstrated bacterial proliferation considered as the pathogen.

**Conclusion:** We think that it would be more suitable to use mini-BAL instead of endotracheal aspiration to isolate pathogen causing ventilator-associated pneumonia, due to no positive correlation existing between endotracheal aspiration and mini-BAL culturing techniques, high risk of contamination in endotracheal aspiration culturing techniques and it's being worst technique in obtaining samples from distal airway.

**P1763** Perceptions and attitudes of French general practitioners towards rapid antigen diagnostic tests in acute pharyngitis using a randomised case-vignette study: a cross-sectional study

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**Objectives:** This study had three objectives: (i) to assess the use of Rapid Antigen Diagnostic Tests (RADT) and their impact on the antibiotic prescribing behaviour of GPs in acute pharyngitis; (ii) to study the barriers to the use of RADT; and (iii) to identify GPs' characteristics associated with non-compliance with French guidelines.

**Methods:** We conducted a cross-sectional survey of a representative sample of 369 self-employed GPs in Southeastern France using a randomised case-vignette study.

**Results:** The availability of a RADT allowed a 44% relative reduction in the rate of antibiotic prescriptions. Thirty-four percent of GPs did not use a RADT in our acute pharyngitis vignette and 13% prescribed an antibiotic despite a negative RADT result. Non-compliance with French guidelines (i.e. not using a RADT and/or prescribing an antibiotic despite a negative RADT result) was independently associated with the following factors: less reading of medical journals, less benefits/risks discussion with patients about vaccinations and more perception that clinical examination was sufficient to prescribe antibiotics. The three main declared barriers to RADT use were: time to perform the test, patient expectations regarding antibiotics and the perception that clinical examination was sufficient to decide to prescribe an antibiotic.

**Conclusion:** Rapid Antigen Diagnostic Tests are a useful but not sufficient tool to reduce antibiotic prescribing in general practice. The results of this study increase understanding of the factors underlying clinical decision-making for acute pharyngitis and may contribute to the development of interventions to improve practice.

**P1764** A survey of diagnostic strategy of culture-negative septic arthritis in paediatrics age group amongst United Kingdom microbiologists

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**Background:** Prompt bacteriologic confirmation of the diagnosis and early initiation of appropriate antibiotics are vital to prevent the risk of long-term sequelae in septic arthritis. Improved culture methods and use of 16S r DNA PCR amplification and sequencing has demonstrated that *Kingella kingae* is a common cause of osteoarticular infections in younger children accounting for 48% of cases of septic arthritis in children under 2 years.

**Objectives:** Local practice and experience with culture negative septic arthritis and detection of *Kingella* sp. by 16S r DNA PCR amplification and sequencing prompted us to ascertain the current practice employed by UK hospital laboratories for diagnosing culture negative septic arthritis.

**Method:** We conducted a short national survey in UK asking two questions. An online survey was hosted at [www.surveymonkey.com](http://www.surveymonkey.com) and microbiologists from 109 laboratories in different parts of the UK were contacted via e-mail and asked to take part. Responses were collated by the website, downloaded and analyzed. Cases of culture negative septic arthritis in paediatrics age group in our hospital were identified retrospectively for a period of 12 months (June 2010–May 2011) using laboratory information system.

**Results:** Microbiologists from 52 (48.6%) laboratories requested to participate answered the survey. Only 21.2.3% of the respondents said that they routinely culture synovial fluid in blood culture enrichment. If the cultures were negative 36.5% respondents said that they would send the synovial fluid for 16S r DNA PCR.

We also analysed our local positive rate of *Kingella kingae* in paediatric age group. Three cases were identified in period of 12 months. Clinical, microbiological and molecular sequencing data on the three patients were reviewed. All three cases were diagnosed by a positive 16S r DNA PCR result on the synovial fluid and only one case positive by blood culture enrichment technique.

**Conclusion:** *Kingella kingae* is an emerging causative agent of septic arthritis and osteomyelitis, primarily in infants and children. The bacterium is best detected by rapid inoculation in blood culture systems or by real-time PCR assays. We recommend the use of 16S r DNA PCR amplification and sequencing when initial culture of joint fluid are negative by routine and enrichment culture techniques.

**P1765** Rapid detection of Pantone-Valentine leukocidin in *Staphylococcus aureus* cultures by monoclonal antibodies using a lateral flow assay

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**Objectives:** Pantone Valentine leukocidin (PVL) is a phage born virulence factor of *Staphylococcus aureus*, which is associated with chronic/recurrent skin and soft tissue infections (SSTI) and necrotising pneumonia. Because of its clinical relevance, the detection of *S. aureus* which carry PVL genes warrants more aggressive therapy and infection control measures than PVL-negative strains (see [www.hpa.org.uk](http://www.hpa.org.uk)). However, PVL detection is currently essentially limited to reference centres and specialised laboratories as it is performed by molecular methods.

**Methods:** Recombinant Pantone Valentine leukocidin (F-component) was used to generate a set of monoclonal antibodies by phage display. These antibodies were purified after over-expression in *E. coli*, characterised initially by ELISA and spotted in different dilutions in microtiterstrip-mounted protein microarrays. Results from these microarray assays assisted in the identification of suitable antibodies which then were used to establish a lateral flow assay. This assay was used to detect PVL in overnight cultures of *S. aureus* in 10 minute. Isolates were genotyped by microarray hybridisation in parallel for confirmation and for assignment to clonal complexes.

**Results:** The detection limit for the lateral flow test was determined to be around 1 ng/mL. Overnight cultures from Columbia blood agar, Mueller Hinton agar and a commercial MRSA selective growth medium as well as liquid cultures (in a broth described by Kato&Noda) after as few as 3 hour incubation proved suitable for PVL detection. For evaluation, 231 clinical isolates from patients with skin and soft tissue infections from North America, Europe, Australia, Africa and the Middle East were yet tested. One hundred twenty-three isolates belonging to 26 distinct strains were PVL-positive. One hundred and eight isolates from 33 strains were PVL-negative. This included methicillin-susceptible as well as -resistant *S. aureus*. The sensitivity of the assay in these initial trials was 100%, the specificity was 98.18%. The positive predictive value was found to be 98.41%, the negative predictive value 100%.

**Conclusion:** This test allows the rapid detection of PVL under conditions of a routine bacteriological laboratory. As it utilises cultures from standard media and as it does not require sophisticated equipment, it can easily be integrated into a laboratory's workflow. This might contribute to timely therapeutic interventions in cases of PVL-associated infections.

**P1766** A novel rapid method that enables identification of pathogenic micro-organisms within 3 hours after samples are collected

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**Objectives:** As current blood culture methods require at least several days, empirically selected antibiotics are instead administered until the pathogenic microorganisms are identified. Though mass spectrometry is can be utilized as a rapid identification method of pathogens, it requires the blood culture process, so it takes more than 10 hours after samples are collected. Our objective is to develop a novel rapid method which enables identification of pathogenic microorganisms within 3 hours after collection of patient samples.

**Methods:** To detect pathogenic bacteria by PCR sensitively and correctly, we newly developed "eukaryote-made" Taq polymerase, which is free from bacterial DNA contamination (J Clin Microbiol. 2011 Sep;49(9):3316-20). We also developed a novel rapid method to identify pathogenic microorganisms using seven primer sets, real-time PCR, high resolution melting analysis (RotorGeneQ:Qiagen), and the original web-based identification software (International patent application:2007). To combine the eukaryote-made Taq polymerase and a novel identification method, we developed a rapid identification system of pathogenic microorganisms successfully.

**Results:** We performed the blind tests to evaluate its accuracy and specificity. Using the DNA samples of 60 kinds of bacterial species, a novel method gave the correct answer perfectly (60/60). Using the bacterial colonies of 100 kinds of bacterial species, the identification rate was 96% (four kinds of bacteria were not included in the database of our identification software). Using the 20 patient samples (blood, amniotic fluid, aqueous humor, and artificial valve of the heart) of infectious diseases, a novel method gave the correct answer perfectly within 3 hours.

**Conclusion:** Our group is currently developing this system for practical use and applications in advanced medicine by performing comparative studies between it and conventional microbial testing with more patient samples, as well as repeatedly evaluating its accuracy and specificity. A novel method allows the identification of the pathogenic microorganism species within 3 hours after collection of patient samples, thereby permitting evidence-based early-phase treatment of infectious diseases.

**P1767** F-18 FDG PET/CT for outcome prediction in pyogenic vertebral osteomyelitis

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**Objectives:** To determine validity of a 2-week follow-up F18-fluoro-deoxyglucose positron emission tomography/computed tomography (PET/CT) in predicting treatment success in patients with vertebral osteomyelitis (VO).

**Methods:** In two University Hospitals (Udine and Bologna) in Italy, patients with pyogenic VO March 2007–May 2011 were included in this prospective cohort study. A pre-treatment PET/CT and a follow-up PET/CT within 2–4 weeks from treatment initiation were performed. Microbiological diagnosis was attempted in all cases; antibiotic treatment was standardized according to microbiological diagnosis. Throughout treatment, the patient underwent weekly clinical, biomoral and TDM assessments. Success at the end of treatment was defined as reduction of pain, no signs/symptoms of local/systemic inflammation, CRP stably normal at end of therapy and 3 months thereafter, improved 3-month follow-up MRI. For the PET/CT validity study purposes, treatment success was defined as recovery with the same antibiotic regimen at the time of the follow-up PET/CT; patients changing therapy for toxicity/intolerance were excluded. Absolute and relative standard uptake value (SUV) change from baseline to follow-up PET/CT were calculated. ROC curve analysis for predicting treatment success were used; cut-offs of absolute and relative SUV change were chosen as to maximize sensitivity and specificity.

**Results:** In the study period, 76 patients (52–68%–males, mean age 63.7 years), were included. In 10 (13%) VO was post-surgical. Causative pathogens were *S. aureus* in 18 (24%), *Streptococcus* spp. in 10 (13%), other germs in 21 (16%), not identified in 36 (47%). Antibiotic therapy was levofloxacin ± rifampicin in 39 (51%), teicoplanin ± rifampicin in 11 (14%), other drugs/combination in 26 (34%). At the end of treatment, four (5.3%) were not cured, two (2.6%) were lost to follow-up and 70 (92.1%) were cured; of these, seven changed treatment for toxicity and were excluded, eight changed initial therapy for failure. Patients included for PET/CT analyses were therefore 67 (88.2%), 55 successes and 12 failures. AUC for absolute and relative SUV change were 0.81 and 0.82 respectively. Complete results are in the Table 1.

	AUC (95% CI)	Chosen Cut-offs	Sensitivity % (95% CI)	Specificity % (95% CI)	Positive predictive value % (95% CI)	Negative predictive value % (95% CI)	Correctly classified % (95% CI)
absolute SUV change	0.81 (0.69-0.89)	-0.7	48/55 87.3% (75.5-94.7%)	9/12 75% (42.8-94.5%)	48/51 94.1% (83.7-98.8%)	9/16 56.2% (29.9-80.2%)	85.07% (82.1-17.9%)
relative SUV change	0.82 (0.71-0.90)	-7%	49/55 89.1% (77.7-95.9%)	9/12 75% (42.8-94.5%)	49/52 94.2% (84.0-98.8%)	9/15 60.00% (32.3-83.7%)	86.6% (82.1-17.9%)

**Conclusion:** Although the sample size is limited, our study indicate that including PET/CT scan at baseline and at an early point after initiation of treatment reliably predicts treatment outcome and adds useful information in the clinical management of these patients.

**P1768** Diagnostic value of procalcitonin in febrile neutropenic patients

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**Objectives:** Febrile neutropenic patients represent a very heterogeneous population and the majority have a low risk for developing infectious complications, especially in solid organ malignancy and lymphoma patients. In order to select low-risk patients and provide them with less aggressive and more convenient therapy, we can use MASCC (the Multinational Association for Supportive Care in Cancer) index. The objective of current study is to compare the safety of procalcitonin and MASCC index score in identifying febrile neutropenic patients at low risk for complications.

**Methods:** Procalcitonin levels were estimated prospectively at baseline and 24–48 hours later in 281 chemotherapy induced febrile neutropenic patients with solid tumors and lymphoma.

**Results:** Among 281 febrile episodes, the type of infection was bacteremia in 19 episodes, clinically defined infection in 89 episodes,

microbiologically defined infection in 38 episodes, and unexplained fever in 154 episodes. Fifty-one (18.1%) of these episodes experienced serious medical complications (SMC). Procalcitonin (24–48 hours later) cut-off value of 0.5 ng/mL yielded a sensitivity of 46%, a specificity of 80%, a positive predictive value (PPV) of 45%, and a negative predictive value (NPV) of 92% for the prediction of SMC (AUC = 0.879). MASCC index score >21 identified low risk patients with a sensitivity of 73%, specificity of 90%, PPV of 63% and NPV of 93% (AUC = 0.694). Test characteristics of procalcitonin (24–48 hours later) for the prediction of bacteremia were 84% for sensitivity, 75% for specificity, 20% for PPV, and 98% for NPV at a cut-off value of 0.5 ng/mL (AUC = 0.822). MASCC index score <21 identified bacteremic patients with a sensitivity of 47.4%, specificity of 80.8%, PPV of 15.2% and NPV of 95.5% (AUC = 0.877).

**Conclusion:** Procalcitonin (24–48 hours later) may be useful to predict SMC and bacteremia in febrile neutropenic patients with solid tumors and lymphoma.

## Molecular bacteriology – miscellaneous

### **P1769** Virulence genes profiles and intimin subtypes of shiga toxin-producing *Escherichia coli* isolated from healthy and diarrhoeic calves

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The virulence properties of Shiga toxin-producing *Escherichia coli* (STEC) strains isolated from diarrhoeic and non-diarrhoeic calves were compared. The strains were also tested for O157:H7, O111 and O26 serotypes, using PCR and conventional serotyping methods. *E. coli* strains isolated from 297 faecal samples, from 200 diarrhoeic and 97 non-diarrhoeic calves, were screened by multiplex PCR assay for the *stx1*, *stx2*, *eae* and *Ehly* virulence genes. STECs were recovered from 8% of diarrhoeic calves and 10.3% of non-diarrhoeic calves. The predominant virulence genes profile were *stx1/eae/Ehly* (47.3%) among isolates from diarrhoeic calves and *eae/Ehly* (36.8%) among isolates from non-diarrhoeic calves.

Among three tested serogroups, the predominant serogroup was O26 (18.4%), and O157:H7 was not detected. Intimin subtyping by restriction fragment length polymorphism analysis revealed only three intimin subtypes (beta, gamma and theta). A significant difference was observed in the distribution of Int-theta between two groups. Int-theta was present in 50% of the isolates from diarrhoeic calves and in 11.1% of the isolates from non-diarrhoeic calves; this difference was statistically significant ( $p = 0.01$ ).

### **P1770** Occurrence of individual bacteriocin determinants in *Escherichia coli* phylogroups: microcin producers are predominantly associated with pathogenic strains

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**Objectives:** The association between *Escherichia coli* phylogroups and production of specific bacteriocin types was tested.

**Methods:** A set of 1003 *Escherichia coli* strains isolated from human gastrointestinal tract during years 2007–2010 in the Czech Republic from patients attending regional hospital in Brno was classified to one out of four *E. coli* phylogroups including group A ( $n = 282$ ), B1 ( $n = 83$ ), B2 ( $n = 366$ ) and D ( $n = 272$ ). In each *E. coli* bacteriocin-producing strain, the incidence of 30 bacteriocin-encoding determinants (i.e. 23 colicin and seven microcin encoding genes) was tested.

**Results:** No significant differences were identified between *E. coli* phylogroups with respect to patient's gender, age, primary diagnosis and the year of isolation. The incidence of bacteriocin producers in each phylogroup ranged from 44.6% (phylogroup B1) to 63.1% (phylogroup B2). An increased incidence of microcin producers (51.6%) was identified in the phylogroup B2 when compared to phylogroup A (29.8%,  $p < 0.001$ ), B1 (27.7%,  $p = 0.01$ ), and D (29.8%,  $p < 0.001$ ).

Statistically significant differences were found between groups A + B1 (mainly commensal *E. coli* strains) compared to groups B2 + D (mainly human extraintestinal pathogenic *E. coli* strains) comprising higher incidence of genetic determinants encoding colicin Ib ( $p = 0.01$ ), colicins Ia/Ib and microcin V ( $p = 0.02$ ), colicins B, Ia/Ib, M, and microcin V ( $p < 0.001$ ). In contrast, higher incidence of microcin determinants encoding mH47 ( $p < 0.001$ ), mM ( $p < 0.001$ ), and mB17 ( $p = 0.02$ ), were found in the phylogroups B2 + D.

**Conclusion:** Colicins B, Ia/Ib, M, and microcin V are all encoded on large plasmids and the presence of these plasmids appears to be an important characteristic of commensal intestinal *E. coli* strains. Synthesis of microcins H47, M, and B17 appears to be associated with increased virulence of *E. coli* strains either because of microcin synthesis itself or because of co-associated genes.

### **P1771** In vivo potential transfer of genes involved in SH2 production in *Escherichia coli* isolates implicated in a bacteraemic pyelonephritis

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**Objective:** The aim of this work was to characterise two multiresistant PFGE-related *E. coli* isolates recovered from a same blood culture of an elderly patient with bacteremic pyelonephritis. One isolate (C2535) was SH2-producer (SH2+), and the other one (C2534) non-SH2-producer (SH2-).

**Methods:** Identification of isolates was performed by microbiological and molecular methods. SH2 production was detected in Kligler's iron, Triple Sugar Iron agar medium and API20 system. Molecular typing of isolates was carried out by MLST and PFGE-XbaI. Susceptibility testing to 20 antibiotics was performed by Microscan® and by disk diffusion method (CLSI). Beta-lactamase genes and other 22 resistance genes were analysed by PCR. Mutations in GyrA and ParC proteins, integron characterization, phylogenetic group determination, as well as plasmid replicon typing (PBRT) were performed by PCR and sequencing. Transfer of SH2 + character was assayed by conjugation (receptor *E. coli* CHS26). Number, size and genetic characterization of plasmids were analysed by PFGE-S1 and subsequent hybridization with specific probes.

**Results:** *Escherichia coli* strains C2534 and C2535 showed indistinguishable PFGE-patterns, and belonged to ST448 and phylogroup B1. C2534 was SH2- and C2535 was SH2+. Both strains were resistant to ampicillin, nalidixic-acid, ciprofloxacin, chloramphenicol and streptomycin, whereas C2535 showed additional resistance to co-trimoxazole, tetracycline, gentamicin, tobramycin, kanamycin, and sulphonamides. Both strains showed amino acid changes in GyrA (S83L and D87N) and ParC (S80I) proteins, and presented *floR* and *strA-strB* genes. In addition, *sul2*, *tet(A)*, *aac(3)-II*, and *sul3* genes and also a class 1 integron (*intI1-aadA22-qacED1-sul1*) were detected in SH2+ C2535 strain. C2534 and C2535 carried plasmids type Inc11 and IncW, and C2535 also IncY. Three types of transconjugants were obtained from C2535. Two of them acquired the SH2+ character, the *tet(A)* gene and harboured the plasmids Inc11, IncW and IncY. One of these SH2-positive transconjugants also acquired the integron structure. The *tet(A)* gene hybridized in a 135 kb plasmid in *E. coli* strain C2535.

**Conclusion:** *Escherichia coli* can become SH2-producer by plasmid acquisition that also co-transferred the *tet(A)* gene. This transference could happen in vivo in the course of an infection and could difficult the correct identification of *E. coli*.

### **P1772** Growth dynamics of CC17 and non-CC17 (CC9, CC22, CC94, ST96, ST172) *Enterococcus faecium* revealed inter-and intra-clonal differences in fitness

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**Objectives:** *Enterococcus faecium* (Efm) has emerged as an important nosocomial pathogen specially due to the success of clonal complex

(CC) 17. Other Efm CCs with enhanced ability to colonize humans and/or animals are also frequent. This work analyzes the fitness of Efm CCs to understand the dynamics of Efm lineages among different hosts.

**Methods:** Sixty-one Efm strains of CC17 (eight ST18, five ST117, five ST19, three ST17, three ST203, two ST16, two ST103, one ST63, one ST442), CC9 (four ST25, three ST29, one ST9, one ST10, one ST21, one ST26, one ST97, one ST266), CC22 (two ST22, ST21, ST32, ST71, ST214, ST420 [1 each]), CC94 (two ST178, one ST85), CC69 (ST69), ST102 (2), ST172 (2) ST96 (1) and ST515 (1) were analysed. Isolates are representatives of these lineages for which comprehensive data including mobilome content (plasmids, transposons, GIs) were considered. Growth dynamics experiments were performed at 37°C using Bioscreen C apparatus. Optical Density (OD) was determined every 5 minute for 24 hour, plates being agitated 10 s before each measurement. Fitness was analysed by determining Growth Rates (GR) and Generation Time (GT) for each strain as described.

**Results:** Remarkable inter and intra-clonal variations of bacterial fitness were observed (average GR =  $0.7938 \pm 0.1246$  and average GT =  $53.92 \pm 9.57$  minute; GR ranging from  $0.4510 \pm 0.0225$  for ST19-CC17 to  $1.0336 \pm 0.0265$  for ST85-CC94). Ampicillin resistant (ApR) CC17 strains were consistently less fit than strains belonging to other CCs ( $0.7016 \pm 0.0828$  vs.  $0.8484 \pm 0.1127$ ). Within CC17, a clear difference in fitness was observed between ApR strains and ampicillin susceptible (ApS) strains ( $0.7016 \pm 0.0828$  vs.  $0.9165 \pm 0.0206$ ). Similar results were obtained for ApR and ApS ST102 clones ( $0.6824 \pm 0.394$  vs.  $0.8901 \pm 0.0165$ ). Among non-CC17 strains, CC94 was the best fit ( $1.0180 \pm 0.115$ ) followed by ST96 ( $1.0039 \pm 0.0455$ ), ST172 ( $0.8437 \pm 0.0152$ ), CC9 ( $0.8395 \pm 0.0592$ ), CC22 ( $0.8349 \pm 0.1229$ ), ST102 ( $0.7862 \pm 0.1039$ ), ST515 ( $0.755 \pm 0.191$ ) and ST69 ( $0.7365 \pm 0.1800$ ) isolates.

**Conclusions:** Non-CC17 strains, mainly CCs associated with human or animal colonization (CC94, CC9, CC22, ST96 and ST172), showed better fitness than CC17 strains. Acquisition of ApR either by CC17 or ST102 has a high cost for the bacteria ( $\pm 23\%$ ) while other ABR genes and plasmids did not influence bacterial fitness. Ecological conditions linked to the amplification and persistence of CC17-AREfm, which account for the majority of nosocomial associated infection caused by Efm, remains to be clarified.

#### **P1773** Comparative assessment of faecal bacteria composition in ulcerative colitis patient vs. healthy individuals

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**Objectives:** Ulcerative colitis (UC) is a class of chronic aggressive inflammation of gastrointestinal tract (GIT). Resident gut microflora has been proposed to play an important role in pathogenesis of human inflammatory bowel disease (IBD). We aimed to compare the composition of fecal flora under in situ conditions by evaluating the change in the diversity of gut flora from healthy to diseased state in northern Indian population.

**Methods:** The fecal flora composition of controls (n = 13) and UC patients (n = 25) with different disease activity was analysed by using fluorescent in situ hybridisation (FISH) in combination with Flow Associated Cytometry (FACS) by eight group specific probes belonging to predominant and subdominant genera. Controls under antibiotic treatment before three months of sample collection and the patients under probiotic treatment were excluded from the study. The data was statistically analysed by Student's *t*-test.

**Results:** We observed significantly lower representation of Bifidobacteria, ( $p = 0.0001$ ), *Clostridium coccooides* ( $p = 0.0001$ ) and *Clostridium leptum* ( $p = 0.0001$ ) while *Bacteroides* ( $p = 0.0001$ ), *Lactobacillus/Enterococcus* ( $p = 0.0003$ ), *Atopobium* ( $p = 0.0001$ ), and *Desulfovibrio* ( $p = 0.0011$ ) showed significant increase in severe UC conditions as compared to healthy individuals. However, the members of family Enterobacteriaceae did not show any significant change. During remission stage, except the members of *C. leptum* and *Desulfovibrio* others reverted back to normal level. Hundred percent

incidence rate of *Lactobacillus/Enterococcus* and *Desulfovibrio* members was observed in contrast to previous reports.

**Conclusion:** Dynamics of fecal bacteria composition changed during UC. Here the microbial imbalance during disease state is characterized by an increase in the ratio of gram positive to gram negative bacteria and consequent decrease in Firmicutes/*Bacteroides* ratio. This imbalance may contribute to disease development probably by upsetting the crosstalk between host epithelial cells and resident gutflora. The *C. leptum* and *C. coccooides* groups constituting firmicutes produce large amount of butyrate, which is the main energy source for colonic epithelial cells and play an important role in inhibiting the expression of pro inflammatory cytokines in the mucosa. Thus our finding supports the proposed "energy deficiency" hypothesis as a cause of ulcerative colitis.

#### **P1774** Colicin FY – a bacteriocin specifically killing pathogenic strains of *Y. enterocolitica*

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**Objectives:** Three *Yersinia* species (out of 17) are important human pathogens – *Y. pestis*, *Y. enterocolitica* and *Y. pseudotuberculosis*. Out of yersiniae, only one bacteriocin, pesticin I, was characterized on a molecular level so far. In this study, we have mapped the production of antimicrobial agents by 53 environmental yersiniae.

**Methods:** A set of 53 yersinia strains was tested on production of antimicrobial agents (comprising species *Y. frederiksenii*, *Y. kristensenii*, *Y. ruckerii*, *Y. aldovae*, *Y. rohdei*, *Y. intermedia*). Colicin activity assay was used to describe the activity-spectrum of bacteriocins. To identify the bacteriocin- and immunity protein-encoding genes, the whole plasmid DNA was isolated, sequenced and analyzed by transposon mutagenesis. The newly identified colicin FY was His-tagged and purified by Ni-NTA and ionex chromatography. Mechanism of its lethal action was revealed by planar lipid bilayer experiments. Receptor specificity was identified by in vivo chromosomal mutagenesis of sensitive strain *Y. kristensenii* with pNKBOR suicide-plasmid.

**Results:** In the set of yersiniae strains, we have identified six bacteriocin producers – *Y. frederiksenii* (one strain), *Y. intermedia* (one strain) and *Y. ruckerii* (four strains). Bacteriocin produced by *Y. frederiksenii* 27601 was further analyzed. This bacteriocin seems to specifically target yersinia strains, as it does not act against any other tested Enterobacteriaceae (20 strains out of five enterobacterial genera have been tested so far). Interestingly, this bacteriocin specifically kills *Y. enterocolitica* (98%, 45 out of 46 strains tested) in contrast to strains of *Y. pseudotuberculosis* (0%, 15 strains tested). Among the 53 environmental yersiniae, this bacteriocin was active on 14 strains (24%). Genes encoding the colicin (1317 bp) and immunity protein (338 bp) have been identified on a plasmid. Functional tests revealed its pore forming activity. Moreover, receptor binding domain interacting with yersinia-specific outer membrane protein YiuR was identified together with TonB-system responsible for its translocation.

**Conclusion:** We have described a novel colicin FY, isolated from a strain of *Yersinia frederiksenii*, its complete plasmid sequence (pYF27601), mechanism of its toxicity, corresponding receptor (YiuR), and translocation routes into a susceptible bacterium. This is the first colicin characterized in detail, which is active mainly against strains of *Y. enterocolitica*. Work was supported by NS9665-4.

#### **P1775** Taking the bull by the horns – a new high content molecular assay addressing complex ESBL detection

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Prompt initiation of appropriate antibiotic treatment and patient management strategies benefit hugely from rapid diagnostics.



Molecular assays offer the possibility for rapid testing but require a high degree of complexity if large numbers of molecular targets are to be covered. Today, a steadily growing number of 900 beta-lactamase variants is known and especially Extended Spectrum Beta Lactamases (ESBLs) and carbapenemases (e.g. NDM-1) pose a serious threat for patients and the healthcare system. To detect the large variety of these enzymes and associated genes a number of molecular strategies have been devised such as multiplex PCR, DNA microarrays and DNA sequencing but the challenge of upcoming new mutations and high demands for clinical routine use remain pressing.

We present a novel generic molecular assay principle demonstrated on the example of ESBL (TEM, SHV, CTX-M) and carbapenemase (NDM-1) caused antibiotic resistance which has the demonstrated potential to overcome these limitations. The test is rapid, equally compatible with point of care and automated high throughput lab based testing and provides an exceptional amount of sequence information with single nucleotide resolution without having to open the reaction tube. Most importantly, the test has the inherent capability to detect new variants which were not initially identified as targets of the assay reducing the likelihood of false negative results. The assay principle is based on Linear-After-The-Exponential (LATE)-PCR [1] and a novel Thermalight™ probe chemistry. It uses a characteristic fluorescence pattern if melting curve analysis is applied to a single strand amplicon produced by LATE-PCR. We designed individual assays for blaTEM, blaSHV, blaCTX-M, and blaNDM and tested them using fully characterised reference strains and clinical isolates. All tested strains and isolates were genotyped with our previously reported DNA microarrays system [2] and DNA sequencing and subjected to standard antibiotic susceptibility testing.

The Thermalight assay principle offers a fundamentally novel approach to diagnostic genotyping and is especially suited for the complex ESBL and carbapenemase detection. We will discuss its profile in light of the existing technologies and present opportunities to engage in wider epidemiological and multicenter studies towards the clinical diagnostic routine.

**P1776 Health-related quality of life (SF-36 Health Survey) and *Brucella melitensis* DNA levels in patients with chronic brucellosis**

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After antibiotic treatment of *Brucella* infection, a percentage of patients have nonspecific symptoms. The aim of this study was to assess differences in the health-related quality of life (HRQoL) and *B. melitensis* DNA levels in both symptomatic and asymptomatic subjects with a well-documented history of brucellosis.

**Material and Methods:** Sixty-one subjects with a well-documented history of brucellosis were recruited from the University General Hospital of Albacete (Spain) and screened for their HRQoL assessed by SF-36 questionnaire Health Survey (SF-36). Peripheral blood and serum samples from these patients were analyzed using quantitative real-time PCR (QrtPCR) to detect and quantify *B. melitensis* DNA (Clin Infect Dis 2006;42:1266-73). According to their clinical outcome, subjects were classified into two groups. Group A consisted of 23 patients who had been diagnosed of focal disease and/or presented nonspecific symptoms. Such patients are defined here as chronic brucellosis patients. Group B comprised 38 asymptomatic subjects.

**Results:** The internal consistency reliability of the SF-36 (assessed by Cronbach's alpha) was high, with a range 0.9–1 for group A and 0.8–1 for group B. By comparing the standardized scores of both groups, we observed that group A had diminished HRQoL compared to group B. We found statistically significant differences between groups in the following dimensions: physical functioning, role-physical, bodily pain, general health perceptions, vitality and mental health (*t*-Student,  $p < 0.05$ ). Asymptomatic subjects showed an overall HRQoL similar to that of the Spanish general population. In group A, 16 patients (70%) had bacterial DNA in blood and/or serum samples. In group B, 13 (34%) had a QrtPCR positive result in blood and/or serum. The

percentage of patients with QrtPCR positive results was significantly higher in group A than group B (chi-square;  $p = 0.007$ ). However, there was no significant difference between groups in *B. melitensis* DNA levels neither blood (Group A =  $797 \pm 934$  copies/mL (mean,SD); Group B =  $755 \pm 792$  copies/mL; *t*-Student;  $p = 0.88$ ) nor serum samples (Group A =  $1067 \pm 888$  copies/mL; Group B =  $756 \pm 820$  copies/mL; *t*-Student;  $p = 0.30$ ).

**Conclusions:** Chronic brucellosis patients included in this study showed an overall diminished HRQoL compared to those subjects with a history of brucellosis that remained asymptomatic. This group of patients with chronic brucellosis had a higher percentage of QrtPCR positive results than asymptomatic patients.

**P1777 Characterisation of viridans and non-haemolytic streptococci isolated from infective endocarditis patients with correlation to the clinical and echocardiographic presentations**

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**Background:** Viridans streptococci form a significant part of the normal flora of the oral cavity. They may become an opportunistic pathogen. They are major causative organisms of bacterial endocarditis occurring on native valves. The viridans group includes a variety of streptococcal species, including *Streptococcus sanguis*, *mitis*, *salivarius* and *mutans*.

**Objectives:** Characterization of viridans streptococci isolated from patients with infective endocarditis (IE). The evolved species were correlated with the echocardiographic data and the clinical outcome.

**Methods:** The study was conducted on 40 patients with acquired rheumatic valvular heart disease and ongoing IE. Typing of the evolved Viridans isolates was carried out both phenotypically and genotypically. Phenotyping was done using the API20-STREP kit as well as antibiogram. Genotyping was done using random amplified polymorphic DNA analysis (RAPD).

**Results:** Thirteen patients (32.5%) were proved to have definite viridans streptococcal IE according to the Duke university criteria. Of the 13 viridans streptococcal blood isolates, *S. salivarius* was the most frequent species (five isolates [38.4%]). *Streptococcus sanguis* ranked the second (four isolates [30.7%]). Three (23.3%) of the isolates were identified as *S. mutans* and one (7.6%) isolate was identified as *S. mitis*. Complications were seen in five cases (38.5%) and included persistent infection in two and peripheral emboli in three. In-hospital death occurred in one of them. Two-dimensional echocardiograms demonstrated vegetations in all of them and flail mitral valve in one. None of our patients had perivalvular abscesses. The frequency of complications was three in the *mutans* species(all), one in the *mitis* (all) and one in the *sanguis*. Antibiogram results revealed no specific patterns among strains. RAPD analysis of the 13 viridans strains resulted in 13 different RAPD patterns.

**Conclusions:** Viridans streptococcal endocarditis can at times produce serious disease similar to that associated with organisms considered to be more virulent. Despite being infrequently isolated; *mutans* and *mitis* biotypes were associated with major complications. While RAPD analysis has a superior discriminatory power, yet biotyping of *Streptococcal viridans* endocarditis strains might be more useful in identifying patients at risk for unfavorable clinical outcome.

**P1778 Adhesion to epithelial cells, capsule production and transcription of fimbriae associated genes in *Porphyromonas gingivalis***

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**Objectives:** Isolates of the periodontopathogenic specie *Porphyromonas gingivalis* exhibit differences in the expression of surface components involved in interaction of bacteria with epithelial cells. This study aimed to evaluate the effect of interaction with epithelial cells on the transcription of fimA, encoding the fimbrial

subunit, in capsulated and non capsulated isolates of *P. gingivalis* of different fimA genotypes.

**Methods:** Fifteen clinical isolates of *P. gingivalis* and two reference strains (ATCC33277 and W83) were evaluated. Capsule was detected in exponential grown of anaerobic cultures by negative staining and microscopy. Adhesion efficiency to gingival epithelial cells (OBA09) was determined after 2 hour interaction. FimA genotyping was performed by PCR using type specific primers. fimA relative transcription was evaluated by RT-qPCR after interaction with epithelial cells and compared with control condition.

**Results:** Eight strains exhibited a detectable capsule. Adhesion efficiency ranged from 1.95% to 5.66% for the capsulated and from 1.19% to 6.12% for the non-capsulated isolates, with no difference in mean adhesion levels between capsulated and non-capsulated isolates. The two non-capsulated type II isolates, but not the capsulated ones, exhibited statistically significant higher adhesion efficiency than the others. Transcription of fimA was very low for most capsulated isolates, in both tested conditions (adhesion and control) except for two strains, with fimA mRNA levels >ATCC 33277 in control. Adhesion to epithelial cells resulted in no fold change/increased transcription of fimA in two out of eight non-capsulated isolates, whereas this condition led to a decrease in fimA transcripts levels in four out of nine capsulated. However, the fimA mRNA levels in one capsulated strain increased after epithelial cell interaction, suggesting a role of fimbriae in this strain.

**Conclusion:** fimA is positively regulated after interaction with epithelial cells in most non-capsulated isolates, confirming the role of the main fimbriae in interaction of *P. gingivalis* with epithelial cells. However, regulation of fimA seems to be strain specific, since it is transcribed at very low levels in most capsulated isolates, even after adhesion to epithelial cells, indicating that other components besides the main fimbriae may be involved in this interaction. FAPESP grants 09/50191-1 and 09/53958-1.

#### **P1779** Possible association between bacterial infections and HPV in cervical lesion progression

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**Objectives:** High risk human papillomaviruses (hr-HPV) are known to be the etiological agents of cervical cancer disease. On the other hand, other cofactors are considered to be important in cervix carcinogenesis.

**Methods:** In order to evaluate the possible role of certain sexual transmitted bacterial pathogens in cervical lesions progression, cervical specimens (from 140 women, 18–55 years old) were investigated. DNA was isolated (QuickGene DNA tissue kit; FujiFilm) from cervical cells from patients with different cytology (normal cervical epithelium, LSIL-Low-Grade Intraepithelial Lesion, HSIL-High-Grade Intraepithelial Lesion and SCC-Squamous Cell Carcinoma) and tested for HPV DNA presence (Seeplex HPV4A ACE Detection kit, Seegene and Innolipa Genotyping kit; Innogenetics). To elucidate a causative role of *Neisseria gonorrhoeae* (NG), *Chlamydia trachomatis* (CT), *Mycoplasma hominis* (MH), *Mycoplasma genitalium* (MG), *Ureaplasma urealyticum* (UU) and *Trichomonas vaginalis* (TV) in cervical lesions, Seeplex STD6 ACE Detection kit (Seegene) was used.

**Results:** Our data revealed the fact that 66.6% (20/30 cases) of LSIL patients were positive for HPV DNA, present in single or co-infections. The most prevalent HPV genotypes in this group of patients were HPV6 (20%), HPV11 (13.3%), HPV51 (13.3%) and HPV18 (6.6%) present in single infections. In HSIL patients group, 80% (24/30 cases) of the women presented HPV DNA in single infection (66.5%) or co-infection (33.5%) and HPV16 (33.3%) and HPV31 (20%) were the most prevalent high-risk genotypes. Regarding HPV infection in cervical cancer patients group, 86.6% (52/60 cases) of the patients presented either infection with a single high risk HPV genotype (60%) or a co-infection with multiple HPV genotypes (26.6%). Following STD multiplex detection, 35% (21/60 cases) of SCC cases, 16.6% (5/30) of LSIL cases and 33.3% (10/30) of HSIL cases presented infection with at least one bacterial pathogen. The most frequently found

pathogen was CT (in 18/36 cases), followed by MG (7/36) and UU (5/36 cases).

**Conclusion:** The present study provides evidence that some associated bacterial infections may be cofactors involved in HPV-induced cervical dysplasia. The role of CT, NG, MG, MH, UU and TV in cervical lesion progression is extremely difficult to prove conclusively. These may play a role in cervical precursor lesions and cancer but their role in the mechanism of carcinogenesis remains to be solved.

#### **P1780** Role of multiplex PCR and IL-6 in cerebrospinal fluid for diagnosis of ventriculomeningitis in neurosurgery patients

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**Objective:** The purpose of this study was to assess the performance of a multiplex real-time PCR in combination with interleukin-6 (IL-6) measurements for diagnosis of EVD-related ventriculomeningitis in cerebrospinal fluids (CSFs) from neurosurgery ICU-patients.

**Methods:** In this observational cohort study, 62 CSF samples were obtained from 41 neurosurgical patients with suspicion of EVD-related ventriculomeningitis. Concentration of CSF parameters, including lactate and IL-6, were measured and microbiological CSF cultures were performed. In addition, 1.5 mL from the CSF was subjected to a commercial multiplex real-time PCR assay (SeptiFast, SF; Roche Diagnostics, Mannheim, Germany) which detects simultaneously 25 nosocomial-relevant bacteria and fungi within 6 hours. For comparison of IL-6 and lactate concentrations with pathogen detection by PCR, receiver operating characteristic (ROC) curves were used, and the optimal cut-offs and odd ratios were calculated.

**Results:** Seventeen CSF samples were culture and PCR positive which results in a concordance of 91%. In one case a pathogen (coagulase-negative staphylococci, CoNS) could only be detected by SF. Four samples were CSF culture positive but SF negative from which three isolates (CoNS) were considered to be contaminations. ROC analysis demonstrated an area under the curve (AUC) of 0.90 for intrathecal IL-6 and 0.77 for lactate. The calculated cut-off for IL-6 was 3100 pg/mL, resulting in a sensitivity of 88% and specificity of 98% with an odds ratio of 9.60 (95% CI: 2.35–39.11) for pathogen detection in CSF by multiplex PCR.

**Conclusion:** The results of the present study indicate that performing multiplex PCR in CSF samples with increased IL-6 concentrations (>3100 pg/mL) might be powerful diagnostic tool for the rapid etiologic diagnosis of EVD-related ventriculomeningitis in neurosurgical ICU-patients.

#### **P1781** Direct sequencing and RipSeq analysis as a tool for identification of polymicrobial bacteraemia

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**Objective:** Direct sequencing has become an important supplementary tool for identification of microorganisms in culture-negative infections. However, the combination of broad-range PCR and direct sequencing is not compatible with polymicrobial samples, as it gives mixed sequencing chromatograms. The commercially available tool RipSeq Mixed separates chromatograms resulting from up to three different species.

In a previous study, 293 blood samples were examined by cultivation based methods and direct sequencing for comparison. For 15 samples direct sequencing was invalid despite that one or more species were identified by cultivation. In this study the chromatograms of these 15 samples were analyzed using RipSeq Mixed to see if this would affect the outcome of direct sequencing.

**Methods:** The BACTEC 9240 system was applied for aerobic and anaerobic blood cultivation, and positive samples were subject to further cultivation steps and colony identification. Concurrently, direct 16S sequencing was performed on culture-positive samples using the

3130xl Genetic Analyzer and MicroSeq system (Applied Biosystems) and chromatograms from invalid sequencing were analyzed with RipSeq Mixed (Isentio). For comparison, clone libraries of nearly full length 16S rRNA genes were constructed for selected samples and species specific real-time polymerase chain reaction (PCR) was carried out.

**Results:** RipSeq analysis of the sequencing chromatograms revealed bacterial DNA in all 15 samples and for 12 samples (80%) DNA from at least two different species was identified. The results of the RipSeq analysis corresponded well with the cultivation data and the clone libraries, and real-time PCR confirmed several of the findings. However for some samples, bacteria were identified by one method that could not be confirmed by any other method.

Table 1. Fifteen blood samples found to be polymicrobial by cultivation but negative by direct sequencing were analyzed by RipSeq Mixed. The RipSeq algorithm assigns a similarity score to each result, and results below 99.3% identity are left out. The RipSeq results are listed according to the score. For selected samples clone libraries were constructed and real-time PCR performed. “(an)” and “(ae)” indicate anaerobic or aerobic blood cultivation, respectively. “CoNS”: Coagulase-negative staphylococci. “n.n.”: nearest neighbor to the BLAST hits referred to as uncultured bacteria

Sample	Species	Similarity Score (%)	Number of Reads	Nearest Neighbor
1	<i>Enterobacteriaceae</i>	99.3	12	<i>E. coli</i>
	<i>Streptococcus</i>	99.3	8	<i>S. pneumoniae</i>
2	<i>Staphylococcus</i>	99.3	15	<i>S. aureus</i>
	<i>Streptococcus</i>	99.3	10	<i>S. pneumoniae</i>
3	<i>Streptococcus</i>	99.3	20	<i>S. pneumoniae</i>
	<i>Enterobacteriaceae</i>	99.3	15	<i>E. coli</i>
4	<i>Streptococcus</i>	99.3	18	<i>S. pneumoniae</i>
	<i>Enterobacteriaceae</i>	99.3	12	<i>E. coli</i>
5	<i>Streptococcus</i>	99.3	22	<i>S. pneumoniae</i>
	<i>Enterobacteriaceae</i>	99.3	18	<i>E. coli</i>
6	<i>Streptococcus</i>	99.3	25	<i>S. pneumoniae</i>
	<i>Enterobacteriaceae</i>	99.3	20	<i>E. coli</i>
7	<i>Streptococcus</i>	99.3	30	<i>S. pneumoniae</i>
	<i>Enterobacteriaceae</i>	99.3	25	<i>E. coli</i>
8	<i>Streptococcus</i>	99.3	35	<i>S. pneumoniae</i>
	<i>Enterobacteriaceae</i>	99.3	30	<i>E. coli</i>
9	<i>Streptococcus</i>	99.3	40	<i>S. pneumoniae</i>
	<i>Enterobacteriaceae</i>	99.3	35	<i>E. coli</i>
10	<i>Streptococcus</i>	99.3	45	<i>S. pneumoniae</i>
	<i>Enterobacteriaceae</i>	99.3	40	<i>E. coli</i>
11	<i>Streptococcus</i>	99.3	50	<i>S. pneumoniae</i>
	<i>Enterobacteriaceae</i>	99.3	45	<i>E. coli</i>
12	<i>Streptococcus</i>	99.3	55	<i>S. pneumoniae</i>
	<i>Enterobacteriaceae</i>	99.3	50	<i>E. coli</i>
13	<i>Streptococcus</i>	99.3	60	<i>S. pneumoniae</i>
	<i>Enterobacteriaceae</i>	99.3	55	<i>E. coli</i>
14	<i>Streptococcus</i>	99.3	65	<i>S. pneumoniae</i>
	<i>Enterobacteriaceae</i>	99.3	60	<i>E. coli</i>
15	<i>Streptococcus</i>	99.3	70	<i>S. pneumoniae</i>
	<i>Enterobacteriaceae</i>	99.3	65	<i>E. coli</i>

**Conclusion:** Analysis of sequencing chromatograms with RipSeq Mixed revealed DNA from 1 to 3 different bacterial species in all 15 samples where direct sequencing was initially invalid. RipSeq Mixed thereby improved the performance of direct sequencing considerably. Generally there is a risk of detecting clinically irrelevant DNA residing in the sample when applying DNA based methods. To make sure that only active microorganisms are detected, the less stable RNA could be targeted instead of DNA. However, this study is based on culture-positive samples and therefore the findings are assumed to result from active bacteria.

**P1782 Molecular monitoring of dominant aerobic and anaerobic bacteria in intestinal microbiota of pre-term infants during first months of life**

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Microbial colonization of human gastrointestinal tract (GIT) starts from first days of life, and depends on several factors i.e. mode of birth, composition of maternal microflora, diet, environmental conditions and use of antibiotics. The gut of full-term infants is colonized with facultative anaerobic bacteria during first 2 weeks of life, next follow anaerobic bacteria. However, the colonisation of intestinal tract of pre-term infants is obscure.

The aim was to assess the differences in the colonization of dominant aerobic and anaerobic bacteria in the GIT of pre-term infants after treatment with combination of gentamicin (GEN) and penicillin (PEN) or ampicillin (AMP) in two Estonian hospitals.

**Methods:** Study group comprised of 20 pre-term infants (mean weight: 868 g; min-max: 574–1130 g) born in two Estonian hospitals (Tartu University hospital; n = 10) and Tallinn Children’s hospital; n = 10). Fecal samples were collected in first week, first and second month. All infants were treated with either PEN (n = 10) or AMP (n = 10) combined with GEN during the first week of life. The molecular diversity of intestinal bacteria was analyzed using denaturing gradient gel electrophoresis of the amplified 16S rRNA genes, followed by cloning and sequencing.

**Results:** Enterobacteriaceae (*E. coli*, *Klebsiella* sp., *Enterobacter* sp., *Citrobacter* sp.), *Streptococcus* sp., *Veillonella* sp., *Bacteroides* sp. and *Clostridium* sp. dominated in the GIT of pre-term infants during first 2 months. Almost half of the samples (9/20) obtained in the first week were bacteria negative. Enterobacteriaceae was detected less (1/5 vs. 5/5) in the first week in the samples of infants treated with PEN + GEN, in 1 month less with aerobic bacteria (3/10 vs. 6/10) and more with anaerobic bacteria (7/10 vs. 4/10) if compared to AMP + GEN group. Comparison in the colonization of infants of two hospitals showed differences in the colonization of Enterobacteriaceae (5/10 vs. 1/10) in the first week, in gram-negative anaerobes (7/10 vs. 2/10) in first month and in gram-positive anaerobes (0/10 vs. 4/10, p = 0.033) in first month. There were no differences in colonization between groups and hospitals in the samples of second month.

**Conclusion:** The different antibiotic treatment regimens (PEN + GEN vs. AMP + GEN) and environments (two hospitals) influence the colonization of gut by aerobic and anaerobic bacteria during first month of life of pre-term infants.

**P1783 Leptospira borgpetersenii in British rodents – a cause for concern?**

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**Objectives:** Our over-arching objective was to elucidate the role of British-caught mice as a reservoir for Leptospirosis.

Previously, the role of *Leptospira borgpetersenii* in mice as a potential reservoir for infection has been largely overshadowed by those strains found in rats. Recent evidence from countries such as Australia and New Zealand have suggested recent expansions of *L. borgpetersenii* infections, which coupled with the demonstration of full pathogenic potential of *L. borgpetersenii* in the guinea pig model and recovery from human cases, underscore the need for surveillance.

**Methods:** We investigated kidney tissues collected from 292 wild-caught rodents from northern England/Scotland. Additionally we assessed (20 bovine; 120 ovine and 60 not stated) livestock cotyledon (countrywide). DNA was extracted from tissues using DNeasy (Qiagen) and used for real-time PCR specific for pathogenic leptospires targeting the 16S rRNA gene and with positives confirmed using a real-time assay for LipL32. The identity of leptospires was disclosed by amplification and sequencing of secY (S10-spc- alpha locus).

**Results:** Of the rodent kidneys tested, 55/292 (19%) were found to be positive, with 31 of these being confirmed using the LipL32 assay. The livestock cotyledon failed to produce any positive findings. Sequencing revealed that the infecting species was *L. borgpetersenii*. As no cultivable isolates were available serotyping was not possible. Phylogenetic analysis of sequences generated showed all clustered closely together amongst other *L. borgpetersenii* strains. Interestingly, *L. borgpetersenii* sequences from GenBank appeared to fall into two different clades.

**Conclusion:** Wild rodent samples revealed that a fifth (19%) of samples were positive for *L. borgpetersenii*. Despite this, infection with *L. borgpetersenii* was not apparent among livestock species, possibly resulting from test sample differences or through the poor ability of *L.*

*borgpetersenii* to persist within environmental locations. Recent reports of human infections with *L. borgpetersenii* coupled with demonstration of virulence of this strain in the guinea pig model and the apparent increase of *L. borgpetersenii* in some countries, underscore our need to remain vigilant.

**P1784 Infectious endocarditis: 16S rDNA sequencing can help guiding treatment**

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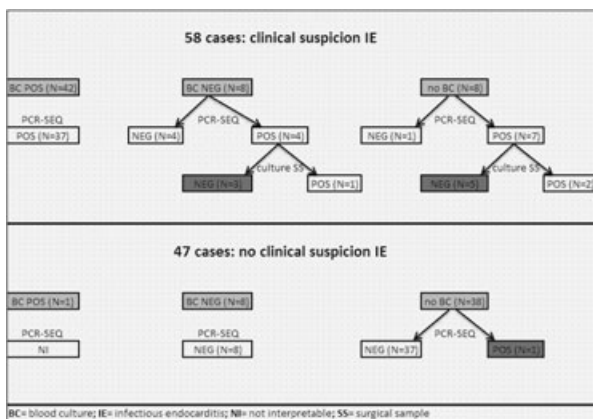
**Objectives:** In order to assess the added value of PCR followed by sequence analysis (PCR-SEQ) to classic microbiological diagnostics of infectious endocarditis (IE), a retrospective study over a 4-year period was conducted.

Originally PCR-SEQ on cardiac surgical samples was performed when IE was clinically suspected. Starting from November 2008, PCR-SEQ was performed on all cardiac surgical samples received at the microbiology laboratory.

**Methods:** From November 2007 to November 2011, 154 surgical samples (heart valves [N = 151], valve rings [N = 3]) were received from 105 patients. All samples were analyzed by nested PCR with primers on 16S-rDNA followed by sequencing and BLAST-analysis. For all patients, medical charts were examined and the presence of positive blood cultures (BC) before surgery, the culture results of the surgical sample(s) (SS), serology, clinical and echocardiographic data were recorded.

**Results:** IE was clinically suspected in 58 patients with positive echocardiographic findings. In 42 of them, blood cultures prior to cardiac surgery were positive. Germ identification with PCR-SEQ was in 37 cases identical with the culture results, no bacterial DNA was detected in three cases, and in two cases a different micro-organism was found. In eight of 58 patients, blood cultures were negative. Here, the etiological agent was found with PCR-SEQ in four patients, of which culture of the SS remained negative for three samples. For these three patients, a history of previous antibiotic treatment was available. An etiological agent was not found for the remaining four patients with negative BC and negative PCR-SEQ. In a third group of patients (8/58), no blood cultures were taken before surgery. The etiological agent was found with PCR-SEQ in seven of eight patients, while the culture of the SS remained negative in five cases. For three out of these five patients, a history of previous antibiotic treatment was available. Altogether, an etiological agent was found exclusively by PCR-SEQ of the SS for 13.8% (8/58). Results are shown in Fig. 1.

In 47 patients from whom a cardiac surgical sample was received, no prior clinical suspicion existed for IE. Here, one case of IE was discovered only by PCR-SEQ of the heart valve.



**Conclusion:** The application of molecular diagnostics on cardiac surgical samples is useful, even sometimes when infectious endocarditis is not suspected on clinical grounds. In 8.6% (9/105) of cases, an etiological agent was found solely by PCR-SEQ of the surgical sample(s).

## Molecular diagnosis of gastrointestinal pathogens

**P1785 First Italian experience in clinical practice of the gastrointestinal pathogen panel using a unique multiplexing technology at a Bologna hospital**

C. Vocale\*, M.P. Landini, V. Sambri (Bologna, IT)

**Objective:** xTAG<sup>®</sup>GPP, produced by Luminex Molecular Diagnostics, is a new qualitative multiplex PCR assay to detect simultaneously 15 different pathogens responsible for hospital acquired infection, such as *Clostridium difficile*, foodborne illness agents like *Salmonella* and common pediatric diarrhea causatives such as a Rotavirus in a single human stool sample. The aim of this study was to establish the clinical performance of the GPP assay to detect the clinically relevant gastrointestinal targets in patients with gastrointestinal infection.

**Methods:** Three hundred eighty-five raw stool/stool in Cary-Blair media, from hospitalized symptomatic patients were collected and tested with GPP using Luminex<sup>®</sup> 200<sup>®</sup> instrument. The samples were pre-treated as described in the package and then underwent to automated nucleic acid extraction using NucliSENS EasyMAG<sup>®</sup> method (BioMérieux<sup>®</sup>, France). The GPP assay includes a RNA based internal control (MS2 bacteriophage) which was spiked into each samples prior to extraction, in order to help users troubleshoot and assess assay performance. The RT-PCR and the following hybridization reaction were performed according to the xTAG<sup>®</sup>GPP manual by a single trained operator. We use the recommended thermal cycler Mastercycler<sup>®</sup> gradient (Eppendorf<sup>®</sup>, Germany). Analysis of signal and data acquisition were carried out using TDAS Software. To establish clinical performance, all specimens were run using various comparator methods too. All clinical specimens were tested following routine algorithm: a culture for bacteria detection, an EIA assay for viruses detection and the microscopic assay for the parasites identification.

**Results:** xTAG<sup>®</sup>GPP has very good sensitivity and specificity and shows an excellent performance against the reference method routinely used. 104/385 samples tested gave a positive results (27%) against 13% tested routinely. Ten percent of positives were co-infected with two different targets while one sample showed the presence of three simultaneous different germs. With the Luminex method, the percentage of positivity in samples tested has increased by almost 50%.

**Conclusion:** xTAG<sup>®</sup>GPP can be considered a hopeful tool for detection and identification of gastroenteritis pathogens, using a simple procedure with a hands-on time of 5 hours. Moreover using xTAG<sup>®</sup>GPP we will be able to identify undiagnosed infection as well as co-infection. Finally, this assay will provide fast and accurate data for hospitalized patients management and epidemiological surveillance.

**P1786 Infectious gastroenteritis: comparison of conventional and molecular methods for detection of pathogens**

D. Ciardo\*, O. Dubuis, D. Burki, C. Noppen, E.H. Viollier (Allschwil, CH)

**Objectives:** Infectious gastroenteritis is a disease with an important impact worldwide. Specific therapy is only possible if the pathogen is known. Two newly developed multiplex PCR assays allow the simultaneous detection of a broad range of pathogens. The accuracy and usefulness of these assays in a diagnostic laboratory was evaluated by comparison with conventional methods.

**Methods:** One hundred and twenty-six routine stool specimens from patients with gastroenteritis were examined. Conventional detection consisted in culture on selective media and in specific EIA. For Noroviruses, RT-PCR on LightCycler was used.

Two commercially available multiplex-PCR assays were tested. The Seplex Diarrhea ACE assay starts with three parallel amplification reactions by dual priming oligonucleotides and detection by auto-capillary electrophoresis. The xTAG GPP assay is based on a single one-step (RT)-PCR followed by target specific primer extension,

hybridisation to specific xTAG beads and laser detection (Luminex technology).

**Results:** In 42 of 126 routine stool specimens, following pathogens were found: *Campylobacter* spp., *Clostridium difficile* Toxin, *Salmonella* spp., *Shigella* spp., *E. coli* Verotoxin, *Yersinia enterocolitica*, Norovirus, Rotavirus. This reflects the pathogens normally found to cause infectious gastroenteritis in Switzerland. For six specimens, only one of three methods was positive. Pathogen detection with two of three methods was possible in six cases, and all three methods gave an equal result in 30 stool samples. The two multiplex assays gave identical results in 116/126 samples. No pathogen was found in 84 specimens.

**Conclusions:** Multiplex PCR allows a rapid diagnosis of a wide range of infectious gastroenteritis causing pathogens within one assay. The definition of a higher cut-off than proposed by the manufacturer allowed a better correlation compared to conventional methods. For less frequent pathogens like protozoa further evaluations are needed. Country-specific spectra of pathogens should be furthermore considered for the choice of an assay.

**P1787 Use of a multiplex molecular assay for the detection of pathogens in stools from diarrhoeic patients**

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**Objectives:** The Luminex Universal Array is a proprietary solution-based microarray that can combine any set of 100 single DNA tests and perform them simultaneously in a single reaction. The xTAG® Gastrointestinal Pathogen Panel (xTAG GPP) is a qualitative molecular multiplex diarrhoea test which can perform the detection of 15 bacteria, viruses or parasites in <5 hours.

The objectives of the present study were:

1 Evaluate the xTAG® Gastrointestinal Pathogen Panel (xTAG GPP) for the microbiological diagnosis of diarrhoeas.

2 Compare the results with those of classical microbiological techniques

**Methods:** 1 Diarrheal stools samples collected from immunocompromised children (53) and adults (113), from children attending the emergency unit (119) and from children hospitalized in neonatology unit (60) were tested for the presence of the xTAG panel pathogens (*Salmonella*, *Shigella*, *Campylobacter*, *Clostridium difficile* toxin A/B, Enterotoxigenic *E. coli* LT/ST, *E. coli* O157, Shiga like toxin producing *E. coli*, *Vibrio cholerae*, *Yersinia enterocolitica*, Adenovirus 40/41, Rotavirus A, Norovirus GI/GII, *Giardia*, *Entamoeba histolytica*, *Cryptosporidium*).

2 Conventional bacteriological cultures, molecular detection of *E. coli* shigatoxin like gene, Exacto Combo Adeno Rota® All Diag and ImmunoCard STAT Norovirus® Meridian Bioscience were used for comparison.

**Results:** 1 fourteen samples from adults were positive for at least one pathogen whereas 113 samples from children were ( $p < 0.0001$ ). Among them 93 were attending the emergency unit ( $p < 0.0001$ ).

2 one hundred and fifty-six pathogens were detected: 98 viruses, 42 bacteriological markers, and 16 parasites. Twenty-nine coinfections were diagnosed; all of them involved a viral pathogen.

3 The xTAG GPP assay was statistically more sensitive ( $p < 0.0001$ ) than culture for *Salmonella* detection, *C. difficile* Toxin detection and viruses immunochromatographic assays. Similar results were observed for *Campylobacter* detection ( $p = 0.02$ ).

4 The xTAG GPP assay was statistically less sensitive ( $p < 0.01$ ) than multiplex PCR detection for shiga like toxin producing *E. coli*.

**Conclusion:** The xTAG GPP assay performed well in comparison to conventional culture or immunochromatographic assays for the detection of gastrointestinal pathogens and provided useful informations in <5 hours. Most of pathogens were detected among children attending the emergency unit. Current results showed that pathogens were unfrequently detected in stools of diarrheic immunosuppressed patients.

**P1788 A novel molecular-based diagnostic screening test utilising the NanoCHIP® microarray technology for simultaneous detection of gastrointestinal protozoan parasites and bacterial infections**

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**Objectives:** Infectious Gastroenteritis is a global health problem associated with extremely high morbidity and mortality rates. Accurate diagnosis is crucial to allow appropriate and timely treatment. Stool testing at the microbiology laboratory is currently a complex, time consuming and cumbersome process, demanding highly qualified personnel and application of a wide range of techniques. Thus, workload, lab space and turnaround time are high and costly. Savyon Diagnostics has recently finalized the development of a novel molecular-based diagnostic screening test for simultaneous detection of eight bacterial and protozoan parasitic pathogens accounting for the majority of infectious gastroenteritis cases on its proprietary NC400 NanoChip® molecular electronic microarray system. The bacterial panel includes *Salmonella*, *Shigella*, *Campylobacter*, *Clostridium difficile*, and its toxins A and B. The parasitic panel is composed of *Entamoeba histolytica*, *Giardia lamblia*, *Dientamoeba fragilis* and *Cryptosporidium* spp. The aim of this work is to demonstrate the utility of the NanoCHIP technology for screening large number of samples for simultaneous detection of pathogenic bacteria and parasites directly from stool.

**Methods:** DNA was extracted from characterized stool samples using a variety of readily available manual and automatic methods. Specific bacterial and parasitic genes were amplified through multiplex PCR and subjected to the NanoCHIP system. The generated amplicons were electronically addressed to discrete loci on the NanoCHIP cartridge, pre-activated with specific capture oligonucleotides. Detection was achieved through specific fluorescent reporter oligonucleotides. Culture, microscopy, EIA or RT-PCR of the corresponding stool samples served as reference methods.

**Results:** The NanoCHIP results were in complete accordance with the characterizations of the tested samples in terms of clinical sensitivity and specificity. The Nanochip multiplex analysis provided clear results about the identity of the pathogen, either bacterium or protozoan parasite, within a working day time frame.

**Conclusions:** The NanoCHIP has proven to be a useful platform for medium-high throughput screening of stool samples for reliably detection of bacterial or parasitic gastrointestinal infections. This technology presents significant advantages, mainly in terms of minimal hands-on time, improved laboratory workflow and turn around time, enabling flexibility and saving costs.

**P1789 Multiplex PCR detection of all major gastrointestinal pathogens employing a novel universal extraction method**

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**Objectives:** Annually in the US acute gastroenteritis (GI) accounts for >1.5 million outpatient visits/year and for 200 000 hospitalisations/year. Rapid, simple and accurate diagnostic methods are required to ease the burden on hospital and pathology labs and provide better patient diagnosis and care. We have developed rapid real time multiplex PCR (mPCR) assays for all major GI pathogens. All assays share a universal sample processing method and incorporate our previously described 3base™ technology.

**Methods:** We developed mPCR panels for bacteria (*Salmonella*, *Shigella*, *Campylobacter*, *Listeria*, *Yersinia* and *Clostridium difficile*), parasites (*Cryptosporidium*, *Giardia*, *Entamoeba histolytica*, *Dientamoeba fragilis* and *Blastocystis hominis*) and viruses (*Rotavirus*, *Norovirus* I/II, *Adenovirus*, *Astrovirus* and *Sapovirus*). A reflex assay can identify any hypervirulent strains of *C. difficile*, such as ribotype 027. A universal sample processing method was devised that lysed bacteria, viruses and protozoan parasites in the primary patient

sample and simultaneously converted all nucleic acids, be they DNA or RNA. The buffer protects the nucleic acids from the harsh conditions required for complete lysis. The method comprises a single tube sample preparation followed by mPCR detection of bacteria, parasites and viruses. The method is compatible with automated laboratory systems such as Roche, Qiagen and Thermo platforms and most real-time instruments including those from Roche, Qiagen, Cepheid, ABI, Biorad and Stratagene.

**Results:** All assays were linear from  $10^{6-10}$  copies and no cross reactivity was observed between individual primers and a larger number of bacterial and fungal agents. Over 400 clinical samples have been assessed and compared to conventional techniques such as culture, EIA and microscopy with excellent concordance. The method developed here is therefore suitable to the rapid and sensitive screening of primary patient material.

**Conclusions:** The assays developed here may be used as a complete system for the diagnosis of all major GI pathogens from primary clinical samples. The assays are simple and use universal sample preparation conditions. All assays have incorporated controls for sample processing and inhibition. The assays can be run on virtually all purification and real time instruments found in major hospital and pathology laboratories. Sample to results time is <3 hours, allowing for rapid patient diagnosis and management.

#### **P1790** Diagnosis of gastroenteric infections: comparison of traditional methods with the new molecular technologies

J.M. Marimón\*, M. Montes, M. Gomariz, G. Cilla, E. Pérez-Trallero (Donostia-San Sebastian, ES)

**Introduction:** Complete diagnosis of infectious gastroenteritis implies the detection of pathogenic bacteria, viruses and/or parasites what requires a specific microbiological procedure for each one (bacterial culture, ELISA or PCR for viruses, microscopy for parasites) being time consuming. New microbiological molecular tools allow the detection of multiple and different pathogens within the same sample.

**Material and methods:** A total of 387 human stool clinical samples collected in 2010 and 2011, 233 retrospective ( $-80^{\circ}\text{C}$  frozen samples) and 154 prospective, were analysed for enteric pathogens. Routine enteropathogen detection included standard stool-culture for bacteria; ELISA (ProSpect, OXOID) or an in-house PCR for rotavirus, an in-house real-time PCR for norovirus, microscopy for *Giardia lamblia* and microscopy and/or an in-house PCR for detection of *Entamoeba*.

All samples were tested with the Luminex xTAG-GPP<sup>®</sup> capable of simultaneous detection of 15 enteropathogens: nine bacteria, three viruses and three parasites. Automated total nucleic acid extraction was done using the Nuclisens EasyMag (bioMérieux) without any previous treatment. Eighty-two percent samples were rectal swabs. Rectal swabs and stools were resuspended in B199 medium prior to extraction.

**Results:** A total of 225 positive stools for enteropathogens by standard techniques (173 retrospective and 52 prospective) including 16 positive to more than one pathogen, were studied. Overall agreement with the Luminex xTAG-GPP<sup>®</sup> was observed in 189 (84%) samples. Most *Campylobacter* (61/63), rotavirus (63/65) and norovirus (25/26) were detected. However 7/42 *Salmonella* were not detected with the Luminex xTAG-GPP<sup>®</sup>, but they could neither be detected using another commercial PCR. In six *Salmonella* positive cultures the Luminex xTAG-GPP<sup>®</sup> detected the *Salmonella* together with an *Entamoeba*.

Of the 162 negative samples (60 retrospective and 102 prospective) agreement was observed in 138 (85%). In 12/24 negative samples the Luminex xTAG-GPP<sup>®</sup> identified a rotavirus, most of them confirmed by an in-house rotavirus PCR.

Performance time for Luminex xTAG-GPP<sup>®</sup> was about 5 hours, working in batches of 24 samples. By standard technologies, more than 48 hours were needed to obtain final results.

**Conclusions:** The Luminex xTAG-GPP<sup>®</sup> technology demonstrated good and quick results in the screening of human enteropathogens. The lack of a gold standard technology makes difficult to assess the complete performance of this new technology.

#### **P1791** Development of a TaqMan array card for the simultaneous detection of 16 pathogens causing gastroenteritis

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**Objectives:** Gastroenteritis is a very common infectious disease syndrome of humans. Yearly, around 5 billion cases of diarrhea occur worldwide. Many microbial pathogens cause acute gastroenteritis making diagnosis a laborious task. In recent years molecular tools like multiplex PCR have been developed which significantly aid in the diagnosis of gastroenteritis. Although multiplex PCR reduces the number of PCR reactions needed, still many tests have to be performed to cover all pathogens. Here, we describe the development and validation of a microfluidic molecular device for the simultaneous detection of 16 pathogens involved in gastroenteritis.

**Methods:** We developed quantitative real-time PCR assays for the detection of three viral pathogens (Adenovirus, Norovirus and Rotavirus), eight bacterial pathogens (*STEC*, *Shigella*, *Salmonella*, *Campylobacter coli*, *C. lari*, *C. jejuni*, *Yersinia enterocolitica*, *Clostridium difficile*) and five parasitic pathogens (*Giardia lamblia*, *Entamoeba histolytica*, *Cryptosporidium parvum*, *Dientamoeba fragilis* and *Blastocystis hominis*) using a proprietary bioinformatics pipeline from Life Technologies. To control for PCR inhibition and inefficient nucleic acid isolation, three different internal controls (*Synechococcus*, PDV and PhHV) are included. We first tested all assays for their sensitivity and specificity on clinical isolates using 96 well plates.

All targets were tested against an inclusion and exclusion panel and PCR efficiencies were determined by serial dilution experiments. In a next step these TaqMan Assays were spotted on 384 well, microfluidic cards that allow the detection of these 20 pathogens in duplicate reactions within 1 hour. A set of several hundred clinical stool samples was analyzed with the new system and compared to conventional methods like culture and microscopy.

**Results:** The results of the in- and exclusions panels showed that all PCRs were specific for the targets they were designed for and all PCR efficiencies were >90%. Testing clinical stool samples showed that the TaqMan Array Card was much more sensitive than conventional techniques like culture and microscopy.

**Conclusion:** We successfully validated real-time PCR tests to detect 16 different pathogens causing gastroenteritis. The TaqMan Array Cards described here provide us with a fast, sensitive and cost-effective way for the simultaneous detection of gastro-intestinal pathogens in clinical stool samples.

#### **P1792** Prospective application of the Luminex xTAG<sup>®</sup>-GPP multiplex PCR in diagnosing infectious gastroenteritis

E. Wessels, L. Rusman, M. van Bussel, E.C.J. Claas\* (Leiden, NL)

**Objectives:** Infectious gastro-enteritis (GE) is a major diagnostic challenge as it can be caused by parasites, bacteria and viruses. The Luminex Gastro-enteritis Pathogen Panel (xTAG<sup>®</sup>-GPP) detects 18 most common GE causing pathogens. A clinical multicenter study performed in 2010, using a preliminary version of the kit, showed promising results. After some modifications in the kit, including more specific detection of *Campylobacter jejuni*, *C. coli* and *C. lari*, discrepant analysis of the LUMC results of the 2010 clinical data has been performed. These data were convincing resulting in the start of a prospective clinical study in November 2011.

**Methods:** Using the adapted version of the kit, all discrepant result obtained in the initial clinical study (presented at the 2011 ECCMID) were evaluated. In the prospective study, all fecal samples submitted for GE diagnosis, are also analyzed by the xTAG-GPP assay.

**Results:** Most discrepancies with the initial kit version were a large amount of *Campylobacter* species positive results. Using real-time PCR, part of these were conformed as high CT-value positive results. Using the adapted version of the kit, only the specific *C. jejuni*, *C. lari* and *C. coli* positives were detected, that all were confirmed by real-time PCR. In addition, the clinically important discrepant results we obtained

with *Entamoeba histolytica*, could not be confirmed with the updated version of the kit. The prospective study that has been initiated will provide data on the specificity of the diagnostic requests on clinical criteria. Most samples only are being submitted for diagnostic virology, bacteriology or parasitology, so most likely additional diagnostic results will be obtained by xTAG-GPP application.

**Conclusion:** The 18-target multiplex of the xTAG®-GPP assay has shown to be a sensitive diagnostic tool for GE. The concordance with current diagnostic molecular methods was good, and the assay was superior to bacterial culture. The prospective study will show data supporting the added value of this approach in comparison to the limitations of diagnostic requests on clinical criteria.

## Molecular diagnosis of sepsis and joint infections

### P1793 Identification and characterisation of bacterial pathogens and fungi causing blood infections (sepsis) by DNA microarrays

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**Objectives:** We have developed a prototype of DNA microarray for accurate and reliable identification of sepsis-causing bacteria and fungi from both samples (positive blood cultures and direct blood) within a working-day using a multiplex-PCR plus microarray based-assay.

**Methods:** DNA was automatically extracted from positive blood cultures and blood using different protocols. Species and Genus-specific primer mixture was designed using conservative region from bacterial and fungal genomes. The primer design allowed DNA amplification method producing labeled, single-stranded DNA suitable for microarray hybridization. The probes on the microarray were designed against species-specific or taxa-specific variable regions flanked by the primers. Specific hybridization were performed in eight well-strip format (CLART-Strip®) containing a microarray at the bottom. Also, mecA-specific primers and probes have been included in the assay to indicate the detection of antimicrobial resistance. Automated data analysis was performed by designed specific software.

**Results:** The assay correctly identifies the most clinically relevant species from the following bacteria (Staphylococcus, Streptococcus, Enterococcus, Listeria, Enterobacter, Escherichia, Klebsiella, Salmonella, Citrobacter, Serratia, Proteus, Haemophilus, Bacteroides, Acinetobacter, Pseudomonas and Stenotrophomonas), the methicillin resistance marker present within Staphylococci, and fungi (Candida albicans albicans, C. glabrata, C. krusei, Candida spp. and generic fungi marker). The application of this assay for the detection of pathogens directly in blood is being determined by generic PCR assays combined by detection on microarray, being early detection of blood infections crucial in clinical settings. In addition genus and species PCR assays and detection in microarray were made from 700 blood cultures. The results from tested samples were in line with the phenotypic and antimicrobial susceptibility tests, showing values of sensitivity, specificity, reproducibility and repeatability higher than 95%.

**Conclusions:** The results from the method were available 5 hours after the positive sample collection. Up to 96 samples could be processed simultaneously. The assay provides rapid and reliable data of accurate identification of microbial pathogens at the early stage of infection, avoiding unnecessary treatment with general antibiotics that promote the appearance of drug resistant bacteria.

### P1794 Evaluation of PNA FISH assays for the rapid diagnosis of sepsis and other severe infections, and identification of *Streptococcus agalactiae* in the screening of pregnant women

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**Objectives:** Identification (ID) of pathogens by conventional methods from liquid culture media requires 24–48 hour. Peptide nucleic acid fluorescence in situ hybridization (PNA FISH) is a new molecular diagnostic tool for the rapid ID of pathogens directly from liquid media. The aims of this study were to evaluate PNA FISH in comparison with conventional methods both from positive blood cultures (BC) and other biological fluids, as well as to evaluate the ID of *Streptococcus agalactiae* (GBS) from vaginal swabs (VS) in pregnant women.

**Methods:** The PNA FISH assays (AdvanDx) were applied on 61 positive BC bottles (Bactec 9240, BD) (56 blood samples and five biological fluids other than blood). On the basis of the Gram stain microscopy results, four different panels were used: one for identification/differentiation of *Staphylococcus aureus* (SA) and other coagulase-negative staphylococci (CNS), one for *Enterococcus faecalis* (EF) and other enterococci (OE), one for *Escherichia coli* (EC), *Klebsiella pneumoniae* (KP) and *Pseudomonas aeruginosa* (PA), and one for *Candida albicans*/*C. parapsilosis* (CAP), *C. tropicalis* (CT) and *C. glabrata*/*C. krusei* (CGK).

For GBS ID, “GBS PNA FISH” assay (AdvanDx) was performed on 25 VS belonging to pregnant women after 24 hour of incubation in enrichment broth.

The results of the molecular assays were compared with those obtained by ID with conventional methods.

**Results:** On all 56 positive BC, PNA FISH assays showed a 100% agreement with the ID obtained by conventional methods (14 CNS, four SA, five EF, one SA + EF, one OE, nine EC, two KP, two PA, three CAP, one CGK, one CT, 14 negative). When PNA FISH assays were tested on the two peritoneal fluids, one cerebrospinal fluid, one bile and one liver abscess, the results agreed with the conventional methods in all cases (one EF + EC, one CGK, one CNS, one OE, one OE + CAP). PNA FISH assays provided species identification in average 2.8 days before the conventional methods.

“GBS PNA FISH” tested on 25 VS, all samples showed a 100% agreement with conventional methods providing species identification in average 1 day before than conventional method.

**Conclusion:** PNA FISH assays showed, even if tested in this study only on a limited number of samples, an excellent efficacy in the rapid identification of main pathogens yielding a significant reduction on reporting time, leading to a more appropriate patient management and therapy in case of sepsis and severe infections and a rapid screening for GBS colonization in pregnant women.

### P1795 Direct identification of major blood culture pathogens by *lucisco*® bacteraemia I and II panel, a beacon based FISH identification

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**Objectives:** Bacteremia is a common cause of morbidity and mortality worldwide. Rapid identification and antibiotic susceptibility testing of the causative agents of bloodstream infections are essential for clinicians to select the most appropriate antimicrobial therapy. Thus, early administration of adequate antibiotic therapy has been shown to reduce mortality. In order to provide timely reports on blood culture isolates, we evaluated the performance of *lucisco*® Bacteremia I and II Panel (miacom® diagnostics), a beacon-based FISH (bbFISH) identification method.

**Methods:** Positive blood culture were contemporary processed using conventional microbiological methods and using *lucisco*® Bacteremia I and II Panel. In this method, which use a simple slide with eight fields, DNA sequences that specifically bind bacterial species-specific ribosomal RNA, are labeled with fluorescent markers, in the so called beacon-based FISH (bbFISH) technology. Bacteremia Panel I is specific for 10 different gram negative bacteria, Bacteremia Panel II is specific for nine different gram positive bacteria. After conventional microscopic examination, useful to choose gram positive or negative panel, 25 µL of each blood culture was used to perform the *lucisco*® test. The evaluation was carried out using a fluorescent microscope and each field was examined in both, the red and the green channel. Positive

and negative control of fluorescence was located in field 1. A clear positive signal in one of the remaining fields means the presence of the respective pathogen.

**Results:** From July to October 2011, 203 blood culture were analyzed, 153 samples were positive and 50 were negative. The comparison between lucesco<sup>®</sup> Bacteremia I and II Panel and the conventional methods for identification showed an optimal agreement (Table 1). Only 10 pathogens (6.5%) were not identified with lucesco<sup>®</sup> technique. No false-positive reaction were found during this study.

Tab.1

Pathogens	Number of samples	Sensitivity	Specificity	Negative Predictive Value	Positive Predictive Value	Concordance
Gram Negative	69	92%	100%	92%	100%	95.8%
Gram Positive	84	95%	100%	95%	100%	97.6%

**Conclusions:** The performance of lucesco<sup>®</sup> Bacteremia I and II Panel, distinguishing to species levels the principal blood pathogens, showed an optimal concordance with conventional blood culture processing. Additionally this beacon-based FISH identification shortens the time to result by at least 1 work day, providing species-level identification within ½ hour from blood culture positivity.

**P1796 Evaluation of the SEPTIFAST real-time PCR for rapid identification of blood pathogens in patients with suspected sepsis: an experience in a northwestern Italy hospital**

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**Objective:** Sepsis is a serious medical condition that requires rapidly administered appropriate antibiotic treatment.

Although blood culture (BC) remains the gold standard for bloodstream infections (BSI) diagnosis, because it allows the execution of antibiotics sensitivity tests, it often lacks sensitivity, especially in patients already treated with antibiotics.

Molecular diagnostic tools can contribute to a more rapid diagnosis in septic patients.

Aim of the present study was to investigate the potential clinical utility of SeptiFast LightCycler (SF) in patients with suspected sepsis, admitted to departments of Infectious Diseases, Amedeo di Savoia Hospital University of Turin from January 2009 to June 2011.

**Methods:** Five hundred thirty-six samples collected from patients with clinically suspected sepsis were analyzed, sampling on the same day, by SF real-time PCR (Roche Diagnostics, Germany) and by BC (automated Bact/Alert 3D BioMérieux). The results were compared in terms of positive identifications obtained individually and in combination.

SF method is able to detect DNA of 25 of the most frequent bacterial and fungal pathogens from whole blood samples in <6 hours.

**Results:** 19.8% of the samples resulted positive to SF while 16.1% to BC ( $\chi^2 = 154.71$ ,  $p < 0.0001$ ); two isolates were detected only by BC and identified as a species not included in SF master list (*N. meningitidis* and *C. neoformans*). The difference between the two percentages were further increased if contaminants were excluded: while the rate of SF contamination is almost undetectable (2/534), the BC contamination rate (19/534) is 3.5%. The positivity rate of combined methods was 25.9%, allowing detection of 19 etiologic agents in monomicrobial and nine in polymicrobial infections (SF = 9, BC = 4). The analysis of concordance showed an overall agreement between the two methods equal to 85.6%. Considering fungal infections, two samples were founded positive to *Aspergillus fumigatus* only by SF. SF results were obtained in <6 hours and BC, assessed on Gram staining evaluation, in an average of 3 days.

**Conclusions:** Our results show that SF method reduces time of pathogens detection and increases microbial identification in patients with clinical suspected sepsis. SF can be considered as a complementary test to BC in the management of critically ill patients.

**P1797 SepsisTest<sup>TM</sup> molecular diagnosis of bacteraemia in febrile paediatric patients**

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**Objectives:** Fever is a common cause of presentation to the paediatric emergency department. In the majority of children, a self-limiting illness is diagnosed after a thorough history and clinical examination, but in 10–15% of children, serious bacterial infection (SBI) such may be present, sometimes without obvious clues. The low blood culture positivity rate in these children means that the aetiology of SBI is not really known. We aimed to enhance aetiological diagnosis using 16S rDNA PCR.

**Methods:** Febrile children <16 year were included presenting to the Emergency Department of Alder Hey Children's Hospital requiring investigation for the presence of SBI. Blood culture (BC) bottles (BacT/ALERT PF, Biomerieux) were inoculated with 1 mL blood and incubated for 5 day if negative. For PCR analysis, 1 mL EDTA blood was analysed using SepsisTest<sup>TM</sup> (Molzym, Bremen, Germany). Amplicons were sequence-analysed (NCBI BLAST; SepsisTest-BLAST).

**Results:** Among the 120 patients showing increased levels of C-reactive protein 16 (13%) were BC+ and 54 (45%) PCR+. Nine of the 11 BC+, PCR+ cases were concordant identical (*N. meningitidis* (2), *S. pneumoniae* (2), *S. pyogenes* (2), *K. pneumoniae* (1), CoNS (1), *C. albicans* (1)). Eight of the nine aetiologies were associated with sepsis patients. With other diseases, BCs were less frequently positive than PCR. Among oral-respiratory tract infections, 3/59 patients were BC+ in contrast to 20/59 PCR+ and among gastro-intestinal/urinary tract infections, 2/20 were BC+ vs. 9/20 PCR+. Patients with dermal/viral infections were BC- while 10/17 and 4/6 cases were PCR+, respectively. Most prominent PCR-identified organisms were viridans streptococci (20/54 patients) which were mainly found with oral-respiratory tract infections (9/20) and dermal/viral infections (6/20). PCR further indicated rare aetiologies, including, among others, *Comamonas* spp., *Enterococcus cecorum*, *Fusobacterium* spp., and *Gemella haemolysans*.

**Conclusion:** The study included febrile patients considered to have a possible serious bacterial infection. A high concordance of BC and PCR (diagnostic sensitivity, 82%) was observed with systemic infections. The benefit of SepsisTest<sup>TM</sup> became especially evident for a significant part of patients for which aetiologies (viridans streptococci, rare pathogens) were identified while BCs were negative. SepsisTest<sup>TM</sup> thus proved to be a valuable diagnostic tool as a complement of BC.

**P1798 Evaluation of new real-time PCR test for the detection of bacterial and fungal pathogens in patient with suspected bacteraemia**

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**Objectives:** Early diagnosis and provision of appropriate antimicrobial therapy is known to correlate with positive outcomes of sepsis. The main limitation of standard blood culture (BC) is long time-to-result (2–5 days). Molecular diagnosis is faster for the detection and identification of main sepsis causing pathogens.

**Methods:** A new commercial multiplex RT PCR based assay (Magicplex Sepsis Real-time Test, Seegene) for screening more than 90 and direct detection of 27 different bacterial and fungal pathogens in a whole blood was compared to standard blood culture (BC). 1–3 pairs BC per patient collected within 1 hour and 1 EDTA-blood sample was collected at the same period. Blood samples were cultured in BacT/Alert 3D instrument (bioMérieux).

**Results:** Between February 2011 and October 2011 blood samples from 331 patients were obtained from internal medicine, ICU, infectious diseases and pediatrics department. One hundred and ninety-seven patients were negative by both methods. For 71 patients only contaminants were detected (in most cases *Staphylococcus epidermidis*, *Staphylococcus* spp., *Stenotrophomonas maltophilia*).



Altogether the number of patients positive for a pathogen by at least one method was 63 (19% of all investigated patients). Of them 49 (78%) were PCR positive and 38 (60%) were BC positive in at least one bottle. Of the patients with detected pathogens 24 (38%) were positive by both methods, 25 (40%) patients positive only by PCR and 14 (22%) only by BC. BC detected five additional microorganisms not included in the RT-PCR test menu (*Salmonella enteritidis*, *Yersinia enterocolitica*, *Haemophilus influenzae*, *Gemella* ssp., *Morganella morganii*) and nine of PCR negative and BC positive patients had pathogene growth in only one bottle. Fungi were detected in only two PCR specimens (*Candida parapsilosis*, *Candida krusei*), but not in BC. **Conclusions:** The PCR method and BC supplement each other. The positivity rate of PCR was higher than BC's, but it's detection menu is more limited. Only PCR was able to detect fungi. A single PCR test does not seem to give sufficient results in all cases.

**P1799** Rapid detection of gram-positive bacteria and resistance determinants directly from positive blood cultures using the microarray-based sample-to-result Verigene BC-GP assay

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**Objectives:** Gram-positive bacteria constitute the majority of positive blood cultures. Some of these organisms are associated with serious infection, while others are skin flora associated with lines or improperly collected specimens. Early identification of the organism and appropriate antibiotic treatment are critical to management of the infection and improving patient outcome. We evaluate the microarray-based Verigene Gram-Positive Blood Culture (BC-GP) Assay (Nanosphere, Northbrook, IL, USA) for detection of bacteria directly from positive blood cultures.

**Methods:** A total of 119 positive blood cultures containing gram-positive bacteria were analyzed using the BC-GP within 12 hour of culture positivity. Results were compared to routine biochemical testing as the gold standard. The BC-GP detects 13 bacterial targets including *Staphylococcus*, *Streptococcus*, *Enterococcus*, *Micrococcus* and *Listeria* species. The resistance determinants *mecA* (oxacillin) and *vanA/vanB* (vancomycin) are also detected.

**Results:** Among 114 monomicrobial cultures, the BC-GP was 100% sensitive in detection of *Staphylococcus* spp. (n = 83), *S. aureus* (n = 35), *S. lugdunensis* (n = 1), *Streptococcus* spp. (n = 10), *S. agalactiae* (n = 5), *Micrococcus* spp. (n = 5), and *E. faecalis* (n = 9). BC-GP was 93.9% specific for detection of *S. epidermidis* (n = 33) and 75.0% sensitive for *E. faecium*. Three cultures contained organisms not on the BC-GP panel (*Kocuria*, *Granulicatella*), and were resulted as "Not Detected." In one of four cultures containing two organisms the BC-GP correctly identified both organisms (*S. aureus*, *S. epidermidis*); the remaining three cultures contained *S. epidermidis* and *S. hominis* and were resulted as *S. epidermidis*. The BC-GP correctly predicted oxacillin resistance in 100% (34/34) *S. aureus* or *S. epidermidis* (*mecA* +) and vancomycin resistance in 100% (2/2) *Enterococcus* spp. (*vanA* +). A reproducibility panel of 20 strains was tested 20 times immediately following culture positivity or 8 hour after positivity. The call rate was 97% (388/400) at initial positivity and 96% (384/400) 8 hour after positivity. All samples failing to generate a result at both time points successfully returned an accurate result following a single retest (final call rate 100%).

**Conclusions:** The BC-GP assay identifies 13 gram-positive targets and three resistance markers directly from positive blood cultures. The BC-GP requires only 350 µL of specimen and results are available within 2.5 hour of blood culture positivity.

**P1800** Impact of the Xpert MRSA/SA SSTI<sup>®</sup> assay (GeneXpert<sup>®</sup>) in the choice of the antibiotherapy of patients suffering from bone and joint infections

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**Objectives:** After validation of the Xpert MRSA/SA SSTI<sup>®</sup> assay for the detection of methicillin-susceptible (MSSA) and methicillin-resistant (MRSA) *Staphylococcus aureus* as well as methicillin-resistant coagulase negative staphylococci (MRCoNS) directly on perioperative samples, we evaluated the impact of this assay on the choice of antibiotherapy in bone and joint infections.

**Methods:** We included 72 infected patients and measured the mean time to results (GeneXpert<sup>®</sup> vs. culture) after sampling according to the mapping pathways method. We then compared sensitivity and specificity of the molecular method respect to culture for the detection of MSSA, MRSA and MRCoNS. Furthermore, we calculated the statistical value of the Xpert assay respect to Gram stain which is actually the only technique available to adapt antibiotherapy during the surgical time. Finally, we evaluated the impact of the Xpert assay on the prescription of vancomycin.

**Results:** Mean time to result after sampling was 72 minute (GeneXpert<sup>®</sup>) vs. 79 hours for culture. The MRSA/SA SSTI<sup>®</sup> assay displayed sensitivity, specificity, positive predictive and negative predictive values of respectively 100%, 98.3%, 88.9% and 100% for MSSA, 100%, 100%, 100%, 100% for MRSA and 100%, 95.3%, 85.2% and 100% for MRCoNS. The statistical analysis showed a very good correlation with culture. However, sensitivity of the Xpert<sup>®</sup> assay was higher than Gram stain (p < 0.001). Among the 72 patients, only 21 had a Gram stain indicating the presence of bacteria and only 57 had a positive culture. The Xpert<sup>®</sup> assay allowed a rapid initiation of documented antibiotherapy with vancomycin for 28 patients. On the contrary, the use of vancomycin could be avoided for 26 patients inducing a reduction of costs (central line, cost of the antibiotic and of the dosages).

**Conclusion:** The Xpert MRSA/SA SSTI<sup>®</sup> assay is perfectly correlated to culture and allows a better therapeutical orientation than Gram stain in bone and joint infections due to staphylococci. In this context, it promotes a rapid initiation of documented antibiotherapy and generates in many cases a reduction of health cost by avoiding the use of vancomycin.

**P1801** Comparison of molecular and conventional microbiological diagnostic tools in suspected joint infections

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**Objectives:** The diagnosis of septic arthritis is based particularly on synovial analysis and conventional culture techniques. However, this approach is time consuming and lacks of sensitivity. In this study, a contribution of molecular methods to establishing a definite diagnosis of septic arthritis was evaluated.

**Methods:** Two hundred and seventy-one synovial fluids or periarthral tissue samples from 230 patients, examined in our laboratory from 1 February 2004 to 31 May 2008, were included into the study. Clinical orthopaedist retrospectively sorted samples into four categories like definite, probable, possible infection or non-infectious sample, according to the clinical and laboratory findings including age, sex, presence of prosthesis, fever, redness, swelling, pain, limited articular motion, UZ/RTG images, CRP, macroscopic appearance of sample, microscopic and histopathological report, but not including results of microbiological examination. Microbiological diagnostics was performed using routine conventional cultures and broad-range 16S rRNA PCR followed by sequencing and/or staphylococci-specific multiplex PCR.

**Results:** One hundred and fifty-four samples were classified as definite or probable infection (INF), 95 samples as possible infection (POSS-INF) and 12 samples as non-infectious samples (NON-INF). Eighty-three (53.9%), 8 (8.4%) and 0 samples were culture positive, while 102 (66.2%), 20 (21.1%) and 0 samples were PCR positive in INF, POSS-INF and NON-INF group, respectively. Thus, PCR showed sensitivity 66.2% and specificity 100% compared to sensitivity 53.9% and specificity 100% for conventional culture techniques. A combined sensitivity and specificity using both culture and molecular methods was 79.2% and 100%, respectively.

**Conclusion:** Molecular methods showed higher sensitivity compared to culture and should be routinely used in addition to culture. Combined sensitivity of both PCR and culture reached reasonable 79.2%. A demonstrated high specificity of both classical and molecular methods is of a limited value due to a low number of samples in NON-INF group. Discordant results of both methods need to be evaluated individually with respect to other clinical and laboratory signs of infection and possible contamination, particularly in POSS-INF group.

**P1802** Accurate detection of prosthetic joint infections by multiplex PCR of sonication fluid

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**Objectives:** Causes of prosthetic joints failure can be infectious or non-infectious. It is important to accurately diagnose prosthetic joint infection (PJI) because its management and outcome differs from that of other causes of arthroplasty failure. The purpose was to assess the potential of multiplex PCR for differentiating between PJI and aseptic loosening (AL). Also, we compared multiplex PCR with current diagnosing techniques (periprosthetic tissue culture and sonication fluid culture).

**Methods:** We consecutively included all explanted prosthesis from 8/2010 through 8/2011. Episodes of PJI and AL were included. PJI was defined as visible purulence, acute inflammation on histopathology or sinus tract. AL was defined as failure of prosthesis in the absence of any of these criteria. The removed implants were sonicated and the resulting sonication fluids were cultured aerobically and anaerobically. Aliquots of the fluids were also investigated using multiplex PCR.

**Results:** Ninety explanted prosthesis (63 AL and 27 PJI) from 85 patients were included, including, knee (n = 61), hip (n = 24), elbow (n = 3) and shoulder (n = 2) prosthesis. In sonication culture, the causative organism of PJI was identified in 19 (70%) cases and by multiplex PCR in 26 (96%) cases (p = 0.016). In 17 PJI cases (63%), the results of the three techniques were concordant. Among the 10 discordant PJI cases (37%), in six episodes the causative microorganisms were detected only by multiplex PCR (four of these patients had previously received antibiotics). In two episodes, PCR was positive but one of the culture methods was negative. One case was caused by *P. acnes* which cannot be detected by this multiplex PCR.

	Periprosthetic tissue culture	Sonication fluid culture	Multiplex-PCR
Sensitivity	70.37%	70.37%	96.29%
Specificity	100%	93.65%	100%
Accuracy	0.91	0.86	0.98
PPV	100%	82.60%	100%
NPV	88.73%	88.06%	98.44%
LR+	Infinity	11.08	Infinity
LR-	0.29	0.32	0.04

**Conclusion:** Multiplex PCR of sonication fluid offers the following advantages: it is easy to perform; it allows to distinguish between PJI and AL; it makes possible to detect PJI faster than culture methods; it is more sensitive (96.29% vs. 70.37%) and specific (100% vs. 93.65%) than culture methods and it provides an accurate diagnosis of PJI, which allows a better adjust of antimicrobial treatment.

## Molecular diagnosis of sexually transmitted infections

**P1803** STD-Finder SMART: a new approach for screening for clinical relevant pathogens causing STD

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**Objective:** World-wide sexually transmitted diseases (STDs) present a major public health concern. Most of these diseases are treatable if detected on time. Untreated STDs can lead to infertility, chronic pain

and sometimes even to death. Some diseases are without symptoms and therefore of greater risk of passing the disease to others. At present, routine diagnosis of STD is mainly focused on the detection of the two most common pathogens *Chlamydia trachomatis* and *Neisseria gonorrhoeae* using either culture, serology or PCR. Occasionally, negative samples are subsequently further screened for additional pathogens.

Here we present the STD-Finder SMART assay which is able to detect the seven most prevalent pathogens causing STD in one RealTime PCR reaction.

**Method:** The STD-Finder SMART is based on the SmartFinder technology, a technology that allows a highly complex analysis of up to 13 targets in a single RealTime PCR reaction. The assay can detect and identify Herpes Simplex virus type 1 and type 2, *Chlamydia trachomatis*, *Mycoplasma genitalium*, *Neisseria gonorrhoeae*, *Treponema pallidum* and *Trichomonas vaginalis* in a single reaction

**Results:** Analytical sensitivity for the different targets was determined using commercial reference samples and was for each pathogen between 6 and 30 copies per reaction. Clinical specificity and sensitivity were obtained by screening EQA panels from QCMD and also a large set of clinical samples. Results were compared with the BD ProbeTect™ CT/GC Qx Amplified DNA Assay, a RealTime PCR test detecting *Chlamydia trachomatis* and *Neisseria gonorrhoeae*.

Samples which were found positive by the STD-Finder SMART for an additional pathogen were further validated by pathogen specific RealTime PCR.

The STD-Finder SMART assay has comparable sensitivity to conventional single and duplex RealTime PCR. There was no cross-reactivity observed between any of the seven target species or related species.

**Conclusion:** STD-Finder SMART is able to detect seven different STD pathogens simultaneously and has comparable sensitivity to RealTime PCR. This STD-Finder SMART assay is suitable for the routine detection of a broad spectrum of pathogens causing STD at relatively low cost due to multiplexing. The results of the clinical screening show that when only testing for the two most prevalent pathogens, *C. trachomatis* and *N. gonorrhoeae*, some patients are misdiagnosed as negative.

**P1804** Comparison of the VERSANT CT/GC DNA assay with Gen-Probe APTIMA COMBO 2 assay using VERSANT swab collection kit

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**Objectives:** This study compared the performance of the VERSANT® CT/GC DNA 1.0 Assay (kPCR)\* (Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA) to the Gen-Probe APTIMA COMBO 2 (AC2) Assay using the fully integrated VERSANT® kPCR Molecular System.\* The VERSANT CT/GC DNA 1.0 Assay (kPCR) is a real-time kinetic, PCR-based test intended to detect the presence of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC) in both symptomatic and asymptomatic individuals from male urethral swab specimens, female endocervical swab specimens, and male and female urine specimens.

**Methods:** In this study, 1317 clinical endocervical, vaginal, and urethral swab specimens were collected using the Siemens male and female VERSANT® Swab Collection Kit\* (SCK). The same specimens were also tested in the AC2 assay using Gen-Probe's collection devices. The percent concordance rate of the VERSANT CT/GC DNA 1.0 Assay (kPCR) with the AC2 assay was determined from these results. Specimens with results that were discrepant by the two assays were subjected to DNA sequencing to identify the presence of amplified targets.

**Results:** Data indicated that the estimated overall percent agreement between the VERSANT CT/GC DNA 1.0 Assay (kPCR) and the AC2 assay for CT and GC detection was >97%, and the negative percent agreement was >98%. Furthermore, the positive percent agreement was >96% for urethral, endocervical and vaginal swabs tested.

**Conclusion:** The VERSANT CT/GC DNA 1.0 Assay (kPCR) was both sensitive and specific for detecting CT and GC targets in male urethral and female endocervical and vaginal swabs using the VERSANT Swab Collection Kit for male and female samples. The positive and negative percent agreements between the VERSANT CT/GC DNA 1.0 Assay (kPCR) and AC2 assay were high, exceeding 95%. \*The VERSANT CT/GC DNA 1.0 Assay (kPCR) and the VERSANT kPCR Molecular System (both CE marked) are not commercially available in the U.S. VERSANT Swab Collection Kits are not commercially available.

**P1805 Evaluation of a multiplex real-time PCR system (DX CT/NG/MG assay, Bio-Rad) for the detection of *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Mycoplasma genitalium* in urogenital specimens**

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**Introduction:** *Chlamydia trachomatis* and *Neisseria gonorrhoeae*, if untreated, can cause pelvic inflammatory disease (PID) in women and can lead to infertility in both women and men, while *Mycoplasma genitalium* presence in genital specimens is associated with urethritis or cervicitis in women and urethritis in man.

It therefore seems important in this context, the development of methods for screening of sexually transmitted diseases (STDs).

**Objective:** Aim of this study was to evaluate the performance of the real-time PCR multiplex system "DX CT/NG/MG Assay" Bio-Rad for the contemporary research of *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Mycoplasma genitalium* in different samples.

**Methods:** We analyzed 461 different kind of genital samples provided from different hospital departments, including a group of patients at high-risk for STDs. Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* were compared in all samples against the Abbott CT/NG real-time PCR, in addition, all samples positive for *Neisseria gonorrhoeae*, were compared with the culture method.

The presence of *Mycoplasma genitalium* was confirmed through the use of multiplex PCR kits "STD6 ACE detection" – Seegene.

**Results:** Of the 461 samples tested, 14.32% were positive for *Chlamydia trachomatis*, 6.94% for *Neisseria gonorrhoeae* and 3.04% for *Mycoplasma genitalium*. Two samples tested for *Chlamydia trachomatis*, showed different results compared with the Abbott kit, regarding *Neisseria gonorrhoeae*, all results compared were identical, these results were confirmed by culture method in 21/32 samples (66%). Detection of *Mycoplasma genitalium* was confirmed in all samples. The values of sensitivity and specificity of Multiplex Real-Time PCR System were respectively 98.48% and 99.75% for *Chlamydia trachomatis*, 100% and 100% for both *Neisseria gonorrhoeae* and *Mycoplasma genitalium*. Specificity value regarding *Neisseria gonorrhoeae* dropped to 97.50% if compared against culture while sensitivity remained unvaried.

**Conclusions:** Our results show that the Multiplex Real-Time PCR System (DX CT/NG/MG Assay, Bio-Rad) has sensitivity and specificity values comparable to other systems also when used in the testing of different kinds of samples providing from low and high risk STDs population.

**P1806 Evaluation of the Roche Cobas® 4800 CT/NG test for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in men**

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**Objective:** Second generation nucleic acid amplification tests (NAAT) are the method of choice for screening and diagnosis of infections with *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG). This study compared the performance characteristics of the Roche cobas®

4800 CT/NG test (c4800) to the Becton Dickinson ProbeTec™ CT/GC Qx assay (Qx) and Gen-Probe Aptima Combo 2 (AC2) assay for the detection of CT and NG in men using patient-infected status (PIS).

**Methods:** The c4800 is a new diagnostic assay utilizing an automated workstation to isolate nucleic acids from clinical specimens and to perform real-time PCR based amplification of both CT and NG dual target DNA. Urine and urethral swabs were obtained from men attending STD, family planning, or OB/GYN clinics from 11 geographically distinct locations in the U.S. Aliquot order was randomized for urine specimens between AC2, c4800, and Qx. Urethral swab collection was randomized between AC2 and Qx. Urethral swabs were used only to define PIS and were not tested on the c4800. A participant was considered infected if the two comparator assays with different molecular targets had positive results from either sample type.

**Results:** Of 790 men screened, 768 were evaluable for CT and NG and symptoms were reported in 296 (37.5%). For urine, when compared to PIS, the overall sensitivity and specificity of the c4800 assay for CT were 97.6% and 99.5%, respectively. Sensitivity and specificity for NG were 100% and 99.7%, respectively. The overall prevalence of CT was 16.4% (ranging from 1.4% to 26.1%); the prevalence of NG was 9.2% (ranging from 0% to 20%). The c4800 showed high positive and negative predictive values for CT and NG.

**Conclusions:** The cobas® 4800 CT/NG test has excellent sensitivity and specificity when compared to PIS. Assay performance was similar in symptomatic and asymptomatic populations, and was equivalent to assays that are currently on the market.

**P1807 Clinical study of Cobas® 4800 CT/NG as detection kit for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* compared to Aptima Combo2® TMA, and ProbTec® ET SDA assays**

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**Objectives:** We investigated the performance of the new cobas 4800 CT/NG Test for the detection of *C. trachomatis* and *N. gonorrhoeae* in urine, endocervical swab, and throat samples.

**Methods:** We performed a clinical trial of new cobas CT/NG Test detection rate of *C. trachomatis* and *N. gonorrhoeae* in a total of 1902 clinical samples of male and female urines, endocervical swabs and throat samples which were collected in urology and gynecology clinics in Japan. Simultaneously, we also processed the same samples using the Aptima Combo2® TMA, and ProbTec® ET SDA and compared the positive rate of *C. trachomatis* and *N. gonorrhoeae*. We also investigated the performance of urines vs. endocervical swab, and of gargled liquid vs. throat swabs for detection of *C. trachomatis* and *N. gonorrhoeae* in female cases.

**Results:** The prevalence of *C. trachomatis* was 15%, the prevalence of *N. gonorrhoeae* was 6% overall. Positive and negative concordance rates were high (*C. trachomatis* 98.8%, *N. gonorrhoeae* 99.6%) for results obtained in urine vs. endocervical samples using the cobas® CT/NG Test. The accuracy of the cobas® CT/NG test did not differ significantly from that of the Aptima Combo2® TMA and ProbTec® ET SDA assays. In throat samples, concordance rates for results in the cobas® CT/NG and ProbTec® ET SDA assays were 98.8% for *C. trachomatis* and 95.1% for *N. gonorrhoeae*.

**Conclusion:** Very high concordance rates were observed for the comparison of cobas® CT/NG, Aptima Combo2® TMA, and ProbTec® ET SDA tests for the detection of *C. trachomatis* and *N. gonorrhoeae*. Urine samples are the specimen of choice for screening of genital infections with *C. trachomatis* and *N. gonorrhoeae* thereby avoiding the need to obtain endocervical swabs. Screening of throat infection with *C. trachomatis* and *N. gonorrhoeae* can be performed using gargled liquid instead of throat swabs using the cobas® CT/NG test. Thus, the cobas® CT/NG test is a reliable and highly accurate diagnostic tool for the detection of *C. trachomatis* and *N. gonorrhoeae* in male and female urine, genital swab and gargled specimens.

**P1808** Detection of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Mycoplasma genitalium* in uro-genital samples by the real-time Dx CT/NG/MG<sup>TM</sup> PCR assay

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**Background:** *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) are the most prevalent bacteria responsible for sexually transmitted infections (STIs). However, there is a recent growing interest in the pathogenic role of *Mycoplasma genitalium* (MG).

**Objectives:** 1 To evaluate the contribution of the Dx CT/NG/MG<sup>TM</sup> assay (Bio-Rad, France) for the diagnosis of STIs and to determine the prevalence of MG.

2 To compare the Dx CT/NG/MG<sup>TM</sup> assay to the Roche Cobas TaqMan CT<sup>TM</sup> test for CT detection.

3 To compare the Dx CT/NG/MG<sup>TM</sup> assay to culture when available for NG detection.

**Methods:** We analysed 840 clinical samples (456 first-void urines, 339 vaginal swabs, and 45 swabs from other origins) prospectively collected from 1 September 2011 to 25 October 2011. Swab specimens were collected in M4RT<sup>TM</sup> transport medium according to manufacturer's recommendations. Automated nucleic acids extraction was performed with NucliSens easyMAG platform (bioMérieux, France). The Dx CT/NG/MG<sup>TM</sup> real-time multiplex PCR was performed in parallel to the Cobas TaqMan CT<sup>TM</sup> test according to manufacturer's procedures. Discrepant CT results were retested by both methods. The NG primers and probe of the Dx CT/NG/MG<sup>TM</sup> assay target a sequence shared with some *Neisseria meningitidis* strains. Thus, all NG positive results were checked with an in-house *N. meningitidis* PCR assay to confirm the specificity of the PCR product.

**Results:** The mean age of patients was 29 years (range: 2 days–79 years). The sex ratio M/F was 0.94. In women (n = 433), the prevalence of CT, NG, and MG was 6.9%, 1.4%, and 3%, respectively. In men (n = 407), the prevalence of CT, NG, and MG was 4.9%, 1.2%, and 1.5%, respectively. Co-infection was present in seven patients (0.8%). For CT detection, the agreement between the two assays was excellent (100%). The Dx CT/NG/MG<sup>TM</sup> system enabled the detection of 13 NG positive samples, whereas only five were culture positive. However, among these 13 NG PCR positive, two only were false positive due to the presence of *N. meningitidis*.

**Conclusions:** This study demonstrates that the Dx CT/NG/MG<sup>TM</sup> assay is an easy to use, rapid, and valuable test for the simultaneous detection in a single sample of the three major sexually transmitted pathogens. CT remains the most frequent agent recovered. We show that Dx CT/NG/MG<sup>TM</sup> assay is more sensitive than culture for NG detection. Of note, NG positive PCR must be confirmed by another method as recommended by recent studies. Interestingly, the prevalence of MG was higher than that of NG.

**P1809** Validation of COBAS<sup>®</sup> TaqMan<sup>®</sup> CT Test v2.0 (Roche) on the Rotor Gene Q platform

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**Objectives:** Validation of the COBAS<sup>®</sup> TaqMan<sup>®</sup> CT Test real-time PCR assay, de facto designed to be used with the COBAS<sup>®</sup> TaqMan<sup>®</sup> 48 Instrument, on the Rotor Gene Q platform (Qiagen) for detection of *Chlamydia trachomatis* in genital swabs and urine specimens.

**Methods:** Two hundred microliter M4RT<sup>®</sup> (Remel) transport medium (swabs)/urine was used for extraction using the MagNA Pure platform (Roche, DNA I High Performance protocol). Elution in 110 µL elution buffer. The proposed reaction volume was reduced from 100 µL (50 µL DNA + 50 µL mastermix) to 50 µL (25 µL DNA + 25 µL mastermix). The assay was checked for analytical sensitivity, specificity, accuracy and precision following the Belgian guidelines (Raymaekers et al, Acta Clinica Belgica, 2011).

**Results:** Analytical sensitivity: M4RT<sup>®</sup> transport medium was spiked with the positive control of the kit and a negative urine specimen was

spiked with a Vircell DNA control (Serovar L2) to determine the limit of detection (LOD with a 95% hit rate). The lowest concentration was 860 and 880 copies/mL M4RT and urine respectively, correlating with 40 copies/PCR. This met our validation criteria and is comparable with other commercial kits (1000 copies/mL).

**Specificity:** Although the specificity was sufficiently documented by the manufacturer, the specificity was checked against 43 negative samples. There was no cross reaction found with other organisms.

**Accuracy:** Seventy-seven specimens were tested (36 positive and 43 negative samples). The panel composition consisted of 50 patients tested against a reference method (AMPLICOR<sup>®</sup> CT Test/BD ProbeTec<sup>TM</sup>) and 27 external quality controls. The external quality controls included the Swedish variant, Serovars J, E and L2. There was a 100% agreement.

**Precision:** Two pools of 0.4 and 15 DNA copies/µL elution buffer were analysed in double during 10 days. The standard deviations (SD) for the weak positive and positive sample were 0.66 and 0.84 Cq. This met our validation criteria of SD < 1 Cq.

**Conclusion:** The COBAS<sup>®</sup> TaqMan<sup>®</sup> CT Test met all our validation criteria and was implemented in our routine diagnostic laboratory. The kit can be used in combination with the Rotor Gene Q platform and costs can be saved by reducing the reaction volume without any loss of sensitivity.

**P1810** Prevalence of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections among women and men in Madrid, Spain

M.J Uria\*, A. Gonzalez, E. Saez, A. Ballesta (Madrid, ES)

*Neisseria gonorrhoeae* (NG) and *Chlamydia trachomatis* (CT) infections are the two most common sexually transmitted diseases worldwide. The objective of this study was to establish the prevalence of NG and CT infections among women and men in Madrid, Spain.

**Methods:** We present a retrospective study including samples obtained from six different hospitals in Madrid between 2009 and 2011 involving symptomatic women and men. NG and CT were detected in cervical and urethral specimens by polymerase chain reaction (PCR) (COBAS<sup>®</sup> AMPLICOR CT/NG, Roche and Versant CT/GC kPCR; Siemens).

**Results:** In this study we have analysed a total number of 5607 samples. The overall CT prevalence infection was 9.2%. The distribution was 40% in women and 60% in men. The prevalence of NG infection was 7% with a distribution of 27% in women and 87% in men. In addition, co-infection of both bacteria was found in 60 patients.

**Conclusions:** The results of our study demonstrate that prevalence of either CT infection or NG infection was high according to national epidemiological surveillance data. The incidence of CT infection between men and women was similar suggesting that there is no a pattern of infection by gender. However, a marked increase in the incidence of infection was found for NG in men suggesting that men have a higher risk of developing sexual transmitted disease by NG than women. Comparison of data from PCR and culture by standard methods for NG will be analysed.

**P1811** Comparison of clinical specimens collected with ESwab to affirm collection kits for the detection of bacterial vaginosis using the the Affirm<sup>TM</sup> VPIII microbial identification assay

M. Favaro\*, A. Mauti, J. Dianetti, C. Fontana (Rome, IT)

**Background:** Currently molecular platforms for the detection of sexual transmitted infections utilize their own collection devices that are unsuitable for culture or other testing methods. Collection devices that allow multiple testing methods are practical for confirmatory testing or testing for other targets with the same specimen. ESwab, (a tube with 1.0 mL liquid Amies and a flocced swab) is a Liquid Based Microbiology device that can be used with the Walk Away

Specimens Processor (WASP) and is also compatible with molecular assays. The study objective was to compare the performance of ESwab to the Affirm collection kit with the BD Affirm™ VPIII Microbial Identification Test, a DNA probe test, for the detection of *Candida* spp. (C), *Gardnerella vaginalis* (GV) and *Trichomonas vaginalis* (TV)

**Method:** In this study were analysed 250 vaginal samples collected in duplicate, one with the Affirm kit and another with the ESwab for bacterial culture. Both samples were tested with the the Affirm™ VPIII. Each positive result was confirmed by culture and wet mount microscopy for TV. Twenty-six ESwab samples were tested using 100 and 200 µL to find the optimal sample testing volume. Forty eight samples, 12 each, were used to investigate alternative testing variables like the flocced swab, the pellet from 200, and 200 µL after 48 hours at 40 and -200°C storages. The last 176 ESwab samples were tested using 200 µL following the same procedure as the Affirm samples.

**Results:** in the first analysis 21/26 samples were positive and 5/26 were negative with both the Affirm collection kit and 200 µL of ESwab; 13/26 tested with 100 µL of ESwab, were concordantly positive, while 8/26 GV were not detected. Concordant results were found when testing samples within 48 hours or stored frozen at -200°C after 48 hours. Inhibition was found in nine out of 12 samples when using the pellet obtained from centrifuging 200 µL of ESwab sample. In the 250 samples tested, 45 C, 16 TV, 162 GV and 27 negatives were detected with both collection kits, showing 100% concordance.

**Conclusion:** Copan ESwab can be used for the collection of vaginal specimens for the detection of C, GV and TV with the BD Affirm™ VPIII. Using 200 µL of ESwab within 48 hours or stored frozen after 48 hours proved to be the optimal testing method with the Affirm. Sample collected with ESwab can be used for antigen detection, Gram smear preparation and culture with manual and automated inoculation methods.

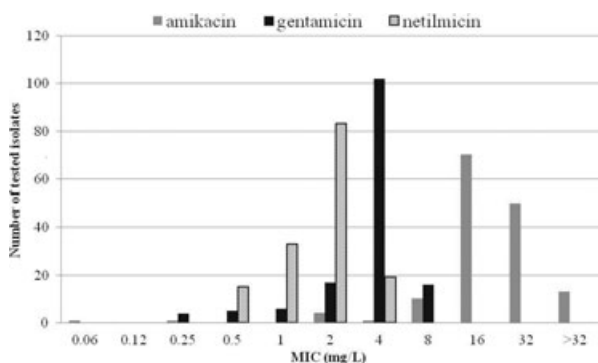
**P1812** Antimicrobial activities of three aminoglycosides: amikacin, gentamicin and netilmicin against *Neisseria gonorrhoeae*

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**Objective:** Antimicrobial resistance in *Neisseria gonorrhoeae* has shown to be increasing globally. As more treatment failures were documented for current treatments like cephalosporins, an alternative is crucial for future therapeutic use. In this study, we report the susceptibility testing results for three aminoglycosides against the clinical isolates of *Neisseria gonorrhoeae* collected in Hong Kong.

**Methods:** Clinical isolates of *Neisseria gonorrhoeae* (n = 150) had been collected from the Yaumatei Social Hygiene Clinic in Hong Kong from 1998 to 2010. Minimum inhibitory concentrations (MICs) of three aminoglycosides: amikacin, gentamicin and netilmicin were determined by agar dilution method according to CLSI (M100-S21).

**Results:** The MICs of the aminoglycosides are presented in the figure below. MIC50 of amikacin, gentamicin and netilmicin was 16, 4 and 2 mg/L while MIC90 was 32, 8 and 4 mg/L respectively.



**Conclusion:** This study demonstrated the MICs of three aminoglycosides on clinical samples isolated in Hong Kong. Comparing the results of MIC90 with the once daily dose and multiple daily doses, amikacin and gentamicin may not be good candidates to be considered. Considering the MIC distribution and MIC90 of netilmicin and its achievable physiological concentration, these data highlight the potential use of netilmicin in treatment of gonorrhoea. Further studies and clinical trials are needed for investigation.

**P1813** Epidemiological and laboratory specificities of *Trichomonas vaginalis* infection in men with and without urethritis syndrome

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**Objectives:** Infection by parasite *T. vaginalis* represents a serious global public health threat. Manifestation in men can entail a clinical diagnosis of urethritis, which today is one of the most common clinical entities in men encountered by microbiologists. The aims of this study were to analyze the socio-demographic and behavioral specificities of men with (group of patients) and without urethritis (control group); to study trichomonosis prevalence in both groups, to examine the value and applicability of PCR in the diagnostics of trichomonosis; and, finally, to define the variables increasing the risk of infection.

**Methods:** The study has included 500 men with and 200 without urethritis symptoms. Every respondent filled out a questionnaire asking for some general data, as well as specific information about habits, behaviors and symptoms. Sample for trichomonosis diagnosis was the sediment of first void urine, while the diagnostic methods used were wet smear microscopy, Diamond's medium cultivation and real-time PCR. Obtained data was analyzed using the SPSS 13.0 program.

**Results:** *Trichomonas vaginalis* infection was documented in 2.4%, 4.8% and 8.2% of the respondents manifesting urethritis symptoms, while the control group tested positive for the same parasite in 1.0%, 1.5% and 2.0% cases. The sensitivity of real-time PCR in trichomonosis diagnosis was greater than those of microscopy and culturing. It was statistically proven that trichomonosis is significantly more common in heterosexual men, men who have had sexual intercourse outside of Croatia, men who have purchased sexual services and men who were of relatively older age during their first sexual intercourse. No statistically relevant association was found between trichomonosis and place of residence, level of education, employment or frequency of use of condoms during sex. Likewise, marital status, parenthood, number of sexual partners over the last year, drug/alcohol use prior to intercourse, smoking and HIV antibody test did not prove to be risk factors for trichomonosis.

**Conclusion:** Having considered the numerous undesirable consequences of untreated trichomonosis and its notable prevalence, the authors believe that the present day neglect of *T. vaginalis* infection is likely change soon, especially in light of the availability of novel noninvasive, fast and highly sensitive methods for the diagnosis of trichomoniasis.

**P1814** Validation of Copan CAT medium for the detection of *Trichomonas vaginalis* by wet mount, culture, antigen detection methods and nucleic acid amplification assays for other STDs

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**Objectives:** Trichomoniasis, is a sexually transmitted disease (STD) caused by *Trichomonas vaginalis* (TV), an obligate parasite that lacks the ability to synthesize nutritive macromolecules, but uses the vaginal secretions or the phagocytosis of host and bacterial cells. TV requires a medium rich with proteins to support the essential macromolecules, vitamins, and minerals to support its growth. Copan has developed the CAT medium that contains all specific nutrients to support

*Trichomonas vaginalis* and *Candida* culture. The objectives of this study are to validate the performance of the CAT medium for the collection of vaginal specimens for the detection of: (i) of *Trichomonas vaginalis* by wet mount, culture, and antigens and (ii) other STDs like *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC) by nucleic acid amplification tests (NAATs).

**Methods:** Sets of CAT medium tubes (n = 10) were spiked with 200 µL of a freshly prepared ATCC culture strains of TV (10-1), CT (10-3) and GC (10-4). After 24 hours incubation at 37°C TV spiked CAT medium tubes were tested by wet mount and with the Inverness/Alere rapid antigen test and for CT/GC with the BD ProbeTec (PT), the GenProbe APTIMA AC2 (AC2) and the Roche Amplicor (RA) assays. TV was also tested by an in-house real time PCR. Two hundred microlitre volume of CAT medium was used for each testing assay. One set of negative CAT medium was used to test for interference or inhibition. Twenty vaginal specimens positive by wet mount for TV, collected from patients with bacteria vaginosis, were inoculated in a tube of CAT medium, incubated at 37°C and checked daily by wet mount until found positive. All samples were also tested by rapid antigen and NAATs as above.

**Results:** The ATCC TV spiked CAT medium tubes were found positive by wet mount, antigen detection and PCR. The ATCC CT/GC spiked CAT Medium were found positive for CT and GC with the PT, AC2, and the RA. No interference or inhibition was found with any of the molecular assays. In the CAT medium inoculated with TV positive swabs, 19/20 samples were positive by wet mount, antigen detection and by PCR after 24 hours. One sample showed positive by wet mount and antigen after 48 hours.

**Conclusions:** Copan CAT Media allows detection of TV by wet mount, culture rapid antigen and PCR from ATCC and clinical specimens. Vaginal specimens collected in CAT medium can be tested for the detection of CT, and GC, with the BD ProbeTec, the GenProbe AC2 and the Roche amplicor.

#### **P1815** Evaluation of APTIMA *Trichomonas vaginalis* assay performance with paediatric genital specimens

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**Objectives:** APTIMA *Trichomonas vaginalis* (ATV) assay is a FDA-cleared nucleic acid amplification test for the laboratory detection of *T. vaginalis*. We describe the performance of ATV assay with several types of urogenital specimens obtained from adolescent and young adult females <21 years old enrolled from nine US centers during the prospective, multi-center clinical trial for FDA submission.

**Method:** Specimens collected following consented patient enrollment included; a urine specimen, three vaginal (Vag) swabs (for wet mount analysis, culture and ATV), endocervical (EC) swab and PreservCyt solution (PCyt) liquid Pap. The wet mount and broth culture was performed at participating US sites. ATV testing on all specimens was performed in select reference laboratories using the automated TIGRIS DTS system. A patient was classified as infected if one or both culture and/or wet mount was positive and non-infected if both test results were negative.

**Results:** A total of 274 subjects with a median age of 18 years (range 14–20) were enrolled. Of 274 specimen-sets collected, the following number of specimens were evaluable following removal of samples that were expired or obtained following inadequate sampling: Urine (n = 211), EC (n = 253), Vag (n = 243) and Pcyt (n = 228). A total of 29 patients were considered infected based on positive test result with wet mount analysis (n = 22) and/or culture (n = 28). Infection was diagnosed in 13% (21/165) of symptomatic and 9% (8/93) of asymptomatic subjects. Sensitivity of the ATV assay for detecting TV was 100% for vaginal, endocervical, and PCyt specimens, and 91.3% for urine specimens. Two urine samples false-negative in the AHPV assay were also negative by PCR and alternate TMA assays for TV. Specificity of ATV assay was 100% for PCyt samples, and 97.9%, 98.1% and 99.1% for urine, vaginal, and endocervical samples, respectively.

**Conclusion:** The ATV assay is a highly sensitive and specific test for detection of trichomoniasis in adolescent girls using various urogenital specimens.

## Multidrug-resistant Gram-negative organisms – old and new strategies

### **P1816** Comparison of antimicrobial susceptibility of *Acinetobacter baumannii* from European inpatient and outpatient isolates: TEST 2007–2011

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**Background:** *Acinetobacter baumannii* has been recognized as an important opportunistic pathogen responsible for pneumonia, septicemia, urinary tract infections and meningitis, and is often associated with nosocomial outbreaks. Due to their capacity to acquire and accumulate resistance determinants, clinical isolates of *A. baumannii* are often multi-drug resistant and difficult to eradicate. The Tigecycline European Surveillance Trial (TEST) has been monitoring antibiotic susceptibilities in Europe since 2007. This study investigated the activity of tigecycline and comparator antibiotics against clinical isolates of *A. baumannii* from in-patients (IP) and out-patients (OP) in Europe originating from multiple infection sources collected during 2007–2011.

**Methods:** All isolates were collected from European countries during the TEST surveillance program. MICs were performed at each site following CLSI guidelines and interpreted according to EUCAST guidelines where available. CLSI breakpoints were used where EUCAST breakpoints do not exist.

**Results:** Results are shown in the following table (MIC<sub>50</sub>/90 in mg/L, %S = % susceptible).

	In-patient (n=3403)			Out-patient (n=278)		
	MIC <sub>50</sub>	MIC <sub>90</sub>	%S	MIC <sub>50</sub>	MIC <sub>90</sub>	%S
Amikacin	8	>64	55.1	4	>64	75
Cefepime	16	>32	49.3	8	>32	66
Ceftazidime	16	>32	42.6	≤8	>32	62
Ceftriaxone	64	>64	23.4	16	>64	32
Levofloxacin	4	>8	41.2	0.25	>8	63
Meropenem	2	>16	57.2	1	>16	73
Minocycline	≤0.5	8	84.5	≤0.5	4	91
Pip Tazo	128	>128	40.9	16	>128	54
Tigecycline	0.5	2	na	0.25	2	na

**Conclusions:** Tigecycline and minocycline exhibited the highest in vitro activity against *A. baumannii*, with MIC<sub>50</sub>/90 values for tigecycline of 0.5/2 mg/L (OP) and 0.25/2 mg/L (IP), and minocycline values of ≤0.5/8 mg/L (IP) and ≤0.5/4 mg/L (OP). Minocycline was the only compound tested with susceptibility >80% for both IP and OP. All compounds exhibited a significant difference (p > 0.001, Fisher's exact test) in %S between IP and OP isolates.

### **P1817** Colistin in combination with daptomycin enhances the effect against *A. baumannii*

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**Objectives:** Multi-drug resistant (MDR) Gram-negative bacteria such as *Acinetobacter baumannii* are currently a major problem in

healthcare, and considering the lack of new antibiotics under development the situation will only worsen during the next ten years. To counter this threat we need to extend the lifetime of current antibiotics by e.g. finding suitable candidates for combination therapy. We chose to screen for novel colistin interactions since it is one of the few remaining active drugs against MDR *A. baumannii*. Drugs picked from each major antibiotic group including unconventional candidates were tested with colistin, in total 10 different combinations.

**Methods:** Synergistic effects were screened for by in vitro time-kill for the combinations at static concentrations. To increase the chances of finding clinically applicable combinations the drug concentrations were free steady state serum concentrations from literature pharmacokinetic data. The test strain was an *A. baumannii* clinical isolate with an MIC of colistin slightly below the colistin concentration used in the time-kill experiment. The most significant interaction found was further characterized with 15 clinical isolates of *A. baumannii*.

**Results:** At the chosen concentrations, the combination with clearest increase in effect against the test-strain was colistin and the Gram-positive-specific lipopeptide-antibiotic daptomycin. As expected, daptomycin by itself had no effect, but in combination with colistin an increase in initial kill rate (1–4 hour) compared to colistin alone was seen. However, regrowth by phenotypically resistant cells occurred at a similar frequency in both cases. The effect of the combination was seen in a majority (13 of 15) of the additional isolates, and at 1 hour the average difference in killing for all strains was 2.3 log (CFU/mL).

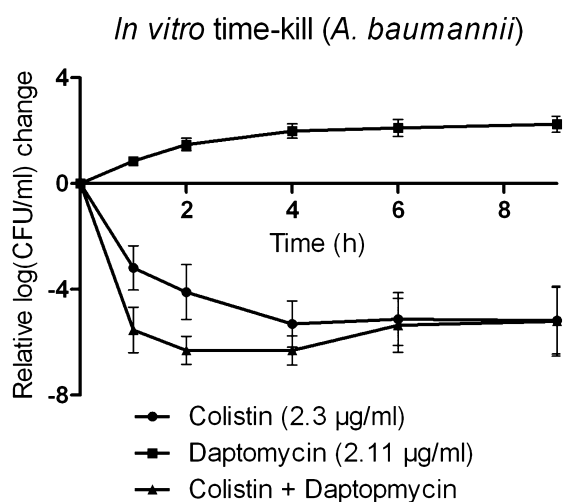


Fig.1: The mean value log(CFU/ml) change relative to the start inocula of *A. baumannii* in the presence of colistin, daptomycin and colistin-daptomycin in combination, on 15 clinical strains of *A. baumannii*. Start inocula  $\sim 10^7$  CFU/ml. Error bars denote 95% confidence interval (n=15).

**Conclusions:** Daptomycin can enhance the initial effect of colistin against *A. baumannii* and could be useful to achieve faster killing rates in a clinical setting, but more importantly a novel target for further antimicrobial development has been found. We speculate that the daptomycin effect is caused by the disruption of the outer cell membrane by colistin, allowing daptomycin to access the otherwise protected peptidoglycan layer, as in Gram positive cells. Studies are needed to characterize the mode of action of the combination.

#### P1818 Various antibiotic combination tests against MDR *A. baumannii* and *K. pneumoniae*

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**Objectives:** The aim of this study was to investigate the potential of antibiotic synergy against MDR *A. baumannii* and *K. pneumoniae* isolates in a tertiary teaching hospital.

**Methods:** We evaluated double-drug combinations of tigecycline (TG)/colistin (COL), TG/gentamycin (GN), TG/rifampicin (RIF), RIF/COL against *A. baumannii* and TG/COL, TG/GEN, COL/GEN against *K. pneumoniae*, using the E-test method, according to CLSI guidelines. We tested 42 *A. baumannii* strains and 30 *K. pneumoniae* isolated from various specimens of ICU patients, throughout 2011. All isolates showed carbapenem resistance and decreased susceptibility or resistance to TG (MIC  $\geq 2$ ). Synergy was defined as a fractional inhibitory concentration index (FIC)  $\leq 0.5$ , additive effect 0.5–1.0, indifference 1.0–4.0 and antagonism  $>4$ . Regarding resistance mechanisms, as tested by PCR, 27/30 (90%) of *K. pneumoniae* strains harbored blaKPC and 3/30 (10%) blaVIM. All *A. baumannii* strains harbored one or more blaOXA genes.

**Results:** Against 42 MDR *A. baumannii* strains, synergy and additive effect of TG with COL, GN and RIF was 78.6% and 21.4%, 40.5% and 59.5%, 100% and 0% respectively, while no antagonism phenomenon was observed; synergy of RIF/COL was 78.6% and additive effect 21.4% against the same strains. Against 30 MDR *K. pneumoniae* strains, synergy and indifference of TG/COL was 40% and 50% respectively, while 3/30 (10%) strains showed antagonistic phenomenon for this combination; synergy and additive effect of TG/ GN was 60% and 40%, with no antagonism or indifference; finally synergy and additivity of COL/ GN was 30% and 60% respectively, with antagonism of 10%.

**Conclusion:** Tigecycline was synergistic or additive in combination with all antibiotics tested, for MDR *A. baumannii* strains, while the most effective combination was TG/RIF for the strains tested. The next most effective combination was TG/COL and RIF/COL, for the above mentioned strains. Regarding MDR *K. pneumoniae* strains, the most synergistic effect was that of TG/GEN, with no indifferent or antagonistic phenomena, while TG/COL and COL/ GN showed both antagonism, however less common. Antibiotic synergy tests should be included in MDR susceptibility reports in clinical microbiology laboratories, as combination of TG/RIF and TG/GEN seems promising for patient treatment, although further studies are needed to correlate clinical outcome.

#### P1819 In vitro activity of carbapenems against multidrug-resistant gram-negative organisms at a tertiary care centre in Lebanon

G.F. Araj, F.A. Jaber\* (Beirut, LB)

**Background:** Globally, multi-drug resistant (MDR) Gram-negative bacteria have been on the rise including those showing carbapenem-resistance. Infections caused by such carbapenem-resistant organisms have a great impact on mortality, morbidity, hospital stay and cost. In our region, there is a dearth of information on such MDR pathogens.

**Objective:** The purpose of this study is to determine the in-vitro activity of carbapenems against MDR Gram-negative isolates at a tertiary care center in Lebanon.

**Methods:** A total of 270 isolates of *E. coli* (n = 101), *K. pneumoniae* (n = 57), *P. aeruginosa* (n = 40) and *A. baumannii* (n = 72) were recovered from various specimens of patients tested at the American University of Beirut Medical Center (AUBMC) during 2010. Etest-MICs were determined for doripenem, meropenem and imipenem according to the guidelines of CLSI testing and result interpretation criteria.

**Results:** The MIC<sub>90</sub>s for doripenem, meropenem and imipenem respectively were: 0.047, 0.064 and 0.75 mg/L for *E. coli* and 0.064, 0.047 and 1.5 mg/L for *K. pneumoniae*. Against *P. aeruginosa* and *A.*

*baumannii*, all three carbapenems had MIC90s of  $\geq 32$  mg/L. The susceptibility rates for doripenem, meropenem and imipenem respectively were as follows: 99%, 99% and 96% for *E. coli*, 98.2%, 98.2% and 91.2% for *K. pneumoniae*, 65%, 47.5%, and 27.5% for *P. aeruginosa*, and 38.9% and 36.1% and 16.7% for *A. baumannii*. Susceptibility to doripenem was observed in 46.2% of *P. aeruginosa* isolates that were resistant to imipenem and/or meropenem.

Table 1. Carbapenem in vitro activity vs. MDRO

MDRO	Antibiotic agent	MIC (mg/L)			% Sensitive*	% Sensitive*
		MIC range	50%	90%		
All isolates (n=270)	Doripenem	0.006–132	0.047	732	77.0%	73.5%
	Meropenem	0.012–132	0.047	732	74.4%	74.4%
	Imipenem	0.064–132	0.75	732	63.7%	63.3%
Enterobacteriaceae (n=151)	Doripenem	0.006–132	0.023	0.047	90.7%	100%
	Meropenem	0.012–132	0.032	0.047	90.7%	99.3%
	Imipenem	0.064–132	0.5	1	94.3%	94.2%
<i>E. coli</i> (n=451)	Doripenem	0.006–4	0.023	0.047	99.0%	100%
	Meropenem	0.012–6	0.023	0.064	99.0%	100%
	Imipenem	0.064–132	0.5	0.75	96.0%	97%
<i>K. pneumoniae</i> (n=67)	Doripenem	0.012–132	0.032	0.064	90.2%	100%
	Meropenem	0.023–132	0.032	0.047	90.2%	90%
	Imipenem	0.23–132	0.5	1.5	36.2%	32%
<i>P. aeruginosa</i> (n=40)	Doripenem	0.023–132	2	732	65.0%	50.5%
	Meropenem	0.012–132	6	732	47.5%	47.5%
	Imipenem	0.30–132	732	732	27.5%	27.5%
<i>A. baumannii</i> (n=72)	Doripenem	0.016–132	12	732	30.9%	16.9%
	Meropenem	0.012–132	16	732	36.1%	36.1%
	Imipenem	0.25–132	732	732	16.7%	16.7%

\*According to CLSI breakpoints of 2013.

\*According to CLSI breakpoints of 2010 for meropenem and imipenem and FDA breakpoints for doripenem. CLSI breakpoints for doripenem were not available back then.

**Conclusion:** Doripenem and meropenem has at least four-fold better activity than imipenem against Enterobacteriaceae. Doripenem was more active than meropenem or imipenem against *P. aeruginosa* with doripenem-susceptibility observed for some meropenem- and/or imipenem-resistant isolates.

#### P1820 Selection of biocide-resistant mutants among *Escherichia coli*, *Klebsiella pneumoniae* and *Salmonella* after pre-exposure to biocides or antibiotics

T. Curiao\*, J.L. Martinez, F. Baquero, T.M. Coque and the BIOHYPO Consortium (Brussels)

**Objectives:** The combined use of biocides and antibiotics in particular industrial, medical or veterinarian settings is a risk for developing cross resistance to both classes of compounds. The aim of this study is to determine the effects of pre-exposure to biocides (triclosan, TRI; benzalkonium chloride, BKC; chlorhexidine, CHX), or antibiotics (ampicillin, Amp; ciprofloxacin, Cip) on the selection of antibiotic/biocide-resistant bacteria.

**Methods:** Three antibiotic susceptible strains, B23 *-Escherichia coli* (Ec), *Klebsiella pneumoniae* (Kpn), *Salmonella enterica* SL1344 (Sal), were used. Cultures in plain Luria Bertani (LB) broth and LB supplemented with subinhibitory concentrations (1/2xMIC) of biocides or antibiotics were incubated overnight at 37°C. Aliquots of 100 µL were sub-cultured at 30°C on LB plates containing TRI, CHX, BKC, Amp or Cip (4xMIC), and viable counts determined. CLSI procedures were applied for susceptibility testing. Stability of mutants was evaluated after serial passages in non-selective LB broth (>3 passages).

**Results:** Stable combined TRIR/BZCR mutants of Ec, Kpn and Sal (MICTRI and MICBZC stably increased 2–16 fold and two-fold respectively) were recovered in the absence of pre-exposure, but more frequently with previous exposition to antibiotics (ciprofloxacin) or biocides. Stable TRIR mutants with increased susceptibility (HS) to CHX were observed for Ec after with and without previous biocide exposure. Stable combined TRIR/BZCR/CHXR, TRIHS/BZCR/CHXR and single BZCR mutants (MICBZC and MICCHX increased two-fold, MICTRI increase/decreased 16-/two-fold) were only obtained for Sal after exposure and selection with different antibiotics and biocides. A variety of stable mutants exhibiting increased susceptibility to TRI, CHX and BZC (2-fold MIC) was obtained for Sal (TRIHS) or Kpn (TRIHS/BZCHS, TRIHS/CHXHS) after pre-exposure to different compounds.

**Conclusions:** The exposure of Ec, Kpn, or Sal to either biocides or antibiotics (ciprofloxacin, ampicillin) results in the emergence of a diversity of biocide resistant mutants, indicating a trade-off of selective processes shaping the evolution of combined antibiotic-biocide resistance.

#### P1821 Bactericidal activity of fosfomycin against NDM-1 producing Enterobacteriaceae

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**Background:** Therapeutic options against the recently emerged New Delhi metallo-beta-lactamase-1 (NDM-1) producing Enterobacteriaceae are limited. With very few newer antibiotics in the pipeline, there is an urgent need to re-explore the older agents against emerging resistant strains.

**Methodology:** Overnight cultures of eight well characterised strains of NDM-1 producing Enterobacteriaceae (two *E. coli*, two *K. pneumoniae*, and four *K. oxytoca*) and one control strain (*E. coli* NTCC 13353) were selected. The fosfomycin (FM) MIC for all the isolates was determined by micro dilution method. Out of eight isolates, the MIC of four of the isolates was >128 mg/L (resistant group), and the MIC of the remaining four isolates were between 0.5 and 4 mg/L (sensitive group). The MIC of control strain *E. coli* NTCC13353 was <0.12 mg/L. Time-kill curves using initial inoculums of 10<sup>6</sup> CFU/mL in Muller-Hinton broth over 48 hours at following time points 0, 1, 3, 6, 12, 24, 36 and 48 hours. We used following FM concentrations (Cmax/Cmin/Coverage expressed as mg/L) of 250, 20, and 5 respectively along with a growth control for each isolate. These concentrations reflected serum concentrations achieved by intravenous FM therapy (dose 4–8 g) in published clinical studies.

**Results:** FM produced a concentration dependent bactericidal activity against all isolates at peak concentration with a mean log drop in viable count of 2–4 at 3–6 hour, and >4 at 12 hours against sensitive strains, and 2–3 log drop at 3–6 hour, and 1 log drop at 12 hour against resistant strains. Only one out of five sensitive isolates showed any growth beyond 12 hours of incubation as compared to grow back above baseline amongst all isolates in resistant group. At average (Cs) concentrations there was 2–3 log drop in viable count between 3 and 12 hour against sensitive strains, and 1–2 log drop against resistant strains, with a grow back above baseline level beyond 24 hour in both sensitive as well as resistant strains. At trough concentrations, there was modest cidal activity of 1.5–2.5 log drop at 3–6 hour only against sensitive strains.

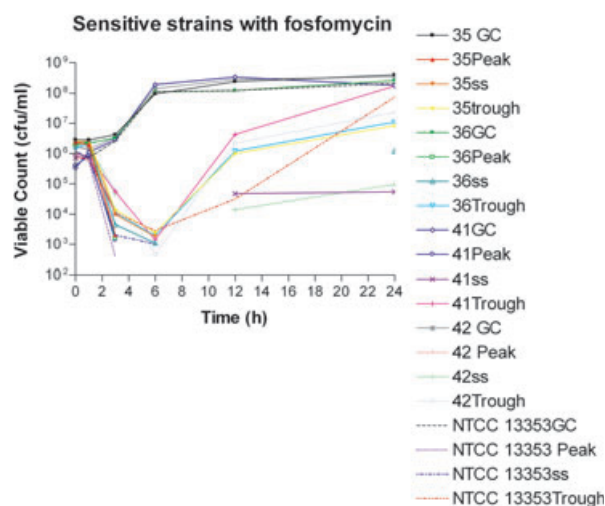


Fig. 1. Sensitive strains with fosfomycin.



**Conclusions:** Fosfomycin showed a concentration dependent bactericidal activity against NDM-1 producing Enterobacteriaceae, especially against sensitive strains. Hence FM would be a useful systemic therapeutic option against sensitive NDM-1 producing strains.

**P1822 High-dose doripenem and polymyxin B are bactericidal against pan-drug resistant *Pseudomonas aeruginosa* and suppress the emergence of resistance beyond 24 hours in a hollow fibre infection model**

T.P. Lim\*, W. Lee, S. Sasikala, N. Rafida, T.Y. Tan, L.Y. Hsu, J. Teo, T.T. Tan, A.L. Kwa (Singapore, SG)

**Background:** Pan-drug resistant (PDR) *Pseudomonas aeruginosa* (PA) resistant to polymyxins is increasing in Singapore; leaving no therapeutic options available. Polymyxin B (P) is the mainstay in Singapore although there is limited clinical experience around the globe. Doripenem (D) is a new carbapenem that had increased potency in-vitro against PA. We had previously elucidated the percentage of XDR PA that is susceptible to D + P (exhibited bactericidal activity of  $\geq 3$  log decrease from baseline at 24 hour) via time-kill studies to be 25%. We aim to further evaluate the activity of D + P against XDR PA beyond 24 hour in a hollow-fibre infection model (HFIM).

**Methods:** A HFIM was used to simulate clinically relevant D & P dosing regimens against XDR PA which are susceptible to D + P. Two isolates with D MIC =  $\geq 64$  mg/L & P MIC  $\geq 16$  mg/L was studied. These isolates harboured VIM-like metallo-beta-lactamases and VEB-1 genes. D was simulated at 1 g every 8 hour given as a 4 hour infusion and P was simulated at 1 MU every 12 hour alone and in combi with an initial inocula of 5 log CFU/mL. Quantitative counts over 120 hour were conducted in duplicate. Further emergence of resistance of the isolates against P in the HFIM were quantified using drug-free and selective (P at 3x MIC) media. D & P drug levels were taken at periodic intervals for analysis by Liquid Chromatography tandem Mass Spectrometry (LCMS).

**Results:** Against the 2 XDR PA isolates, P alone did not exhibit any killing effect despite repeated dosing every 12 hour and reached 9 log CFU/mL at 24 hour, as expected. Repeat MIC testing of the resistant isolates confirms further P resistance (MICs 64–128 mg/L). D alone was bacteriostatic initially till 8 hour before reaching 9 log CFU/mL at 24 hour despite repeated every 8 hour. D + P exhibited bactericidal activity and achieved sustained bactericidal killing up to 120 hour with no regrowth and suppressed the emergence of resistance to P. The pharmacokinetics of D & P, simulated at 1 g every 8 hour given as a 4 hour infusion and 1 MU every 12 hour alone respectively, were validated by LCMS analysis.

**Conclusions:** D + P may be a potential antibiotic combination as pre-emptive therapy for XDR PA infections. The in-vivo relevance of our results warrants further investigations.

**P1823 Antimicrobial susceptibility testing against multi-resistant Enterobacteriaceae isolates**

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**Objectives:** The number of infections due to multiresistant Enterobacteriaceae is rising. Mechanisms of resistance can be production of extended-spectrum beta-lactamase (ESBL), AmpC beta lactamase, carbapenemase or impermeability of antibiotics. Treatment options are scanty and therefore “forgotten” antibiotics have recently been reconsidered. Treatment alternatives comprise fosfomycin, colistin, nitrofurantoin, chloramphenicol and tigecycline. A rather unknown alternative might be temocillin which has a remarkable beta-lactamase stability and resilience to all classical extended-spectrum TEM, SHV and CTX-M enzymes. There is little clinical experience for temocillin treatment of multiresistant till now.

**Methods:** Multiresistance was defined as being susceptible to only one class of first-line antimicrobials (penicillins, cephalosporins, quinolones and carbapenems) and being intermediate or resistant to all classes of

second-line antimicrobials (aminoglycosides, tetracycline, cotrimoxazole and tigecycline) or as being intermediate or resistant to first-line antimicrobials (susceptibility to second-line antimicrobials not relevant).

Phenotypically multidrug-resistant strains were further characterized and tested for their antimicrobial susceptibility against several antibiotics of reserve.

**Results:** The production of ESBL, AmpC beta lactamase, carbapenemase or impermeability of antibiotics were detected among the multiresistant strains using CLSI methodology and molecular diagnosis.

Antimicrobial susceptibility testing revealed different patterns of resistance among the tested strains. There was no clear association between the mechanism of resistance and the antimicrobial susceptibility testing.

**Conclusion:** The results of our study for in vitro susceptibility testing of multiresistant Enterobacteriaceae strains show that in case of multiresistance even “forgotten” antibiotics should be taken into consideration.

Tigecycline, fosfomycin and colistin are antibiotics which are already frequently used as treatment alternatives. Nitrofurantoin, chloramphenicol and temocillin seem to be less frequently used.

Especially temocillin might be a promising treatment option if tested susceptible.

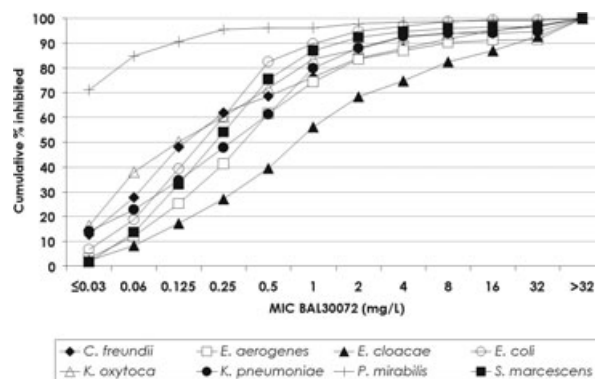
**P1824 In vitro activity of BAL30072 against European and North American Enterobacteriaceae clinical isolates**

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**Objectives:** BAL30072 is a new monosulfactam that is stable to a wide range of beta-lactamases including metallo-beta-lactamases, and is active against a broad spectrum of Gram-negative pathogens, including multidrug resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. We investigated the in vitro activity of BAL30072 and comparator antibiotics against 2442 recent clinical isolates of Enterobacteriaceae.

**Methods:** Isolates of *Citrobacter freundii* (287), *Enterobacter aerogenes* (305), *E. cloacae* (301), *Escherichia coli* (310), *Klebsiella oxytoca* (305), *K. pneumoniae* (303), *Proteus mirabilis* (330) and *Serratia marcescens* (301) were collected in 2007–2009. Isolates were collected in approximately equal proportions from Europe and USA from a variety of body sites and hospital locations. MICs were determined by broth microdilution methodology in CAHMB using susceptibility panels prepared by TREK Diagnostics.

**Results:** The antibacterial activity of BAL30072 against each species is shown in the Figure as the cumulative percentage of strains inhibited. According to EUCAST breakpoints, 20% and 2.3% of the tested isolates were non-susceptible to ceftazidime and imipenem, respectively. BAL30072 inhibited >90% of all strains and 75% (42/56) of the imipenem non-susceptible strains at a concentration of 4 mg/L. BAL30072 exhibited slightly less potent activity against *C. freundii* (88% inhibited at 4 mg/L), *Enterobacter aerogenes* (87% inhibited at



4 mg/L) and *E. cloacae* (75% inhibited at 4 mg/L), possibly due to overexpression of chromosomal AmpC in isolates of these species.

**Conclusion :** BAL30072 offers a potential therapeutic benefit for infections caused by Enterobacteriaceae, including multidrug-resistant and carbapenem non-susceptible strains. Further investigation of the clinical efficacy of BAL30072 is warranted.

**P1825** **Establishing interpretative breakpoints for ceftipime-tazobactam: a novel B-lactam/B-lactam inhibitor combination**

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**Objectives:** The increasing prevalence of ESBLs necessitates need for alternative therapeutic options. Cefepime (PM), a 4th generation cephalosporin, when combined with tazobactam (TZ) would provide a good cover against ESBL and Amp C producers. The normalized resistance interpretation (NRI) method was used to generate interpretative species specific break points for this novel PM/TZ combination.

**Methods:** We determined susceptibility to PM/TZ as part of the ongoing EXXTEND study. The combination was tested on 1155 bacterial isolates [*E. coli* (n = 672), *Klebsiella* spp. (n = 404) and *Enterobacter* spp. (n = 79)] that were collected from various multicentric surveillance studies co-ordinated by our center. The strains were recovered from the following clinical specimens; blood (n = 385), urine (n = 349), pus (n = 251) and respiratory tract (n = 170). Majority of study strains were ESBL producers (n = 1103) Disc diffusion (DD) to PM/TZ was done by Kirby-Bauer method using 30 µg (PM):10 µg (TZ) discs (Hi Media, India). Minimum Inhibitory Concentration (MIC) was determined by agar dilution (PM range 128–0.062 µg/mL) at a fixed TZ concentration of 4 µg/mL. A lower DD and upper MIC limit at 2.5 SD from the theoretical mean of the normalized distribution was used for setting break point values.

**Results:** NRI analysis of DD and MIC distributions was performed species wise generating results for both *E. coli* and *Klebsiella* spp. For disc diffusion, NRI results showed a susceptible peak with a lower limit of 18 mm. Regular regression lines were also constructed which showed a clear clustering of susceptible isolates from resistant ones at a break point value of 18 mm for both species. Accordingly, the susceptible break points for PM/TZ was set to ≥18 mm. The equivalent MIC break point by both scatter plots and regression line calculations was determined to be 8 µg/mL.

**Conclusions:** The following DD breakpoint values are recommended; susceptible ≥18 mm, intermediate 15–17 mm and resistant ≤14 mm. Corresponding MIC breakpoints to be used are; susceptible ≤8 µg/mL, intermediate 16 µg/mL and resistant ≥32 µg/mL. The above breakpoints may be considered by clinical microbiology laboratories while testing the PM/TZ combination.

**P1826** **High-dose doripenem and polymyxin B are bactericidal against extreme drug-resistant *Acinetobacter baumannii* and suppress the emergence of resistance beyond 24 hours in a hollow fibre infection model**

T.P. Lim\*, W. Lee, S. Sasikala, N. Rafida, T.Y. Tan, L.Y. Hsu, J. Teo, T.T. Tan, A.L. Kwa (Singapore, SG)

**Background:** Extreme-drug resistant (XDR) *Acinetobacter baumannii* (AB), with decreased susceptibility to polymyxins is increasing in Singapore; leaving few therapeutic options available. Polymyxin B (P) is the mainstay in Singapore although there is limited clinical experience around the globe. Doripenem (D) is a new carbapenem that had increased potency in-vitro against AB. We had previously elucidated the percentage of XDR AB that is susceptible to D + P (exhibited bactericidal activity of ≥3 log decrease from baseline at 24 hour) via time-kill studies to be 26%. We aim to further evaluate the

activity of D + P against XDR AB beyond 24 hour in a hollow-fiber infection model (HFIM).

**Methods:** A HFIM was used to simulate clinically relevant D & P dosing regimens against XDR AB which are susceptible to D + P. Two isolates with D MIC = ≥64 mg/L & P MIC = 1 & 2 mg/L were studied. Both harboured OXA-23-like & OXA-51-like genes. D was simulated at 1 g every 8 hour given as a 4 hour infusion and P was simulated at 1 MU every 12 hour alone and in combi with an initial inocula of 5 log CFU/mL. Quantitative counts over 120 hour were conducted. Resistance selection of the isolates against P in the HFIM were quantified using drug-free and selective (P at 3x MIC) media. D & P drug levels were taken at periodic intervals for analysis by Liquid Chromatography tandem Mass Spectrometry (LCMS).

**Results:** Against the 2 XDR AB isolates, P alone was bactericidal at 4 hour before regrowth occurred at 24 hour to baseline and reached 9 log CFU/mL at 48 hour despite repeated dosing. Regrowth was seen due to selective amplification of resistant sub-population(s) on P supplemented plates. Repeat MIC testing of the resistant isolates confirmed P resistance (MICs 64–128 mg/L). D alone was bacteriostatic initially till 8 hour before reaching 9 log CFU/mL at 24 hour despite repeated dosing. D + P exhibited bactericidal activity and achieved sustained bactericidal killing up to 120 hour with no regrowth and suppressed the emergence of resistance to P. The pharmacokinetics of D & P, simulated at 1 g every 8 hour given as a 4 hour infusion and 1 MU every 12 hour alone respectively, were validated by LCMS analysis.

**Conclusions:** D + P may be a potential antibiotic combination as pre-emptive therapy for XDR AB infections. The in-vivo relevance of our results warrants further investigations.

**P1827** **In vitro activity of isepamicin against 6296 Enterobacteriaceae isolates collected at a tertiary-care general hospital in Greece**

S. Maraki, G. Samonis, D. Karageorgopoulos\*, M. Mavros, K. Tsokali, D. Kofteridis, M. Falagas (Heraklion, Athens, GR)

**Objective:** Although antimicrobial resistance in Enterobacteriaceae is increasing, few novel antibiotics against these pathogens have been developed. In the meantime, the reevaluation of older antibiotics may identify new therapeutic options. We sought to investigate the role of isepamicin in this regard.

**Methods:** We retrospectively evaluated the in vitro antimicrobial susceptibility to isepamicin and other clinically relevant antibiotics of unique-patient Enterobacteriaceae isolates, which were collected at the microbiological laboratory of the University Hospital of Heraklion, Crete, Greece, between 2004 and 2009. Susceptibility testing was done with the automated Vitek 2 system. The breakpoints for susceptibility to isepamicin were those proposed by the Comité de l'antibiogramme de la Société Française de Microbiologie. The Clinical and Laboratory Standards Institute breakpoints were used for all other antibiotics tested.

**Results:** A total of 6296 isolates were studied, including primarily 3401 (54.0%) *Escherichia coli*, 1040 (16.5%) *Klebsiella pneumoniae*, 590 (9.4%) *Proteus mirabilis* and 460 (7.3%) *Enterobacter* spp. isolates. The most frequent culture specimens were urine (57.3%), pus (13.8%), lower respiratory tract specimens (5.4%), and blood (5.3%). Outpatients constituted 33.1% and patients hospitalized in intensive care units constituted 7.8% of the 6296 source patients. Isepamicin was the most active of the antibiotics tested against all isolates: 6103 (96.9%) of the 6296 isolates were susceptible to isepamicin, followed by meropenem (5890, 93.6%), imipenem (5874, 93.3%), amikacin (5492, 87.2%), gentamicin (5444, 86.5%) and ceftipime (5422, 86.1%). Susceptibility rates for the 1040 *K. pneumoniae* isolates were highest for isepamicin (95.3%), followed by colistin (89.3%) and meropenem (63%). Regarding *K. pneumoniae* isolates with resistance to other antibiotics, 91% of the 392 carbapenem-resistant isolates, 88% of the 375 isolates that were non-susceptible to all other aminoglycosides and 86% of the 111 colistin-resistant isolates remained susceptible to isepamicin.

**Conclusion:** Isepamicin exhibited high in vitro activity against almost all of the major Enterobacteriaceae species. It could be a therapeutic option against carbapenem-resistant, KPC-producing *K. pneumoniae* that is endemic in our region, as it does not show considerable cross-resistance with other aminoglycosides or with colistin.

**P1828 Potential old and new drugs for treatment of multidrug-resistant gram-negative infections**

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**Objectives:** Multidrug resistance Gram-negatives (MDRGN) have become the main problem of nosocomial infections in the last decade. Therefore, potentials old and new drugs to treat these bugs must be constantly evaluated. The aim of this study was to evaluate the susceptibility profile and the presence of resistance genes among nosocomial infections caused by MDRGN.

**Methods:** One hundred and eighty-two strains (23 *A. baumannii* resistant to carbapenem, 48 *S. maltophilia* with 67% of resistance to levofloxacin and/or trimethoprim-sulfamethoxazole, 61 *Enterobacter* spp. and 50 *K. pneumoniae* resistant to at least one carbapenem) from four different hospitals were tested to determine their MIC by agar dilution (fosfomicin) and broth microdilution (minocycline, ampicillin-sulbactam, tigecyclin, colistin and polymixin B) according to the CLSI and FDA criteria. PCR for different resistance genes for each genus were performed (adeB, adeE, bla<sub>oxa</sub>23, bla<sub>oxa</sub>NDM, bla<sub>oxa</sub>143 and bla<sub>IMP</sub> for *A. baumannii*; sul-1, sul-2 and qnrMR for *S. maltophilia* and blaKPC for *Enterobacter* spp. and *K. pneumoniae*) with specific primers for each reaction.

**Results:** Table attached presents detailed results. In concerning of resistance phenotype, 52% of *A. baumannii* were resistant to ampicillin-sulbactam however none of them were resistant to minocycline or tigecyclin. This data may be related to the prevalence of bla<sub>oxa</sub>143 (70%) and adeB (66%) gene, since 75% of the resistants to sulbactam-ampicillin had adeB, also 75% the bla<sub>oxa</sub>143 and 53% had both genes. Most of strains of *S. maltophilia* were susceptible to all antibiotics tested: only 8% presented resistance for tigecyclin, 19% for fosfomicin and only one intermediate resistance for minocycline, but apparently that is not related to the presence of resistance genes, since qnrMR appears in almost all strains and sul-1 and sul-2 gene was shown in more susceptible than the resistant ones. One strain presented resistance phenotype for both tigecyclin and fosfomicin, but were susceptible to minocycline and had none of the genes tested. The majority of *K. pneumoniae* and *Enterobacter* spp. were also susceptible to all drugs tested; however, polymyxins resistance among KPC positives strains was detected.

**Conclusion:** Fosfomicin, minocycline, tigecyclin and polymyxins showed a good in vitro activity against most of strains tested, however, break points and PK/PD issues need to be better addressed to avoid misuse of these potential drugs and the emergency of resistance.

**P1829 In vitro activity of rifaximin against enteropathogenic bacteria isolated from travellers returning to the United Kingdom**

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**Objectives:** Rifaximin is a semi-synthetic rifamycin antibiotic licensed for treatment of travellers' diarrhoea (TD) in many European countries and the US. Although TD is usually regarded as a self-limiting condition, ciprofloxacin or azithromycin are commonly used if required. In contrast to these two agents, rifaximin is poorly absorbed from the gut and potentially offers an alternative with fewer systemic effects. The sensitivity of enteropathogens isolated from American tourists with TD is well described in the literature and has demonstrated an increase in resistance to first line therapies. Currently little is known with regards to UK travellers; therefore we assessed the in-vitro activity of rifaximin and other comparators against 450 isolates of enteropathogenic bacteria from travellers returning to the UK with diarrhoea.

**Methods:** The isolates tested comprised 90 isolates each of *Escherichia coli*, *Shigella* spp., non-typhoidal *Salmonella*, typhoidal *Salmonella* and *Campylobacter* spp. MICs were determined by CLSI agar dilution methodology. Comparator agents were rifampicin, ciprofloxacin, azithromycin, co-trimoxazole and doxycycline. Selected isolates non-susceptible to rifamycins (MICs > 32 mg/L) were screened by PCR for arr genes encoding rifamycin ADP-ribosyltransferases.

**Results:** Rifaximin was active at ≤32 mg/L (mode = 32 mg/L) against 352/360 (97.8%) Enterobacteriaceae. Rifaximin MICs were 64 mg/L for 6/90 (6.6%) typhoidal salmonellae and ≥128 mg/L for 2/90 (2.2%) *Shigella* isolates. Among the Enterobacteriaceae, 0.6% were non-susceptible to rifampicin (MICs > 32 mg/L vs. mode = 16 mg/L), 5.6% to azithromycin (MICs > 16 mg/L; BSAC ECOFF for *S. Typhi*), 9.2% to ciprofloxacin (MICs > 0.5 mg/L), and 39.4% to co-trimoxazole (MICs > 2 mg/L). For doxycycline, 54.4% of *E. coli* *Shigella* isolates were non-susceptible (MICs > 4 mg/L; EUCAST ECOFF for *E. coli*), as were 23.9% of *Salmonella* spp. (MICs > 8 mg/L; EUCAST ECOFF for *Salmonella*). Most (81/90) *Campylobacter* spp. were resistant to rifaximin at ≥128 mg/L. The six typhoidal salmonellae and two *Shigella* isolates with rifaximin MICs ≥64 mg/L were PCR-negative for arr genes.

**Conclusion:** Although high-level rifamycin resistance was detected in two isolates of *Shigella*, rifaximin showed good in-vitro activity against diverse Enterobacteriaceae isolated from travellers returning to the UK with diarrhoea. Rifaximin was inactive against *Campylobacter* spp., which have intrinsic, efflux-mediated resistance to rifamycins.

**P1830 In vitro activity of the novel monosulfactam antibiotic BAL30072 alone and in combination with meropenem against a diverse collection of clinically important Gram-negative pathogens, including colistin-resistant strains**

M. Hornsey\*, W. Stubbings, D.W. Wareham (London, UK; Basel, CH)

**Objectives:** The monosulfactam BAL30072 is a novel monocyclic beta-lactam antibiotic that is currently in Phase I of clinical development. We investigated the in-vitro activity of BAL30072 alone and in combination with meropenem against a collection of 30 Gram-negative isolates including type strains and clinical isolates with defined resistance mechanisms. The activity of BAL30072/meropenem in the presence of colistin was also investigated using a subset of the isolate collection.

Genus/species	<i>Acinetobacter baumannii</i> (n=23)	<i>Demingtonella multivaha</i> (n=4)	<i>Enterobacter</i> spp. (n=41)	<i>Escherichia parvularum</i> (n=5)
Ampicillin-sulbactam	Range	4 to 64	NT	NT
	MIC <sub>50</sub>	8	NT	NT
	MIC <sub>90</sub>	16	NT	NT
Minocycline	Range	<=0.06 to 0.5	<=0.25 to 1	NT
	MIC <sub>50</sub>	<=0.06	0.5	NT
	MIC <sub>90</sub>	0.25	2	NT
Tigecyclin	Range	0.25 to 4	0.25 to 16	0.12 to 4
	MIC <sub>50</sub>	0.5	1	0.25
	MIC <sub>90</sub>	1	4	2
Fosfomicin	Range	NT	32 to 256	1 to 128
	MIC <sub>50</sub>	NT	128	8
	MIC <sub>90</sub>	NT	256	64
Polymyxin B	Range	<=0.25 to 2	NT	1 to >=16
	MIC <sub>50</sub>	<=0.25	NT	2
	MIC <sub>90</sub>	0.5	NT	16
Colistin	Range	NT	NT	<=0.25 to 64
	MIC <sub>50</sub>	NT	NT	0.5
	MIC <sub>90</sub>	NT	NT	16
Resistance genes	70% bla <sub>NDM1</sub> 10% bla <sub>NDM2</sub> 0% bla <sub>NDM5</sub> 0% bla <sub>NDM10</sub> 0% bla <sub>NDM11</sub> 2% bla <sub>NDM12</sub>	0% qnrMR 0% sul-1 0% sul-2	10% bla <sub>KPC</sub>	10% bla <sub>KPC</sub>

**Methods:** Isolates were identified by MALDI-TOF mass spectrometry. MICs were determined by broth microdilution and agar dilution using IsoSensitest media. Potential synergy of the BAL30072/meropenem combination in the presence of colistin was assessed in checkerboard assays. Fractional inhibitory concentrations indices (FICI) were calculated.

**Results:** BAL30072 displayed good in-vitro activity both alone and in combination with meropenem against *Acinetobacter baumannii* including colistin- and tigecycline-resistant representatives of international clone II (MIC range,  $\leq 0.06$ –2 mg/L), with the exception of a single representative isolate of the “Burn” clone; a PFGE-defined UK lineage (international clone II) (MIC, 16 mg/L). The compound alone exhibited MICs of 4–16 mg/L against *Pseudomonas aeruginosa* strains with virulence profiles for acute and chronic infection and displayed good activity in combination with meropenem for all isolates including a VIM-2 producer (MIC range, 0.25–2 mg/L) except one CF isolate belonging to the “Liverpool Epidemic” lineage (MIC, 64 mg/L). The BAL30072/meropenem combination was also highly active against the majority of the Enterobacteriaceae isolates tested (MIC range,  $\leq 0.06$ –2 mg/L) including ESBL-producing, carbapenem- and colistin-resistant strains, although MICs were higher for NDM-producing *Escherichia coli* isolates (MICs, 8 mg/L) and a single AmpC-hyperproducing isolate of *Enterobacter aerogenes* which also displayed porin loss (MIC, 4 mg/L). Neither synergy nor antagonism was observed between BAL30072/meropenem combination and colistin.

**Conclusions:** BAL30072, alone and in combination with meropenem, displayed good in-vitro activity against a broad range of important Gram-negative bacteria, including multidrug resistant *A. baumannii*, *P. aeruginosa* and Enterobacteriaceae species, counting clinical isolates with diverse resistance mechanisms. Further in-vitro and in-vivo studies of BAL30072 alone and in combination are warranted.

## Multidrug-resistant *Pseudomonas aeruginosa*

### P1831 Multiple-locus variable-number tandem repeat analysis for multidrug-resistant *Pseudomonas aeruginosa* strain diversity survey in Bulgarian hospitals

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**Objectives:** The aim of the study is to investigate the genotype diversity by MLVA6 and antimicrobial resistance of *Pseudomonas aeruginosa* isolates from Bulgarian hospitals.

**Methods:** A total of 83 non-repeat *P. aeruginosa* isolates from four university and two regional hospitals during 2004–2008. The identification and antimicrobial susceptibility was investigated by conventional methods and VITEK 2 (Bio Merieux, France) system. Six previously described VNTR loci were combined in the MLVA assay. PCR products were separated on “QiAxcel” capillary electrophoresis system. Data and cluster analysis was carried out in Bionumerics v4.5 (Applied Maths, Belgium). The reproducibility, typeability and discriminatory power (Simpson’s Index of Diversity – D) were evaluated. A multiplex PCR for various types of ESBL and carbapenemase coding genes was performed as described before.

**Results:** According to a MLVA6 all 83 tested *P. aeruginosa* are grouped in 19 main genotypes and eight subtypes. Loci ms010 and ms061 are the most variable and provided a highest discriminatory power. The predominant types are as follow: GT4/4A (13 isolates : hospitals A,C,D); GT12/12A (11 isolates: hospitals A,B,C,D,H); GT2/2A (nine isolates : hospital A,C and D) and GT23/23A (nine isolates : hospital B and F). The genotype distribution appeared to be non-hospital dependent. Most of the genotypes were associated with different wards but several types were located in all hospitals. MLVA6 is characterized by 100% typing ability and 100% reproducibility studies to isolate set. The investigations for genes IMP, VIM, SIM,

GIM, SPM, KPC, OXA-48, GES are without any positive results. Some ESBL coding genes like VEB, PSE, OXA-2-like and OXA-10-like were already detected but the carbapenem resistance still remains uncharacterized.

**Conclusions:** By selected conditions it is possible amplification of the six VNTR loci in a single reaction which reduces analysis time and cost of test. The MLVA is the method of choice for typing hospital *P. aeruginosa* isolates as well as characterization of outbreaks.

Carbapenem resistant *P. aeruginosa* is an emerging problem in Bulgaria. The widespread occurrence of epidemic, multidrug resistant, including carbapenem resistant strains is a serious threat to hospitals. The establishment of carbapenem-resistant clusters in *P. aeruginosa* population is a dynamic process and requires molecular epidemiological studies.

### P1832 Evaluation of risk factors for nosocomial multidrug-resistant *Pseudomonas aeruginosa* infections

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**Objectives:** *Pseudomonas aeruginosa* (Pa) causes serious infections that lead high mortality and morbidity. Gradually increasing multidrug resistance (MDR) is a problem of concern in treatment. In this study, the risk factors for nosocomial MDR-Pa infections were evaluated at intensive care units (ICUs).

**Methods:** A retrospective case–control study was conducted in 670-bed Ankara Research Hospital between January 2008 and July 2011. The patients with MDR-Pa were detected by laboratory based surveillance of nosocomial infections in ICUs (Neurology, Neurosurgery and Anesthesia-Reanimation). Resistance to at least two different antimicrobial groups (carbapenems, beta lactam + beta lactamase inhibitors, fluoroquinolones and aminoglycosides) is used for definition of MDR-Pa. The patients with a nosocomial MDR-Pa infection constituted the study group, and patients hospitalized in the same units and with non MDR-Pa infections served as the control group. The detailed history of hospitalization, APACHE II scores, invasive procedures and previous antibiotic usage were recorded. The identification and antibiotic susceptibility of *P. aeruginosa* were made by VITEK II automated system (BioMérieux, France). The risk factors for MDR-Pa acquisition were analyzed with univariate and multivariate logistic regression tests using SPSS v.15.0 software.

**Results:** There were 37 patients in the study group and 83 patients in the control group. Results of univariate analyses for risk factors between two groups were presented in Table. On multivariate analyses, stay in neurology ICU (OR = 3.57, 95% CI = 1.38–9.18, p = 0.008) and previous use of meropenem (OR = 6.53, 95% CI = 2.39–17.82, p = 0.000) were detected as independent risk factors.

Table. Results of univariate analyses for risk factors between two groups

Risk factors	Cases (n=37) (n-%)	Controls (n=83) (n-%)	P value
Male sex	20-54.1	41-49.4	0.638
Age (mean)	58.6±19.9	58.2±19	0.907
Stay in Neurology ICU	16-43.2	14-16.9	0.002
Stay in Anesthesia-Reanimation ICU	15-40.5	42-50.6	0.308
Stay in Neurosurgery ICU	6-16.2	27-32.5	0.065
> 60 days stay in ICU	31-83.8	51-61.4	0.015
Days between admission and isolation (mean)	69.3±80.3	49.8±44.1	0.090
APACHE II score at admission (mean)	22±3.6	22.2±4.3	0.748
Infected with another organism	33-89.2	57-68.7	0.017
Mechanical ventilation	32-86.5	64-77.1	0.236
Tracheostomy	9-24.3	20-24.1	0.979
Total parenteral nutrition	31-83.8	56-67.5	0.065
Central venous catheter	34-91.9	74-89.2	0.645
Previous use of meropenem	36-97.3	69-83.1	0.030
Previous use of imipenem	3-8.1	8-9.6	0.788
Previous use of ciprofloxacin	6-16.2	4-4.8	0.037

**Conclusion:** The patients with the history of stay in neurology ICU and previous use of meropenem have increased risk of infection with MDR-Pa.

**P1833** Extensively drug-resistant *Pseudomonas aeruginosa* are increasing and are associated with invasive infections in the Netherlands

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**Objectives:** Treatment of infections due to *Pseudomonas aeruginosa* (PA) is often complicated by multidrug resistance (MDR). We determined the rate and prevalence of MDR and extensively drug-resistance (XDR) in PA and the association between resistance and the development of invasive infections in the Netherlands from 2008 to 2010.

**Methods:** All isolates submitted to the Dutch Infectious Diseases Surveillance Information System on Antibiotic Resistance (ISIS-AR) with susceptibility data available for gentamicin or tobramycin, imipenem or meropenem, and piperacillin or piperacillin/tazobactam, ciprofloxacin and ceftazidime were included. MDR was defined as non-susceptible to  $\geq 1$  agent in  $\geq 3$  antimicrobial classes using EUCAST breakpoints. XDR was defined as non-susceptible to  $\geq 1$  agent in all five classes. For prevalence and rate calculations we only included the first isolate per patient per year. Rates were calculated per 100.000 hospital admissions. Trends over time were analyzed by chi-square and generalized linear models with a negative binomial distribution. The association between bacteraemia and the presence of an MDR or XDR isolate was analyzed by multivariate logistic regression including age, gender, intensive care unit (ICU) admission and isolation site.

**Results:** Almost three percent of the 35 792 PA isolates were MDR and 0.3% were XDR. There was no significant increase over time in MDR-PA rate and prevalence, while the rate of XDR-PA increased from 3.8/100.000 admissions in 2008 to 5.3/100.000 admissions in 2010. Of the patients with an XDR isolate 2.8% developed a blood stream infection compared to 0.9% of the patients with an isolate that was not XDR ( $p < 0.001$ ). In multivariate logistic regression analysis ICU admission, aged between 18–65 years, urine as the first site of isolation and XDR (Odds Ratio: 2.0, 95% confidence interval: 1.1–3.0) were independently associated with a higher risk for the development of bacteraemia.

**Conclusion:** XDR PA are increasing in the Netherlands and although the numbers are still small, the independent significant association of the presence of an XDR isolate with subsequent development of bacteraemia illustrates the threat of these isolates for patient care and the need for timely and appropriate antimicrobial treatment.

**P1834** Detection of ESBLs among *Pseudomonas aeruginosa* clinical isolates in Greece: use of a modified double-disc synergy test with antibiotic discs containing boronic acid

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**Objectives:** *Pseudomonas aeruginosa* is a nosocomial pathogen implicated in serious infections. Although MBL-producing *P. aeruginosa* isolates are commonly detected in Greek hospitals, ESBL producers have been scarcely documented. In the present study we investigated the occurrence of ESBLs among *P. aeruginosa* isolates referred to an acute care Greek hospital.

**Methods:** During January 2008–July 2011, 347 isolates of *P. aeruginosa* with reduced susceptibility or resistance to ceftazidime and/or aztreonam (MIC > 8 mg/L) were screened for ESBL production. For the phenotypic detection of ESBLs a modified double-disc synergy test (DDST) using antibiotics discs supplemented with boronic acid was implemented. Phenotypically ESBL-positive isolates were subjected to PCR with specific primers for ESBL (PER, VEB, SHV, CTX-M, TEM, GES, OXA-2, OXA-10), MBL and KPC genes and sequencing analysis. ESBL-positive isolates were tested by agar dilution MICs and PFGE. Conjugation experiments using *E. coli* 2OR764 (Rif<sup>R</sup>) and *P. aeruginosa* PU21 (Rif<sup>R</sup>) as recipients were performed to test transferability of ESBL genes.

**Results:** Nine *P. aeruginosa* clinical isolates were prospectively found ESBL-positive using the modified DDST. PCR and sequencing identified PER-1 ESBL in five isolates and SHV-5 ESBL in the remaining four isolates. The latter isolates also coproduced VIM-17 metallo-beta-lactamase. All ESBL producers exhibited resistance to carbapenems, aminoglycosides, quinolones but remained susceptible to colistin; PER-1-producers were also susceptible to piperacillin/tazobactam. Conjugation experiments failed to transfer ESBL genes. PFGE revealed that four of the five PER-1 producers were clonally related, although they were recovered from separate hospital wards and in epidemiologically distinct periods of time. VIM-17/SHV-5 producers belonged to the same clonal type and were epidemiologically related.

**Conclusions:** The study documents the dissemination of PER-1-producing *P. aeruginosa* isolates in Greece and for the first time a hospital outbreak due to *P. aeruginosa* co-producing VIM-17 carbapenemase and SHV-5 ESBL. The coproduction of both ESBL and MBL in *P. aeruginosa* is worrisome since the therapeutic options are very limited. Phenotypic screening with the modified DDST was found an excellent tool to detect ESBL-producing *P. aeruginosa* isolates in the clinical laboratory, even in cases where additional multidrug-resistant genes are coproduced.

**P1835** Rapid spread of *Pseudomonas aeruginosa* clonal complex 235 throughout Russia: implications in increasing antibiotic resistance

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**Objectives:** In the last years, the use of modern typing techniques, particularly MLST, for the analysis of epidemiology and population structure of *Pseudomonas aeruginosa* (PA) has led to the identification of international epidemic clones and clonal complexes (CCs). In particular, the sequence type (ST)235 and related STs representing the CC235 have been reported from many countries and often associated with multidrug (MDR) and extensive drug resistance (XDR). This study aimed to assess the trend in prevalence of CC235 and its impact on antibiotic resistance in nosocomial PA in Russia.

**Methods:** A total of 2331 consecutive non-duplicate nosocomial PA isolates collected in 32 Russian cities as part of three national surveillance studies in 1997–1999 (n = 540), in 2002–2004 (n = 1012) and in 2006–2007 (n = 779) were analysed. Susceptibility testing was performed by agar dilution and interpreted by EUCAST

Table 1. Prevalence of antibiotic resistance in isolates of CC235 and other STs.

Antibiotic	% Non-Susceptible		p value ( $\chi^2$ test)
	CC235 (n=576)	Other STs (n=1755)	
Piperacillin-Tazobactam	92.9	60.1	<0.0001
Ceftazidime	78.8	33.4	<0.0001
Cefepime	85.2	40.0	<0.0001
Imipenem	79.0	58.5	<0.0001
Meropenem	94.2	46.8	<0.0001
Ciprofloxacin	99.3	54.8	<0.0001
Gentamicin	100.0	67.3	<0.0001
Amikacin	97.4	31.4	<0.0001
Colistin	4.2	6.8	0.1181
MDR	99.5	60.7	<0.0001
XDR	74.4	13.4	<0.0001
PDR	0.5	1.2	0.2349

criteria. Isolates belonging to CC235 were identified using a newly designed multilocus single nucleotide polymorphism (SNP) typing assay. To design the assay, a set of seven SNPs diagnostic for CC235 was identified in six chromosomal loci (*acsA*, *aroE*, *guaA*, *mutL*, *ppsA*, *trpE*) following analysis of the available sequence data from MLST database. Two multiplex real-time PCRs with fluorescent probes were developed and used to detect the above SNPs in all isolates studied. Strains of the known STs were used as controls. Selected isolates assigned to CC235 were typed by MLST.

**Results:** A total of 8 (1.5%), 232 (22.9%) and 336 (43.1%) isolates collected, respectively, from two cities in 1997–1999, 13 cities in 2002–2004 and 23 cities in 2006–2007 were identified as CC235. Ten CC235 isolates selected to represent different time periods, regions and antibiotic resistance phenotypes were all typed by MLST as ST235. Notably, the prevalence of resistance to almost any drugs, except for colistin, as well as of MDR and XDR was significantly higher among CC235 isolates than among isolates of other STs (Table 1). Furthermore, the increase in total resistance rates of PA to cephalosporins, carbapenems, aminoglycosides and fluoroquinolones over the course of the study closely corresponded to the gradual increase in prevalence of resistant CC235 isolates.

**Conclusion:** To our knowledge, this study provides the first evidence, obtained from longitudinal analysis, of the epidemic nature of CC235 and its key role in spreading antibiotic resistance in Russia, the world's largest country by its area.

#### P1836 Identification of a new integron-encoded OXA-type beta-lactamase, OXA-205 in a clinical isolate of *Pseudomonas aeruginosa*

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**Objectives:** Investigation of the prevalence of beta-lactamases encoding genes and characterization of class I integrons in a collection of multidrug-resistant (MDR) *Pseudomonas aeruginosa* isolates from Lithuanian hospitals.

**Methods:** A total of 111 MDR *P. aeruginosa* isolates were recovered from various clinical specimens collected in six regional hospitals in Lithuania during period 2005–2007. Susceptibility testing to 18 antibiotics was performed by disk diffusion method according CLSI. PCR and RFLP based analysis were used for the detection of class I integrons and beta-lactamase encoding genes. Gene cassette array structure was determined by DNA sequencing of variable parts of integrons. The OXA-205 gene was analysed by comparison with a known beta-lactamases encoding sequences deposited in databases.

**Results:** A detailed results on the prevalence of beta-lactamase genes, integrons and their gene cassette array structure as well as susceptibility data are shown in Table 1. Analysis of a novel 1503 bp gene cassette array of integron (named as In671) from imipenem resistant *P. aeruginosa* isolate P16 revealed two cassettes, including *aadB* and a cassette encoding a new OXA-type beta-lactamase, assigned as a OXA-205. Within deduced 266 amino acid sequence, all the conserved motifs typical of class D enzymes were found, namely, 72STFK75, 120SXV122, 146YGX148, 159W and 210KTG212. Comparison of amino acid sequence of OXA-205 with other class D beta-lactamases revealed that it belongs to the OXA-2 sublineage, showing the highest sequence similarity with OXA-type enzymes found in uncultured bacteria from a wastewater plant and clinical isolate of *Burkholderia cepacia* (97% and 96% sequence identity, respectively). It exhibits 91% sequence identity with well-described narrow spectrum class D beta-lactamase OXA-46. Although *P. aeruginosa* isolate P16 represents resistance to imipenem, OXA-205 shares weak relationship with carbapenem-hydrolyzing class D beta-lactamases (<40% sequence identity). The 59-base element of the *bla*OXA-205 gene cassette is in fact 55 bp long and includes all the elements typical for the *attC* sites and appears to be one of the shortest 59-be element ever described.

**Table 1.** Prevalence of antimicrobial resistance, beta-lactamase and aminoglycoside resistance enzymes coding genes and class I integrons among 111 clinical *P. aeruginosa* isolates from Lithuanian hospitals

Antibiotic tested	Antimicrobial resistance		No of isolates		Class I integrons					
	Gene	%	Gene	No	Gene	Size (bp)	No of isolates			
Antibiotic tested	CAZ	25%	<i>bla</i>	1	Gene cassette array					
	GEN	42%	<i>cat</i>	1						
	PIP	37%	<i>pse</i>	4						
	PT4	34%	<i>aca-1</i>	8				<i>acaA4-8</i>	800	12
	AMI	10%	<i>aca-2</i>	-				<i>acaB2</i>	750	5
	FEP	18%	<i>aca-3</i>	7				<i>acaA6-gruD</i>	1300	7
	FOT	65%	<i>catA1</i>	2				<i>acaA4-blaOXA-1-like</i>	1700	3
	CIP	34%	<i>catA2</i>	1						
	IMI	32%	<i>per</i>	1						
	TIM2	47%	<i>tes</i>	-				<i>acaB-blaOXA-205</i>	1500	1
	TOB	41%	<i>ges</i>	-				<i>acaB2</i>	750	1
	SXT	96%	<i>imp</i>	1				<i>acaA6-gruD</i>	1300	1
	AS2	5%	<i>vim</i>	1				<i>acaA7-imp-gruD</i>	1500	1
	FCP	31%	<i>ant(2)-Ia</i>	4				Total	30	
	LOM	40%	<i>aca(6)-Ib</i>	6						
	TIC	45%	<i>aph(6)-Ia</i>	-						
	AXO	0%	<i>aca(3)-Ib</i>	-						
	LEVO	36%	<i>int1</i>	46						

\* CAZ - ceftazidime, GEN - gentamicin, PIP - piperacillin, PT4 - piperacillin/tazobactam, AMI - amikacin, FEP - cefepime, FOT - ceftazidime, CIP - ciprofloxacin, IMI - imipenem, TIM2 - ticarcillin/clavulanic acid, TOB - tobramycin, SXT - trimethoprim/sulfamethoxazole, AS2 - ampicillin/sulbactam, FCP - cefepime, LOM - lomefloxacin, TIC - ticarcillin, AXO - ceftazoxime, LEVO - levofloxacin.

**Conclusion:** this study reports the new OXA-type beta-lactamase, OXA-205, harboured by integron In671 in clinical *P. aeruginosa* isolate. Further investigation of this enzyme is necessary to clarify its properties and clinical significance.

#### P1837 The saga of the extended-spectrum oxacillinases (ES-OXA) continues in *Pseudomonas aeruginosa*

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**Objectives:** Development of resistance to 3rd generation cephalosporins such as ceftazidime (Caz) and cefepime (Fep) mainly results from constitutive overproduction of intrinsic  $\beta$ -lactamase AmpC in *Pseudomonas aeruginosa*. However, emergence of isolates expressing extended-spectrum  $\beta$ -lactamases (ESBL) that derive from OXA-type penicillinases is increasingly reported worldwide. This study describes four novel extended-spectrum oxacillinases (ES-OXA) detected in France by the National Reference Center for Antibiotic Resistance.

**Material and methods:** Strains 10 380, 10 396, 11 581, and 11 599 were isolated in 2010 and 2011 at Besançon, Saint Denis de la Réunion (Indian ocean), Marseille, and Orléans, respectively. Double-disk synergy tests with Caz-clavulanate and Caz-imipenem were positive for these bacteria. In 10 396, 11 581 and 11 599, the  $\beta$ -lactamase encoding genes were amplified by PCR with consensus primers and then sequenced. Resistance of 10 380 to Caz was first transferred by conjugation to *P. aeruginosa* PU21 (Rif R). Then, the 40-kb plasmid conferring the resistance was purified and used to construct a BamHI library in *Escherichia coli*.

**Results:** The four strains of *P. aeruginosa* were all highly resistant to Caz (MIC  $\geq 64$   $\mu$ g/mL). 10 380 contained a class I integron carrying the gene cassettes *aacA4*, *blaPSE-1*, and *aadA2*. This isolate also harboured a conjugative plasmid carrying *blaOXA-2* (that codes for the narrow-spectrum penicillinase OXA-2) flanked by a mutated copy (W159R) of that gene determining a novel ESBL hydrolyzing Caz. Two new extended-spectrum variants of penicillinase OXA-10 were identified in strains 11581 (OXA-220: Q144P, W154G) and 11599 (OXA-222: W154R). Strain 10 396 turned out to produce a variant of OXA-35 (OXA-221: W154R).

**Conclusion:** Penicillinases OXA-2, OXA-10, and OXA-35 are known progenitors of many ESBL in *P. aeruginosa*. The genes coding for these enzymes are often located in class I integrons, sometimes along with carbapenemase genes (*blaVIM-2* in 11599). The duplication and then mutation of gene *blaOXA-2* in strain 10 380 seems to be a rare event in the emergence of ES-OXA. Positions G157 and W154 in OXA-10 (G162 and W159 in OXA-2) are crucial for the extension of the hydrolytic activity to 3rd generation cephalosporins.

**P1838** Porin OprD sequence variations and resistance to imipenem in clinical strains of *Pseudomonas aeruginosa*

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**Objectives:** To investigate the role of quantitative and qualitative variations of specific porin OprD in the resistance of cystic fibrosis (CF) and non CF strains of *Pseudomonas aeruginosa* to carbapenems (imipenem MIC > 4 mg/L, EUCAST breakpoints).

**Material and methods:** The oprD gene was sequenced in 144 carbapenemase negative, imipenem non susceptible *P. aeruginosa* recovered in 2009–2010 from 36 French hospitals. The gene and protein sequences were compared to that of wild-type reference strains PAO1, PA14 and LESB58. The relative expression of gene oprD was measured by real-time reverse transcription-PCR (RT-qPCR) and the amounts of porin OprD in the outer membrane were assessed by western-blotting (WB) in selected strains. The PyMOL software was used to map amino-acid substitutions in the 3-D structure of OprD.

**Results:** A first group of 119 isolates (32 CF, 87 non-CF) was found to contain single point mutations, nucleotide insertions or deletions generating frameshifts or premature stop codons in the oprD gene. An insertion sequence disrupted oprD in six bacteria (one CF, five non-CF). In a second group of 15 strains (five CF, 10 non-CF), the amino acid sequence of OprD turned out to be identical to that of PAO1, PA14, or LESB58. RTqPCR and WB experiments demonstrated that all of these strains were strongly deficient in OprD production. Finally, a third group of 10 isolates (five CF, five non-CF) exhibited amino acid variations (up to 7) in OprD as compared with the reference strains. Most of the substitutions mapped outside the pore, in the beta-sheets presumed to interact with the peptidoglycan or the lipids of the outer membrane. Importantly, the amounts of the porin in the membrane were dramatically reduced in 7/10 of these isolates despite wild-type oprD gene expression.

**Conclusion:** In CF and non CF *P. aeruginosa*, development of resistance to imipenem predominantly results from mutations inactivating the oprD gene (82.7%) or turning down its expression (10.4%). This study demonstrates for the first time that in some strains (6.9%), mutational variations in the sequence of the porin can affect its insertion into the outer membrane and thus carbapenem penetration. Mutants of that type are difficult to detect as they require WB experiments for their characterization.

**P1839** Reactive oxygen species and antibiotic pressure leads to multidrug resistance in *Pseudomonas aeruginosa*

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**Objectives:** *Pseudomonas aeruginosa* are the most common cause of nosocomial infections. Hospital infection by strains acquired multidrug resistance (MDR) has been problem worldwide. Recently, it was reported that oxidative stress has been implicated occurring antibiotic-resistant mutants. The aim of the present study is to evaluate whether sub-MIC levels of antibiotics exposure and reactive oxygen species (ROS), such as hydroxyl radicals, can lead to MDR in *P. aeruginosa*.

**Methods:** *Pseudomonas aeruginosa* PAO1 standard strain was used in this study. Antibiotic susceptibilities of amikacin, meropenem, levofloxacin, piperacillin, piperacillin/tazobactam, and ceftazidime were determined using the Etest method. These six antibiotics were investigated for in vitro inducement of resistance and cross-resistant to other antibiotics. Reference PAO1 strain was incubated and transferred five times after being exposed to 1 mM ROS in addition to 1/2 MIC of each antibiotic by the agar dilution method. When cross-resistant to other antibiotics was confirmed, ampC, mexA, and oprD gene expression and quinolone-resistant mutation were examined.

**Results:** Sub-MIC of piperacillin induced resistant to beta-lactams and fluoroquinolone by stimulating of ROS. It was confirmed by real time PCR that the mechanisms of cross-resistant to beta-lactams were reduction of oprD gene expression, and higher expression of both ampC

and mexA genes. Furthermore, four amino acid changes (S373I, A375D, R377L, R378H) of ParE were detected. These multidrug resistant effects were prevented by the ROS scavenger  $\alpha$ -lipoic acid. The cross-resistant to aminoglycoside was not shown.

**Conclusion:** For multidrug-resistant acquisition in *P. aeruginosa*, stimulation with ROS is important factor as well as exposure to sub-MIC of antibiotic.

## Bacteraemia, sepsis and infective endocarditis

**P1840** Clinical and economic burden of hospitalisation due to *Streptococcus pneumoniae* sepsis and meningitis in Canada from 2004 to 2009

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**Objective:** Understanding the burden of illness associated with *Streptococcus pneumoniae* (SP) is critical to inform public health policy around vaccination programmes. We conducted retrospective analyses of data from 2004 to 2009 to quantify the incidence, mortality, length of stay (LOS), and hospitalisation costs of SP sepsis and meningitis in Canada (excluding Quebec).

**Methods:** A national database was analyzed to identify hospitalisations for SP sepsis and meningitis using International Classification of Diseases-10 codes. Population-at-risk data were obtained from Statistics Canada. Costs were estimated using the Ontario Costing Database.

**Results:** In patients aged 0–4 years, from 2004 to 2009 SP sepsis incidence declined from 7.3 to 4.3/100 000, whereas mortality rates were similar at 2.1% and 1.6%. In patients aged 0–17 years, average LOS increased from 6.5 to 10.1 days with an average total cost in 2009 of Canadian \$17 803. In adults aged  $\geq 65$  years, SP sepsis incidence did not change from 2004 to 2009 (9.0 and 9.2/100 000, respectively), whereas mortality increased slightly from 26.9% to 29.3%. LOS in this age group was similar (15.3 and 16.2 days in 2004 and 2009, respectively). In patients aged  $\geq 70$  years, the average total cost of hospitalisation for SP sepsis in 2009 was Canadian \$36 270. Incidence and mortality rate were highest in patients aged  $\geq 80$  years (12.8 and 12.9/100 000 and 36.8% and 32.3% in 2004 and 2009, respectively). From 2004 to 2009, SP meningitis incidence decreased in patients aged 0–4 years from 2.0 to 1.4/100 000; the mortality rate decreased from 7.7% to 0%. In patients aged  $\geq 65$ , the incidence of SP meningitis was 0.4/100 000 in 2004 and 0.6/100 000 in 2009; the mortality rate increased from 7.7% to 10.0%. LOS increased from 11.0 to 17.8 days in patients aged 0–17 years and from 10.9 to 36.2 days in patients aged  $\geq 70$  years.

**Conclusion:** In patients aged 0–4 years, the clinical and economic burden due to hospitalisation for SP sepsis and meningitis decreased concurrently with the introduction of paediatric conjugate pneumococcal vaccine. In adults aged  $\geq 65$  years, no changes in incidence of hospitalisation or mortality for SP sepsis and meningitis were seen over this time frame. Pneumococcal sepsis and meningitis remain a substantial health and healthcare system burden, particularly in the very young and very old, with mortality, LOS and cost varying greatly by age.

**P1841** PIRO score accurately predicts unfavourable outcome in septic patients

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**Objectives:** A new concept for stratification of septic patients based on Predisposition, Insult, Response, Organ dysfunction has been suggested and presented under the acronym PIRO. This concept considers several issues deemed as relevant for a better patient and syndrome description. A new model for a composite PIRO score has been recently proposed by Rubulotta *et al.* and the results of a clinical evaluation in a large

retrospective study have been released. The study concludes that the proposed PIRO score may become an effective model for staging sepsis.

The present study aimed to apply for the first time on a group of Romanian patients this staging system based on the PIRO concept, as well as to analyse its efficiency in predicting patients' death.

**Methods:** We retrospectively reviewed the charts of all septic patient admitted over a period of 42 months (2006–2009) at the Clinic of Infectious Diseases in Cluj-Napoca. Patients were included according to the 1992 ACCP/SCCM Consensus Conference criteria. Patient demographics, clinical and laboratory data were recorded. PIRO score was calculated by summation of the component scores: P, I, R and O, according to Rubulotta *et al.* Severity assessment from the perspective of organ failure at admission was performed using the SOFA score.

**Results:** Fifty-five out of the 250 studied patients (22%) died during hospitalisation. The composite PIRO score had a median value of 4 (0–10) while SOFA score had a median value of 4 (0–13).

The univariate logistic regression revealed that each of the components of PIRO score was an independent prediction variable for death. The multivariate logistic regression having each of the PIRO score components as independent variables and patients' death as the dependent variable showed that the O score was the most significant for mortality.

The composite PIRO score was significantly higher in deceased patients by comparison with survivors ( $p < 0.0001$ ). The composite PIRO score highly correlated with mortality rate ( $r = 0.933$ ;  $p < 0.0001$ ). The majority of the deceased patients (76.36%) had a PIRO score  $>4$ , while the majority of survivors (71.28%) had a PIRO score  $\leq 4$ . We also found a positive correlation between PIRO and SOFA ( $r = 0.61$ ;  $p < 0.0001$ ).

**Conclusion:** The composite PIRO score is predictive for patients' death and correlates with the mortality rate, a PIRO score of 4 representing a possible cut-off value in mortality prediction.

#### **P1842** Cohort study of mortality among hospital inpatients with sepsis

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**Objectives:** Sepsis outcome figures in the literature vary widely and are often derived from routine database studies in the US. The aim of this study was to analyse mortality among patients with sepsis in an NHS teaching hospital in Scotland.

**Methods:** Patients had been identified prospectively for a sepsis management intervention study in two cohorts from September 2008–February 2009 and October 2009–March 2010. Clinical data were linked to routine outcome data by anonymised record linkage via the Health Informatics Centre, University of Dundee. Univariate then multivariable binary logistic regression was used to calculate odds ratios for likelihood of death, within 30 and 90 days of sepsis onset, associated with demographic and clinical variables.

**Results:** Among the 640 patients in the combined cohort, 124 (19%, 95%CI 16–22%) died within 30 days, and 180 (28%, 95%CI 25–32%) died within 90 days.

In univariate analysis, older age, more co-morbidity (Charlson Index), having been admitted as an emergency, being in a medical ward at sepsis onset, and longer time from admission to sepsis onset, were associated with increased odds of death at 30 and 90 days. Higher scores on several generic and sepsis-specific severity scoring systems were associated with increased odds of death. Having a positive blood culture was only associated with increased odds of death at 90 days (OR = 1.46 [95%CI 1.02–2.09],  $p = 0.04$ ).

In the multivariable analysis, co-morbidity was no longer significantly associated with increased odds of death at 30 days. The other variables significant in the univariate regression (above) were still significant. In particular, being admitted as an emergency and having sepsis onset  $>3$  weeks after admission were associated with higher odds of death. Being in a surgical or orthopaedic, rather than medical, ward at sepsis onset was associated with lower odds of death (Table 1).

Multivariable logistic regression results for 30 day mortality		
Variable	Category	Adjusted odds ratio (95%CI)
Age group	<60yrs (161)	1.00
	60-69yrs (123)	1.00 (0.41-2.41)
	70-79yrs (173)	2.62 (1.27-5.40)**
	$\geq 80$ yrs (161)	6.16 (3.02-12.59)**
Admission type	Elective (130)	1.00
	Emergency (488)	4.17 (1.39-12.47)**
Ward type	Medicine (272)	1.00
	Surgery (235)	0.44 (0.25-0.77)**
	Orthopaedics (68)	0.19 (0.08-0.49)**
	Other (43)	0.50 (0.20-1.30)
Days from admission to sepsis onset	1-3 (178)	1.00
	4-7 (134)	1.81 (0.89-3.70)
	8-21 (209)	1.82 (0.96-3.47)
	$>21$ (97)	2.93 (1.40-6.16)**
CURB65 score	0 (105)	1.00
	1 (173)	3.63 (0.93-14.09)
	2 (185)	7.30 (1.81-29.36)**
	$\geq 3$ (155)	13.20 (3.21-54.21)**
Standardised Early Warning System score	0-1 (88)	1.00
	2-3 (236)	1.12 (0.53-2.38)
	4-5 (175)	1.44 (0.68-3.08)
	$\geq 6$ (119)	2.40 (1.09-5.27)*
Systemic Inflammatory Response Syndrome criteria	2 (220)	1.00
	3 (269)	1.42 (0.81-2.48)
	4 (127)	2.48 (1.34-4.56)**
Rivers severe	No (573)	1.00
	Yes (45)	2.02 (0.95-4.33)
Institute Healthcare Improvement severe	No (417)	1.00
	Yes (199)	2.66 (1.66-4.26)**
Surviving Sepsis Campaign severe	No (453)	1.00
	Yes (165)	3.29 (2.04-5.30)**
Survive sepsis severe	No (437)	1.00
	Yes (179)	2.70 (1.67-4.36)**

\* $p < 0.05$ , \*\* $p < 0.01$

Of the tested severity criteria the CURB65 score, developed and validated in community-acquired pneumonia, had the strongest association with mortality at 30 days. Meeting the criteria from Rivers *et al.*'s randomised controlled trial in severe sepsis was not associated with increased odds of death (Table 1).

**Conclusion:** This study combined prospective clinical data and routine outcome data and found significant mortality among inpatients with sepsis in a UK teaching hospital. The CURB65 score looks clinically useful for risk stratification but this requires further validation.

#### **P1843** Time to blood culture positivity of follow-up blood cultures: a laboratory predictor for clinical outcomes in patients with persistent *Staphylococcus aureus* bacteraemia

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**Objectives:** Time to blood culture positivity (TTP) has been suggested as one of clinically useful laboratory data in patients with *Staphylococcus aureus* bacteremia (SAB). However, the usefulness has been studied only for TTP of the first positive blood cultures. TTP



of follow-up blood cultures has never been studied for the clinical usefulness in patients with SAB, except a recent case report showing that lack of increase in TTP of follow-up blood cultures predicted failure of antimicrobial therapy early in patients who were being treated for persistent SAB. We investigated the change of TTP of follow-up blood cultures in patients with persistent SAB, and evaluated the clinical usefulness of the serial TTP follow-up in the management of patients with persistent SAB.

**Methods:** A total of 466 episodes of SAB occurred in 280 patients. Among them, 41 patients with persistent SAB were finally included. Persistent SAB was defined as the duration of bacteremia  $\geq 3$  days. Primary therapeutic intervention (PTI) was defined as the first introduction of active antimicrobial agents and/or the first removal of removable foci of infection. Lack of increase in TTP of follow-up blood culture was defined as the presence of decrease in TTP of any positive follow-up blood culture, compared to that of the preceding positive blood culture.

**Results:** Of 39 patients who had available data for clinical outcomes, 16 (41.0%) had 30-day mortality or secondary foci of infection. Patients who had resolution of SAB after PTI (12 of 39, 30.8%) had 30-day mortality or secondary foci of infection less frequently than those who did not have (8.3% vs. 55.6%,  $p = 0.012$ ). Of the patients whose SAB did not resolve after PTI, patients with lack of increase in TTP of follow-up blood cultures after PTI (46.2% of 26) had 30-day mortality or secondary foci of infection more frequently than those without it (83.3% vs. 28.6%,  $p = 0.005$ ).

**Conclusion:** In patients with persistent SAB, persistence of bacteremia after the introduction of active antimicrobial agents and/or the removal of removable foci may indicate poor outcomes or unresolved foci of infection. Also, in the patients whose SAB persist after therapeutic interventions, lack of increase in TTP of the follow-up blood cultures may be one of valuable laboratory predictors for poor clinical outcomes or hidden foci of infection.

**P1844 Results from the European Cubicin® Outcomes Registry and Experience (EU-CORE): high success rates with daptomycin in the treatment of patients with sepsis**

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**Objectives:** Gram-positive pathogens are increasingly recognised as major contributors to sepsis and systemic inflammatory response in hospitalized patients. Daptomycin, is rapidly bactericidal against Gram-positive bacteria without inducing cell lysis, a feature that makes it an attractive agent for the treatment of sepsis. In this analysis, we evaluated the clinical experience of daptomycin to treat patients with sepsis from a retrospective non-interventional study.

**Methods:** This analysis reports EU-CORE data collected from January 2006 to June 2011. Patients diagnosed with sepsis (as defined by investigators) at treatment start included in EU-CORE were assessed for clinical outcome (cure, improvement, failure or non-evaluable) by the investigators. Success was defined as the sum of cured and improved outcome rates. The safety assessments were conducted up to 30 days after the last administered dose of daptomycin.

**Results:** Out of 302 patients with sepsis, 65% were male and 44% were of age  $\geq 65$ . Pretreatment 52 (17%) had creatinine clearance of  $<30$  mL/minute and 59 (20%) were on dialysis. Bacteraemia was reported in 117 (39%) patients, 61 (20%) had complicated skin and soft tissue infections and 49 (16%) had endocarditis. The most frequently identified causative pathogens were *S. aureus* (28%,  $n = 83$ ); MRSA ( $n = 40$ ) and *S. epidermidis* (11%,  $n = 34$ ). With regard to daptomycin dose, 17% of patients received 4 mg/kg, 53% 6 mg/kg, and 14% 8 mg/kg or higher. The overall clinical success rate was 71% (43% cured and 28% improved); the clinical failure rate was 12% and 17% non-evaluable. Clinical success by daptomycin dose was 4 mg/kg, 56%;

6 mg/kg, 78% and  $\geq 8$  mg/kg, 76%. Clinical success in patients infected with *S. epidermidis* and *S. aureus* was 85% and 72%, respectively, with similar rates regardless of methicillin susceptibility. An elevation in creatine phosphokinase ( $>5-10 \times$  ULN) was reported in 3% of patients treated with daptomycin. Adverse events (AEs) possibly related to daptomycin were reported in six (2%) patients and four (1%) patients experienced serious AEs. AEs causing study drug discontinuation regardless of study drug relationship were reported in 25 (8%) patients. **Conclusions:** Daptomycin treatment showed a favourable safety profile and achieved success rates above 70% and 85% in sepsis caused by *S. aureus* and *S. epidermidis*, respectively. This data supports the use of daptomycin in sepsis.

**P1845 Results from a non-interventional study: daptomycin is effective as outpatient parenteral antibiotic therapy**

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**Objectives:** Outpatient parenteral antibiotic therapy (OPAT) is increasingly used in Europe for patients with serious Gram-positive infections (otherwise suitable for non-hospital therapy). OPAT reduces the risk of healthcare-associated infection, promotes quicker return to work, improves quality of life and reduces healthcare costs. Daptomycin has several characteristics that support OPAT, including a once-daily 2-minute administration, no need for therapeutic drug monitoring and a low propensity for drug-drug interactions. Here we report the clinical experience of patients treated with daptomycin via OPAT.

**Methods:** Data were collected from the European Cubicin® Outcome Registry and Experience (EU-CORE), a multicentre, retrospective, non-interventional registry, from January 2006 to June 2011. The registry records clinical characteristics (patient population, infections, pathogens and adverse events [AEs]) and outcomes for patients receiving daptomycin therapy in a "real world" setting. Clinical outcomes were assessed following daptomycin treatment by the investigators using standard definitions (cured, improved, failure, non-evaluable). Success was defined as the sum of the cured or improved outcome rates.

**Results:** A total of 551 patients received daptomycin as OPAT, 44% were  $\geq 65$  years of age, 62% were male and 5% were on dialysis. Daptomycin was most commonly used as OPAT in Spain (20% of all patients treated in the country), UK (24%) and Italy (17%). Complicated skin and soft tissue infection (28%), osteomyelitis (17%), endocarditis (14%), bacteraemia (12%) and uncomplicated skin and soft tissue infection (11%) were the most commonly treated infections. The most frequent causative pathogens were *S. aureus* (40%), *S. epidermidis* (11%), other coagulase-negative staphylococci (8%), *E. faecalis* (4%) and *E. faecium* (1%). Median time on OPAT was 22 days (1–290 days). Eighty-nine percent of patients had a successful

**Table 1. Clinical success rate with daptomycin by infection type and pathogen in OPAT patients**

OPAT	
Infection type	Success rate (%)
Bacteremia (n=67)	96
Endocarditis (n=76)	93
uSSTI (n=58)	88
Osteomyelitis (n=93)	85
cSSTI (n=152)	85
Pathogens	
	Success rate (%)
<i>E. faecalis</i> (n=20)	100
<i>S. epidermidis</i> (n=60)	93
<i>S. aureus</i> (n=213)	89
CoNS (n=43)	88
MRSA (n=114)	88
<i>E. faecium</i> (n=7)	71

outcome. Bacteraemia and endocarditis were the most successfully treated infections (96% and 93% success, respectively- Table 1). High success rates were seen against *E. faecalis* (100%) and *S. epidermidis* (93%). Serious AEs and AEs possibly related to daptomycin were reported in five (1%) and 47 (9%) patients, respectively. Blood creatine phosphokinase elevations were reported in eight (2%) patients.

**Conclusion:** Daptomycin is a useful, effective and well-tolerated in the OPAT setting in adults, including the elderly, and may be used for extended treatment periods when required.

**P1846 Safety of high-dose long-term daptomycin treatment ( $\geq 8$  mg/kg/day over more than 2 or 4 weeks) in the European Cubicin® Outcome Registry and Experience (EU-CORE)**

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**Objective:** Daptomycin antibacterial activity is dose-dependent. A high once-daily dose ( $\geq 8$  mg/kg) of daptomycin (DAP-H8) is often considered for difficult-to-treat infections (e.g. involving biofilms) and high bacterial burden. Here we assessed the safety of patients who received DAP-H8 therapy for more than 2 weeks.

**Methods:** Data were collected from EU-CORE a retrospective, non-interventional, multicenter registry (January 2006–June 2011, as four consecutive reporting periods). The safety data, including adverse events (AEs) and serious AEs, were evaluated for treatment periods of 14–30 days and >30 days.

**Results:** Of 230 patients treated with DAP-H8 for >2 weeks, 219 received  $\geq 8$  to  $\leq 10$  mg/kg and 11 received >10 mg/kg with the frequency of DAP-H8 use increasing between 2006 and 2011 (3%, 8%, 12% and 18%, for the four reporting periods, respectively). Sixty-four percent patients were male with a median age of 65 years (range 9–94). At the initiation and end of DAP-H8 therapy, 23 (10%) patients had creatinine clearance <30 mL/minute. At baseline patients had significant underlying diseases including cardiovascular disease (59%) and diabetes mellitus (20%). Most common infections in DAP-H8 group were endocarditis (31%) and osteomyelitis (17%). *Staphylococcus aureus* (38%; of which 19% MRSA) was the most commonly isolated pathogen. The median duration of DAP therapy was 25 days (range: 14–120) and it was used as empiric treatment in 28.3% patients. The overall clinical success rate was numerically higher with DAP-H8 (88.7%) than in the overall EU-CORE population (80.2%). AEs are summarized in Table 1. AEs possibly related to DAP-H8 were reported in 14 (6.1%) patients and treatment was discontinued in nine (3.9%) patients due to AEs. There were no clinically significant musculoskeletal and connective tissue events reported (Table 1). The rate of serious AEs related to daptomycin was similar in the cohort of patients receiving DAP-H8 (0.9%) and the overall EU-CORE population (0.8%).

**Table 1. Most common AEs experienced by patients who received > 14 days of DAP-H8**

Adverse Event	DAP-H8		Overall EU-CORE™	
	14-30 days N=165 n (%)	> 30 days N=65 n (%)	14-30 days N=1462 n (%)	> 30 days N=458 n (%)
Total	25 (15.2)	12 (18.5)	136 (12.7)	63 (13.8)
Infections and infestations	10 (6.1)	2 (3.1)	58 (4.0)	11 (2.4)
Blood CPK increased	5 (3.0)	4 (6.2)	25 (1.7)	7 (1.5)
Cardiac disorders	3 (1.8)	0 (0.0)	14 (1.0)	6 (1.3)
Renal and urinary disorders	3 (1.8)	1 (1.5)	18 (1.2)	7 (1.5)
Gastrointestinal disorders	1 (0.6)	2 (3.1)	17 (1.2)	6 (1.3)
Musculoskeletal and connective tissue	1 (0.6)	0 (0.0)	6 (0.4)	2 (0.4)

**Conclusion:** Daptomycin, administered as high dose ( $\geq 8$  mg/kg) for an extended period (>2 weeks) exhibited a favorable safety profile. This profile was comparable in periods of 14–30 days and >30 days,

confirming the overall good safety profile of daptomycin reported in previous studies.

**P1847 A 6-year study of daptomycin in vitro activity against linezolid-resistant coagulase negative staphylococci recovered from blood cultures of intensive care units patients**

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**Objective:** The purpose of our study was to evaluate the in vitro activity of daptomycin against linezolid resistant coagulase negative staphylococcus (LR-CNS) derived from blood cultures of ICU patients.

**Methods:** During a 6 year period (from April 2005 to October 2011) we examined 44 non duplicated LR-CNS isolated from blood cultures of equal numbered patients hospitalized in the ICUs of a tertiary hospital with common and protracted use of linezolid. All patients carried central or peripheral intravenous catheters or indwelling devices. The identification and the susceptibility testing were performed by the automated Vitek 2 system (Biomerieux, France). The resistance to linezolid was confirmed by E-test strips and the estimation of daptomycin MICs was also performed by the same method according to the manufacturer's instructions (AB-Biodisk, Sweden). Mueller-Hinton agar adjusted to contain physiologic levels of free calcium ions (50  $\mu$ g/mL) was used when testing daptomycin susceptibility. Isolates with MIC >4 mg/L were considered resistant to linezolid and those with MIC <1 mg/L susceptible to daptomycin, according to MIC breakpoints determined by CLSI and EUCAST respectively.

**Results:** The identification of the examined isolates showed 18 *S. cohnii*, 15 *S. epidermidis* and 11 *S. capitis*. All the examined LR-CNS isolates were methicillin resistant (MR) too. MIC range to linezolid varied significantly (8 to >256 mg/L), whereas MICs to daptomycin did not (0.25–0.75 mg/L).

**Conclusion:** LR-CNS seems to be endemic in the ICUs of our hospital. The emergence of linezolid resistant CNS, even rare, raises the concern that this kind of resistance could be disseminated. Daptomycin has an excellent activity against linezolid resistant CNS and may be considered as an alternative option for the treatment of infections caused by these multidrug *Staphylococcus* isolates.

**P1848 Treatment of left-sided gram-positive endocarditis with daptomycin**

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**Objectives:** To evaluate the effectiveness of daptomycin in infective endocarditis (IE) patients.

**Methods:** Fifteen patients, all but one with left heart endocarditis, monitored with a diagnosis of IE according to modified Duke criteria between July 2010 and May 2011, and receiving daptomycin as monotherapy were enrolled in the study. All patients but one had known risk factors for developing infective endocarditis. The success of daptomycin in these patients was revealed with improvements in microbiological, biochemical and radiological findings, as well as physical examination findings.

**Results:** Patients' average age was  $61 \pm 16$  (34–80); nine (60%) were men and six (40%) women. Methicillin resistant *S. aureus* (73%), *Streptococcus mutans* (20%), and methicillin sensitive *S. aureus* (7%) were the pathogens which isolated from our patients. Daptomycin was used in initial treatment in five (33%) patients, while treatment was subsequently modified to daptomycin in 10 (77%) patients because of drug serum level insufficiency, agent sensitivity to the drug administered, drug side-effects or in order to be able to administer outpatient parenteral antibiotic therapy (OPAT). Fourteen patients were discharged in a healthy condition, with successful surgical treatment in five (33%). Only one, an 80-year-old IE patient, was lost due to advanced cardiac failure. No significant side-effect was seen in any patient receiving daptomycin. The most frequent side-effects were

**Table:** Clinical and laboratory features, treatment modalities and outcomes of infective endocarditis cases.

Patient no.	Age	Sex	Site of infection	ECHO findings	Isolate on BC	DA MIC (mg/lE-test)	Initial a.b.	Reason for switch from initial a.b. to DA	Surgery	Result
1	38	M	AV	12x4 cm veg	S. mutans	0.5	VA-gen(7)	VA-levr insufficiency	-	†
2	64	M	SV	11x3 cm veg	S. mutans	0.5	VA-gen (40)	VA susceptibility	+	†
3	80	F	SV	11x3 cm veg	MRSA	0.75	DA (40)	-	+	‡
4	75	M	Left TV	0.8 cm veg	MRSA	0.75	S (40)	MRSA	-	†
5	69	F	SV	1cm (atrial) SEK 0.6cm (ventricle)	MRSA	0.25	VA-gen-CRD (7)	Renal failure	+	†
6	50	M	SV	18x8 cm abscess	MRSA	0.5	VA-gen (7)	Renal failure	+	†
7	70	M	Left ventricle end	0.9x0.5 cm mass	MRSA	0.5	DA (42)	-	-	†
8	34	M	TV	1.9x0.9 cm veg	MRSA	0.25	VA-gen-CRD (7)	OPAT	-	†
9	51	M	SV	0.9x0.5 cm mass	S. mutans	1.0	Pen-G (150)	Pen-R	+	†
10	77	F	AV	1cm (atrial) SEK Dense calcification on AV	MRSA	0.5	TEC (7)	Clinical failure	-	†
11	70	F	SV	0.9x0.9 cm veg	MRSA	0.5	TEC (5)	Clinical failure	-	†
12	50	F	SV	1x0.5cm	MRSA	0.5	DA (42)	-	-	†
13	46	M	AV	1x0.7cm	MRSA	0.5	DA (42)	-	-	†
14	75	F	SV	0.9x0.8 cm	MRSA	0.5	S (3)	MRSA	-	†
15	73	M	SV	1.9x0.8 cm	MRSA	0.5	DA (7)	-	-	†

VA, vancomycin; DA, daptomycin; CRD, ceftriaxone; S, sefazolin  
 Pen-G, penicillin-G; gen, gentamicin; a.b, antibiotic; R, resistance; OPAT, outpatient antibiotic treatment  
 AV, aortic valve; SV, mitral valve; TV, tricuspid valve; ECHO, echocardiogram; SEK, septal aneurysm; ECHO contrast  
 S, mutans, streptococcus mutans; MRSA, methicillin resistant staphylococcus aureus  
 MRSA, methicillin resistant staphylococcus aureus; MIC, minimum inhibitory concentration  
 † infective endocarditis; ‡ day, veg, vegetation; BC, blood culture  
 M, male; F, female; †, Success; ‡ Exitus

minimal rises in serum CPK levels during treatment, these values returning to normal after treatment. Clinical and laboratory features, treatment modalities and outcomes of infective endocarditis cases.

**Conclusion:** Daptomycin can be used successfully in both complicated left heart endocarditis and right heart endocarditis, with no significant side-effects. Studies involving wider patient series are now needed to support the use of daptomycin in left heart endocarditis.

**P1849 Investigation of *Coxiella burnetii* infection in dairy ruminant herds with reproductive disorders in two different regions of Portugal**

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**Objectives:** Recently, an increased number of Q fever outbreaks in some European countries warned the health authorities for the need of knowing its real prevalence in Europe. In Portugal, little is known about the epidemiology of Q fever. The aim of this study was to determine the infection rate and the geographical distribution of *Coxiella burnetii* antibody positive ruminant herds in two different regions from Portugal.

**Methods:** A cross-sectional study was performed on bulk tank milk samples (BTMs) from dairy herds with clinical reports of abortion or other reproductive disorders within 3 months before sample collection. In the first semester of 2010, BTMs were collected from dairy cattle herds in the Northwest region (n = 27) and, between November 2010 and May 2011, BTMs were collected in the Center region from dairy cattle herds (n = 20) and from small ruminant dairy herds (n = 27). All the samples were tested for the presence of anti-*C. burnetii* antibodies by the Enzyme-Linked Immunosorbent Assay, using the LSIVET Ruminant Milk/Serum Q Fever® (LSI; Lissieu, France). Laboratory results were used to determine the infection rate at regional level and by species.

**Results:** Test positive herds were detected in both regions. Considering the Northwest region, a positive result was obtained in 15 (55.5%) cattle herds. In the center region, a positive result was obtained in 10 (50%) cattle herds and in 11 (40.7%) small ruminant herds. Positive titers (S/P) ranged from 34 to 115. In the Northwest region only one (3.7%) herd showed an antibody titer higher than 100. In the center region five (25%) cattle herds and two (7.4%) small ruminant herds showed an antibody titer higher than 100.

**Conclusions:** To our knowledge, this is the first report of the occurrence of *C. burnetii* infection in ruminants in these regions of Portugal. This study demonstrates that infection by *C. burnetii* is important in Portugal. It is crucial to know the real prevalence in ruminants and to investigate the involvement of other species, in order to develop a control program for *C. burnetii*, which does not exist in Portugal, to reduce the economic impact at herd level and the risk for public health.

**P1850 Empirical antibiotic treatment and 3–30 day mortality in hospitalised patients with monomicrobial bacteraemia. A prospective population-based cohort study**

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**Objectives:** In few countries bacteraemia (Ba) is a reportable infection unless the causative agent is subject to national surveillance. Appropriate empirical antibiotic treatment (EAT) is an important clinical goal in order to limit mortality in Ba patients. However, population-based data on appropriateness of EAT and mortality are rare. Therefore, we investigated EAT and 3–30 day mortality in a prospective semi-national Danish cohort.

**Methods:** We included all hospitalized patients with incident Ba 2007–2008 from a collaborative network in Denmark with prospective registration of Ba in a population of ~1.7 mill. inhabitants. Incident Ba was defined as a clinical episode with ≥1 positive blood cultures deemed clinically significant without a prior episode in the preceding 365 days. EAT was defined as the antibiotic treatment given at 1st notification. EAT was recorded as appropriate if given intravenously (except fluoroquinolones, fluconazole and metronidazole) and if the blood isolate/s were susceptible to ≥1 of the antibiotics given. The primary outcome was mortality from day 3 to day 30 after the blood draw; the 2-day interval accounted for the delay of culture reports and precipitous death from sepsis unrelated to antibiotic therapy.

**Results:** We found 6834 patients with Ba. Of these, 4653 had monomicrobial Ba and a record of EAT and were alive at day 3. *E. coli*

Table. 3-30 day mortality of patients with monomicrobial incident bacteraemia by empirical antibiotic treatment status

Department of Clinical Microbiology	Empirical antibiotic treatment (EAT)		3–30 day mortality	
	Appropriate	P value	Appropriate EAT	P value
<b><i>Escherichia coli</i></b>	<b>N (%)</b>		<b>N (%)</b>	
Aalborg	382 (79.1)	0.001	28 (7.3)	0.02
Herlev	482 (86.8)		61 (12.7)	
Hvidovre	512 (86.2)		65 (12.7)	
<b><i>Klebsiella pneumoniae</i></b>	<b>N (%)</b>		<b>N (%)</b>	
Aalborg	68 (80.0)	0.14	9 (13.2)	0.43
Herlev	75 (90.4)		11 (14.7)	
Hvidovre	88 (87.1)		18 (20.5)	
<b><i>Pseudomonas aeruginosa</i></b>	<b>N (%)</b>		<b>N (%)</b>	
Aalborg	25 (51.0)	0.25	3 (12.0)	0.90
Region	26 (66.7)		4 (14.5)	
Hvidovre	26 (65.0)		3 (11.6)	
<b>Anaerobe bacteria</b>	<b>N (%)</b>		<b>N (%)</b>	
Aalborg	32(66.7)	0.77	4 (12.5)	0.37
Herlev	38 (67.9)		4 (10.5)	
Hvidovre	32 (61.5)		7 (21.9)	
<b><i>Staphylococcus aureus</i></b>	<b>N (%)</b>		<b>N (%)</b>	
Aalborg	145 (76.3)	<0.001	28 (19.3)	0.17
Herlev	206 (88.0)		35 (17.0)	
Hvidovre	161 (89.9)		40 (24.8)	
<b><i>Streptococcus pneumoniae</i></b>	<b>N (%)</b>		<b>N (%)</b>	
Aalborg	138 (90.8)	0.30	16 (11.6)	0.83
Herlev	160 (92.0)		16 (10.0)	
Hvidovre	157 (95.2)		19 (12.1)	
<b><i>Enterococcus faecalis</i></b>	<b>N (%)</b>		<b>N (%)</b>	
Aalborg	15 (37.5)	0.008	4 (26.7)	0.98
Herlev	17 (23.3)		5 (29.4)	
Hvidovre	4 (9.1)		1 (25.0)	
<b><i>Enterococcus faecium</i></b>	<b>N (%)</b>		<b>N (%)</b>	
Aalborg	3 (25.0)	0.57	0	
Herlev	4 (16.0)		2 (50.0)	
Hvidovre	2 (10.5)		0	

Chi2 was calculated to determine statistical significance (p<0.05).

Ba accounted for 1632 episodes (35%) followed by *S. aureus* Ba (603, 13%) and *S. pneumoniae* Ba (491, 11%). The proportions of appropriate EAT varied among pathogens and by region for each pathogen (Table 1). The proportions of appropriate EAT varied from 92.7% for *S. pneumoniae* to 22.9% for *E. faecalis*. The 3–30 day mortality was lowest for *S. pneumoniae* (11.2%) and highest for *E. faecalis* (27.8%). Nevertheless, 3–30 day mortality seemed not to be related to the degree of appropriateness of EAT e.g. *E. coli*.

**Conclusion:** Proportions of appropriate EAT and 3–30 day mortality varied between pathogens and appropriate EAT for some pathogens indicating that each region had different empirical antibiotic regimens and/or resistance rates and diverse patient groups. The proportion of appropriate EAT was notably low for enterococcal Ba and the 3–30 day mortality was very high. The finding that 3–30 day mortality did not seem related to appropriateness of EAT suggests bias by indication. Further epidemiological analyses must address potential bias and confounding of these crude results.

#### **P1851** Clinical presentation of patients with acute Q fever to the hospital: a case-control study

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**Study design:** Case-control study.

**Objective:** To evaluate differences in clinical presentation between patients with acute Q fever referred to a hospital and a control group.

**Methods:** The study group consisted of patients presenting to the Radboud University Nijmegen Medical Centre or the Canisius Wilhelmina Hospital in Nijmegen, the Netherlands, between January 2007 and March 2011, with pneumonia, or fever and lower respiratory tract complaints, or fever and hepatitis, and who were diagnosed with acute Q fever based on serology and/or PCR. The same criteria were used for the control group, but Q fever serology had to remain negative >2 weeks after onset of illness. Eighty-two patients diagnosed with acute Q fever were compared to 52 control patients. Data were collected retrospectively.

**Results:** Patients with acute Q fever were younger (mean 53 ± 16 years vs. 59 ± 16 years,  $p = 0.027$ ), less often had a history of lung disease ( $p = 0.001$ ), and were less frequently immunocompromised ( $p = 0.002$ ) while a history of smoking was more frequent ( $p = 0.013$ ). A sore throat was more common in acute Q fever ( $p = 0.008$ ) while sputum production was less frequent ( $p = 0.049$ ). Dyspnea and rhonchi were significantly less frequent in patients with acute Q fever than in controls. None of the other signs and symptoms differed between both groups. Patients with acute Q fever had a higher CRP (mean 167 vs. 117 mg/L,  $p = 0.015$ ) and lower leukocyte counts (mean 9.0 vs. 11.0 × 10<sup>9</sup>/L,  $p = 0.006$ ). In acute Q fever, chest X-ray more often showed an infiltrate (86% vs. 56%,  $p = 0.001$ ). In acute Q fever, both hospitalization (70% vs. 94%,  $p = 0.001$ ) and admission to the ICU were less common (3.5% vs. 18%,  $p = 0.002$ ) than in controls. The duration of hospital-stay was shorter in acute Q fever (9 vs. 17 days,  $p = 0.001$ ). Of 59 patients with available follow-up data, six patients developed chronic Q fever (10%). Fifteen patients had an indication for prophylactic treatment due to risk factors for developing chronic Q fever. None of these patients were diagnosed with chronic Q fever if they received prophylaxis while two out of four patients who did not receive prophylaxis developed chronic Q fever.

**Conclusion:** Due to the absence of typical clinical manifestations, differentiating *Coxiella burnetii* from other pathogens is not possible without Q fever serology or PCR. In endemic areas, the suspicion of Q fever should remain high. In the presence of risk factors for developing chronic Q fever, prophylactic treatment is advised.

#### **P1852** Infective endocarditis in intravenous drug users at a department of infectious diseases, in a university medical centre, Ljubljana, from 1984 to October 2011

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**Introduction:** Infective endocarditis (IE) is one of the most severe complications of parenteral drug abuse. The incidence of IE in intravenous drug users (IVDU) is 2–5% per year. *Staphylococcus aureus* is the most common etiological agent. The remainder of cases is caused by streptococci, enterococci, Gram negative rods, *Candida* spp., and other less common organisms. The tricuspid valve is the most frequently affected, followed by the mitral and aortic valves; pulmonic valve infection is rare. More than one valve is infected in 5% to 10% of cases.

A new pattern of IE in IVDU is emerging, characterized by more frequent left heart involvement, a severe clinical course, and a need for surgery in the active phase. The prognosis of the right-sided endocarditis is generally good; overall mortality is <5%, and with surgery <2%.

**Methods:** IVDU with IE treated at our department from 1984 to October 2011 were included in present report.

**Results:** From 1984 to October 2011 there were 614 episodes of IE at our department. Among them were 42 episodes in IVDU. Basic data are presented in Table 1.

There were four (9.5%) relapses of IE in IVDU. 50.0% of IE in IVDU were isolated right sided IE. Isolated left sided IE was present in 19 (45.2%) episodes. Only one valve was affected in the majority of cases (88.1%). IE in IVDU was caused by *Staphylococcus aureus* in 73.8%, followed by viridans

Table 1. Basic data

	All episodes (614)	IVDU (42)	p
Age median (range)	59 (7–91)	31 (19–44)	0.16
Male	404 (65.8%)	26 (61.9%)	0.62
Female	210 (34.2%)	16 (38.1%)	0.62
Surgery in active phase	158 (25.7%)	12 (28.6%)	0.72
Died	132 (21.5%)	1 (2.4%)	0.001

**Conclusions:** Before 1998 there were no cases of IE in IVDU. In the recent years we noticed a steady increase in the number of IE in IVDU. Only a half of all cases were isolated right sided IE. Only one (2.4%) of our patients died, but 12 (28.6%) needed surgery in active phase of the diseases, relapses were frequent.

There is an increase in incidence of IE in IVDU with the tendency toward more severe course of the disease and more often left sided IE at our department in recent years.

#### **P1853** Aetiology, risk factors and outcome of 899 cases of infective endocarditis: a 25-year national prospective survey, Slovakia

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**Objectives:** The aim of this study was to reassess the aetiology, risk factor and outcome of infectious endocarditis (IE) and to compare two periods, first (1984–1990) where mainly remedial therapy of IE was used and last (2007–2010), where cardiosurgery was introduced as therapy of IE in Slovakia.

**Methods:** A longitudinal observation nationwide survey on aetiology, risk factors and outcome of infectious endocarditis in Slovakia (population about 5 million) was performed during 1984–2010. Thirty-four hospitals participated in the study and 899 cases of infectious endocarditis were reported. Aetiology, risk factors and outcome of infectious endocarditis were compared. Differences (cured vs. died and 1st period – 1984–1990 vs. 5th period – 2007–2010) were assessed by univariate analysis. Chi-square test, Fisher's exact test and *t*-test computerized with the open source statistical package "R" were used and  $p < 0.05$  was considered significant.

**Results:** Concerning risk factors, aetiology and therapeutic strategies of IE in first (1984–1990,  $N = 75$ ) and last periods (2007–2010,  $N = 295$  cases), rheumatic fever, neoplasia, dental surgery, tonsillitis, embolization as complications and staphylococci in aetiology were less frequent in last periods ( $p < 0.001$ ). Negative culture endocarditis,



**P1856** *Streptococcus milleri* group bacteraemia: 21 cases in the last 5 years

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**Objective:** The objective is to provide a retrospective analyse of the last 5-year SGM bacteraemia (2006–2011) in a district university hospital with a catchment population of 250 000 in Madrid, Spain.

**Methods:** A retrospective clinical data review was performed in order to study the clinical features of the patients suffering SGM bacteraemia. The clinical data reviewed were age, sex, underlying diseases, bacteraemia possible source, community/hospital acquisition, coinfection, hospital stay, antimicrobial therapy and clinical outcome. The microbiological characteristics, haemolysis production and Lancefield group agglutination (Oxoid, England), were recorded. Identification of the species was achieved by API Strep (BioMerieux, France). Susceptibility testing was determined using E test (AB Biodisk, Sweden).

**Results:** During the study period, 37,642 blood cultures were processed, and 1,903 were clinically significant. *S. milleri* group accounted for 1.16% of all bacteraemia and caused infection in 21 patients, giving an annual incidence of 1,76 per 100 000 population per year. In 18 (81.7%) patients the infection was community acquired. Twenty-one isolates were studied. *S. constellatus* (42.9%, n = 9) was the most frequent, followed by *S. intermedius* (33.3%, n = 7) and *S. anginosus* (23.8%, n = 5). In the 21 episodes, the bacteraemia was monomicrobial. The majority of patients had underlying diseases. The most frequent were: neoplasia (n = 8), heart disease (n = 6) and diabetes mellitus (n = 5). A primary source of bacteraemia was identified in 19 (90.5%) episodes. The hepatic (23.8%) and dental (23.8%) source were the most common infection sites documented. All of the *S. anginosus* showed alpha-haemolysis, and all but one, carried Lancefield A antigen. *S. constellatus* and *S. intermedius* appear to be diverse in the Lancefield grouping and haemolysis production. All of the strains were susceptible to quinolones, vancomycin and linezolid. One strain showed resistance to erythromycin and ampicillin, and another strain was both erythromycin and clindamycin resistant. The two resistant strains were identified as *S. anginosus*.

**Table 1. Source of bacteraemia and species identification**

Source of infection	Number of patients	Species		
		<i>S. anginosus</i>	<i>S. constellatus</i>	<i>S. intermedius</i>
Hepatic (Liver abscess)	6 (6)	1	2	3
Dental (Dental abscess)	5 (6)	1	3	1
Gall bladder	2			2
Unknown	2		2	
GI tract	1	1		
Ischial abscess	1		1	
Foot finger	1			1
Perianal	1		1	
Vascular ulcers	1	1		

**Conclusions:** The SGM bacteraemia is an unusual event. SGM bacteraemia is often associated with a local site of infection, mostly in the hepatobiliary and respiratory tract location. Our data suggest an association between *S. constellatus* and *S. intermedius* species and the hepatobiliary infection, while *S. anginosus* did not show a characteristic associated infection site.

**P1857** Outcome of aortic graft infection at Christchurch hospital, New Zealand

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**Objectives:** There is a paucity of data on the medical management of infected aortic grafts. This study documents the complications and

outcome of patients treated for infected abdominal aortic grafts at Christchurch Hospital from 1999 to 2010.

**Method:** Clinical data and antibiotic regimens were collected prospectively on a standardized form and held in the ID service database. Additional data was obtained from the hospital notes.

**Definitions:** For inclusion cases required a compatible clinical syndrome and CT scan showing a peri-graft collection, with or without gas. A case was classified as definite if bacteria were isolated from the aortic graft or adjacent tissue, and probable if bacteria were isolated from blood cultures.

**Results:** Eighteen patients (mean age 71 years) were identified (10 definite, eight probable). Organisms isolated at diagnosis were *Staphylococcus aureus* 6 (MRSA 1), beta haemolytic streptococci 2, multiple enteric organisms 9 (viridans streptococci 4, gram negative bacilli 4, anaerobes 2) and there was no isolate from 2. One had complete graft excision and antibiotic therapy at diagnosis. The remainder received intravenous antibiotic therapy (14/17  $\geq$  6 weeks) and 14 indefinite oral therapy.

During follow-up the patient who had graft excision relapsed (viridans *Streptococcus*) and received ongoing antibiotic therapy.

Of the 17 treated conservatively, 11 relapsed (median time 40 months, range 0–98), four underwent graft excision and 10 died (median 40.5 months, range 1–98). Two of four who had graft excision subsequently relapsed and three died, one within a day of operation (median survival/follow-up 22 months, range 6–128). The median survival/follow-up of remaining 13 patients was 45 months (range 1–195). Four died during their first relapse. The remainder had a median survival of 31 months from relapse (range 8–117). Of the 10 who relapsed, four had positive cultures. All isolates were enteric organisms (gram negative bacilli 7, *Enterococci* 5, *Lactobacillus* 1) or *Candida albicans* 2.

**Conclusions:** Conservative management, without graft excision, was successful with 16 of 17 patients surviving for 6 months and median survival was over 43 months. Empirical therapy for the initial presentation should cover both skin organisms and enteric organisms and enteric organisms at relapse. Cultures are essential to direct therapy.

**P1858** Retrospective cohort study of outcome patients with melioidosis treated with cotrimoxazole alone for maintenance therapy

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**Objectives:** We report our experience with cotrimoxazole alone and compare it with cotrimoxazole plus doxycycline as the maintenance therapy of melioidosis.

**Method:** This retrospective cohort study was conducted in Songklanagarind Hospital in southern Thailand from from 1 January 2000 to December 2009. We defined microbiologically confirmed melioidotic patients into two groups, cotrimoxazole alone group comprised of the patients who received cotrimoxazole for maintenance therapy and conventional regimen group comprised of the patients who initially received cotrimoxazole plus doxycycline for maintenance therapy. Follow-up duration was defined from the beginning of maintenance therapy. The outcome was measured with relapse as well as mortality after relapse. The relapse was classified to microbiological relapse and clinical relapse. The patients with character of clinical feature compatible with melioidosis and microbiological evidence were defined as “microbiological relapse” while those who had only clinical feature compatible with melioidosis without microbiological evidence were define as “clinical relapse”. Statistical analysis was performed with Pearson Chi-square and Fisher’s exact tests were used to compare categorized data. The Mann–Whitney *U*-test and *T*-test were used to compare nonparametric data. Statistical differences were deemed significant at the 0.05 level.

**Result:** During the period of study, we saw 186 microbiologically confirmed melioidotic patients at our institute. Of 157 survived patients whose data was eligible, conventional regimen were administered in 109 patients and cotrimoxazole were administered in 31 patients. There were no different baseline characteristics, clinical manifestations or initial intravenous treatments between the two groups. There was insignificant difference between mean duration of maintenance in

cotrimoxazole alone group (31.5 weeks) and in conventional regimen group (29.4 weeks). The microbiologically confirmed relapse among the patients received cotrimoxazole alone (3.2%) and those who received conventional regimen (4.6%) were not significantly different.

**Conclusion:** Cotrimoxazole monotherapy might be the alternative maintenance therapy for melioidosis. Although this study could not demonstrate the difference of relapse between cotrimoxazole alone and cotrimoxazole plus doxycycline, it is necessary to conduct a more powered trial.

**P1859** Virulence factors and phylogroups are not associated with patients' features or source of infection in bacteraemic ESBL-producing *Escherichia coli*: a prospective multicentre cohort

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**Objectives:** We studied the association of phylogenetic groups (PG) and virulence factors (VF) with the epidemiology and clinical features of bloodstream infections (BSI) due by ESBL-producing *Escherichia coli* (ESBLEC).

**Methods:** A prospective cohort including 191 cases of BSI due to ESBLEC from 13 Spanish hospitals (2004–2006) was studied. We analysed the prevalence of different PG and genes codifying for 25 VF by PCR, and their association with epidemiological and clinical features. A VF score (number of VF) was calculated for all isolates. Fisher or chi squared test and Mann–Whitney *U*-test were used for statistical comparisons.

**Results:** The most frequent adherence-related VF was fimH (84%); among toxins, sat (20%); among iron-related, iutA (82%) and iucD (73%); among others, traT (74%) and malX (39%). The average virulence score (SD) was 6.4 (3.5). As regards PG, 27% belonged to D, 16% to B2, and 57% to A or B1. There was no association of specific VF with the source of BSI except for the fact that hlyA, cnf1, and cnf2 were more frequent in biliary than urinary BSI (8% vs. 0,  $p = 0.04$  for each). We found no association between the VF score and acquisition, underlying conditions, local or general predisposing features, previous antibiotic use or source. The average VF score was higher in B2 isolates than in D or A/B1 groups (10.1, 7.5, and 4.9;  $p < 0.01$  for all comparisons), and also in isolates producing CTX-M-1-group ESBLs than in those producing CTX-M-9 and SHV-groups (7.4, 6.5, and 5.0;  $p < 0.01$  for CTX-M-1 vs. SHV); this was in relation with the higher frequency of CTX-M-1-group in B2 isolates. The VF score was significantly higher among ciprofloxacin-susceptible isolates (7.5 vs. 5.9,  $p = 0.008$ ), gentamicin-susceptible (6.7 vs. 5.3,  $p = 0.02$ ), and isolates showing resistance to three or less antimicrobials (7.9 vs. 6.0,  $p = 0.02$ ); this was related to the fact that B2 isolates less frequently showed resistance to ciprofloxacin or gentamicin. We found no association between PG and features of the patients, predisposing features for infection, or source of BSI with the exception of cancer, which was more frequent among B1/A isolates than among B2 (33% vs. 13%,  $p = 0.04$ ).

**Conclusions:** The prevalence of FV in this cohort of BSI due to ESBLEC was lower than in previous series of *E. coli* causing BSI, associated with a higher prevalence of A/B1 isolates. FV score was mainly related to PG. The epidemiological drivers for acquiring BSI due to different PG or VF remain unknown.

**P1860** Characteristics and consequences of inadequate therapy of bacteraemia due to extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae in a Dutch cohort

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**Objectives:** In eight Dutch hospitals, we conducted a retrospective study to determine the characteristics of patients affected by ESBL bacteremia, the appropriateness of initial antibiotic treatment, and the determinants predicting mortality.

**Methods:** From 2008 to 2010 all patients with bacteremia due to ESBL-producing Enterobacteriaceae were included. Epidemiological, clinical and laboratory variables were collected. Outcome was day-30 mortality, analyzed with univariate and multivariate logistic regressions.

**Results:** In total 220 patients (median age 67 years) were included. Twenty-one were children <18 years. Many patients had comorbidities, most frequently malignancy (35%), obstructive urinary disease (18%) and recurrent urinary tract infections (UTI, 19%). Hundred thirty-five (61%) were of nosocomial origin, 53 (24%) were health care-associated and 32 (15%) were community-acquired. Major sources of infection were UTI (47%) and intra-abdominal infection (28%). Within 24 hours after bacteremia onset, 35% of patients received adequate antimicrobial therapy (carbapenems 64%, gentamicin 31%, other 5%). Out of 65 patients with known ESBL-carriage at bacteremia onset, only 47% received adequate therapy within 24 hours. Day-30 mortality was 22%. Age >75 years (OR 3.8, 95% CI: 1.4–10.1), high risk (non-UTI) source of infection (OR 6.0, 95% CI: 2.4–15.4) and the presence of severe sepsis or septic shock at bacteremia onset (OR 11.4, 95% CI: 4.7–27.8), but not adequacy of antibiotic treatment (adjusted OR 2.1, 95% CI: 0.8–5.2), were associated with day-30 mortality.

**Conclusion:** In these Dutch patients 85% of ESBL bacteremia episodes were nosocomial or otherwise health-care associated. Most patients had comorbidities requiring frequent hospital visits. Although inadequate therapy was not associated with day-30 mortality, adequacy of initial treatment may be improved in a significant number of patients by consultation of previous culture results and addition of aminoglycosides.

**P1861** Emergence of *Escherichia coli* O25b-ST131 clone among Hungarian bloodstream isolates

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**Objectives:** The aim of the study was to assess the prevalence of the *Escherichia coli* O25b-ST131 clone among bloodstream isolates and to estimate its contribution to the observed increase of 3rd generation cephalosporin resistance among invasive *E. coli* in Hungary.

**Methods:** All consecutive non-repeat bloodstream *E. coli* (117) isolated between March and November 2010 in major university hospitals of Budapest (BUH) (41), Szeged (SZUH) (27) and Debrecen (DUH) (49) were collected. Ciprofloxacin, cefotaxime and ceftazidime susceptibility was tested by disc diffusion. ESBL production was confirmed by the double disc assay. The phylogenetic lineage and the carriage of the rfbO25b and blaCTX-M genes were determined by PCR. The O25 serotype was confirmed by slide agglutination. The blaCTX-M type was assigned by direct sequencing of the amplicon. The isolates were typed by PFGE analysis and the rfbO25b positive ones also by multi locus sequence typing.

**Results:** Fifty isolates (42.7%) belonged to phylogenetic group B2, 35 (29.9%) to group D, 24 (20.5%) to group A and 8 (6.8%) to group B1, respectively. Fifteen strains (12.8%) represented the O25b-ST131 clone clustering together by PFGE at 74% pattern similarity. The ratios of ST131 strains at DUH, BUH and SZUH were 6.1%, 14.6% 22.2%, respectively. Altogether 26 (22.2%) of the isolates produced ESBL, 26.5%, 17.1% and 22.2% at DUH, BUH and SZUH. Nine of the ESBL producers (34.6%) belonged to the ST131 clone, all carrying blaCTX-M-15, and resistant to fluoroquinolones. Of the 17 non-ST131 ESBL producers 12 carried blaCTX-M-15, 1-1 blaCTX-M-1 and blaCTX-M-14 genes, and three expressed non-cefotaximase type ESBL enzymes. The ratio of the O25b-ST131 clone among ESBL producers recovered at DUH, BUH and SZUH were 0.0%, 57.1% and 83.3%, respectively.

**Conclusions:** Our results confirm that the incidence of 3rd generation cephalosporin resistance among invasive *E. coli*, at least in certain regions of Hungary, was considerably increased by the spread of *E. coli* O25b-ST131 clone carrying blaCTX-M-15.

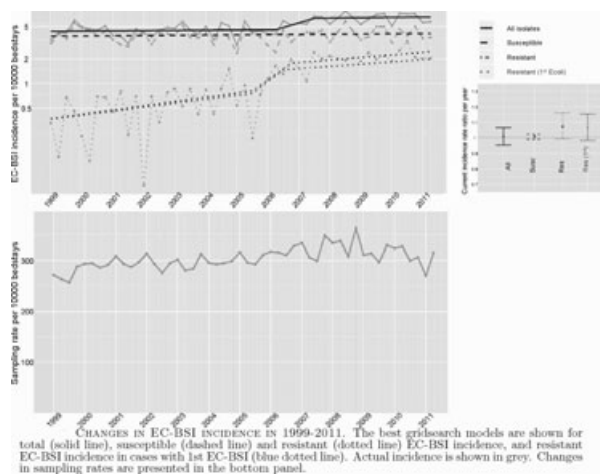
**P1862** Increasing incidence of *E. coli* bloodstream infection is driven by an increase in antibiotic-resistant isolates: electronic database study in Oxfordshire, 1999–2011

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**Objectives:** To investigate the relationship between *E. coli* resistance, bacteraemia rates, and post-bacteraemia outcomes.

**Methods:** Trends in *E. coli* bacteraemia incidence were monitored 1999–2011 using an infection surveillance database including microbiological, clinical risk factor, weather, infection severity and outcome data in Oxfordshire, UK.

**Results:** Two thousand two hundred and forty *E. coli* bacteraemias (2080 patients) were studied, of which 1728 (77%) were susceptible to co-amoxiclav, cefotaxime, ciprofloxacin and gentamicin. *E. coli* bacteraemia incidence increased from 3.4/10 000 bedstays in 1999 to 5.7/10 000 bedstays in 2011. The increase was fastest around 2006, and was driven by organisms resistant to ciprofloxacin, co-amoxiclav, cefotaxime or aminoglycosides. Resistant *E. coli* isolation rates increased similarly in those with and without recent hospital contact. The sharp increase also occurred in urinary isolates, with similar timing. In addition to these long-term trends, increases in ambient temperature, but not rainfall, were associated with increased *E. coli* bacteraemia rates. It is unclear whether resistant *E. coli* bacteraemia rates are currently still increasing (incidence rate ratio 1.07 per annum [95% CI 0.99–1.16],  $p = 0.07$ ), whereas current susceptible *E. coli* bacteraemia rates are not changing significantly (IRR = 1.01 [95% CI 0.99–1.02]). However, neither mortality nor biomarkers associated with mortality (blood creatinine, urea/albumin concentrations, neutrophil counts) changed during the study.



**Conclusions:** *Escherichia* bacteraemia rates have risen due to rising rates of resistant organisms; little change occurred in susceptible *E. coli*. Although severity of resistant infections, and their outcome, appear similar to susceptible *E. coli* in the setting studied, the increasing burden of highly resistant organisms is alarming and merits ongoing surveillance.

**P1863** Cohort study examining the burden of bloodstream infection caused by extended-spectrum beta-lactamase-producing Enterobacteriaceae at a Swiss university hospital using multistate modelling

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**Objectives:** We sought to produce an unbiased estimate of the excess hospital length of stay (LOS) and cost attributable to extended-spectrum

beta-lactamase (ESBL) positivity in bloodstream infections (BSI) due to Enterobacteriaceae.

**Methods:** We performed a retrospective cohort study including patients experiencing BSI due to ESBL-positive and ESBL-negative Enterobacteriaceae from 1 January to 31 December, 2009, at the University of Geneva Hospitals. Patient data were collected from a prospective BSI surveillance system as well as clinical and administrative databases. LOS was defined as time from hospital admission until last discharge from an acute-care ward. Excess LOS was determined separately for both cohorts by inclusion in a multistate model with all patients admitted in 2009 without BSI due to Enterobacteriaceae. The excess LOS attributable to ESBL-positivity was estimated by the difference between these two results. The average cost of a non-psychiatric hospital bed-day was estimated by dividing total annual fixed and variable hospital costs in Swiss francs (CHF) by the total number of patient bed-days in 2009. The economic burden of ESBL-positivity in BSI from the hospital perspective was then extrapolated by multiplying excess LOS by the average bed-day cost.

**Results:** Thirty ESBL-positive and 96 ESBL-negative BSI cases were included, with similar mean age (59 and 63 years, respectively;  $p = 0.36$ ) and gender distribution (70% and 63% male;  $p = 0.45$ ). There were non-significant trends towards less frequent receipt of appropriate antibiotics within 24 hours (59% and 74%;  $p = 0.09$ ) and higher inpatient mortality (20% and 10%;  $p = 0.17$ ) amongst the ESBL-positive group. The ESBL-positive group had a longer LOS (median 22.5 days [IQR, 14–61] and 14.5 days [7–32.5];  $p = 0.02$ ). When included in the multistate model with 47 560 control patients, the excess LOS attributable to ESBL-positive and ESBL-negative BSI, respectively, was 9.44 and 2.63 days, resulting in 6.81 days of excess LOS attributable to ESBL-positivity. Given an average cost per bed-day in 2009 of CHF1391, ESBL-positivity was associated with an attributable cost of CHF9473 per BSI due to Enterobacteriaceae.

**Conclusion:** ESBL-positivity in BSI due to Enterobacteriaceae is associated with a significant health-economic burden. This estimate, based on novel epidemiological methods, has significance in cost-benefit considerations of infection control and public health interventions targeting antimicrobial resistance.

## Klebsiella infections in hospital and community settings

**P1864** Amplification of ST15, ST147 and ST336 *Klebsiella pneumoniae* clones producing different ESBLs in Portuguese hospitals

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**Objectives:** Scarce studies trace the trends in ESBL-types and clones of ESBL-producing Enterobacteriaceae in Portugal. We investigated the shifts in ESBL-types and in population structure of non-*E. coli* Enterobacteriaceae from Portuguese hospitals during the last 5 years.

**Methods:** A total of 97 ESBL-producing non-*E. coli* Enterobacteriaceae isolates (76 *Klebsiella pneumoniae*, 13 *Enterobacter cloacae*, four *Proteus mirabilis*, three *Klebsiella oxytoca*, and one *Serratia marcescens*) recovered from three Portuguese hospitals (north [A] and centre [B, C] regions; 2006–2010) were studied. Bacterial identification and antibiotic susceptibility testing were performed by standard methods. ESBL characterization included DDST, PCR and sequencing. Clonal relatedness was established by PFGE and MLST.

**Results:** Isolates were mainly recovered from urines (65%) and patients at medicine wards (46%). They produced mostly CTX-M-15 (46%) and SHV-12 (21%), and less frequently other SHV-types (19%); SHV-2, -5, -106, -like, TEM (13%); TEM-10, -24, -116, -new variant) or CTX-M-14 (1%) enzymes. Two epidemic clones of *K. pneumoniae* (ST15, ST147) were identified in hospital A. ST15 ( $n = 17/76$ ; three PFGE-types; 2006–2010) carried blaCTX-M-15 (35%), blaSHV-106 (35%) or blaSHV (30%), whereas ST147 ( $n = 8/76$ ; 2006–2010) harboured blaSHV-12. An outbreak of ST336-*K. pneumoniae* ( $n = 34/$



76; one PFGE-type) producing CTX-M-15 (97%) or SHV-12 (3%) was also detected in hospital A (2009–2010). Sporadic *K. pneumoniae* (n = 17/76; 16 PFGE-types) and *K. oxytoca* (n = 3, three PFGE-types) clones produced different ESBLs (TEM-10, -24, -116; SHV-2, -5, -12; CTX-M-14, -15). ESBL-producing *E. cloacae*, *S. marcescens* and *P. mirabilis* were also rare and confined to hospitals from Centre region (2006–2007). *Enterobacter cloacae* isolates (four PFGE-types) harboured blaSHV-12 (n = 10) or blaCTX-M-15 (n = 3), *S. marcescens* (n = 1) contained blaTEM-10, and *P. mirabilis* isolates (three PFGE-types) carried blaCTX-M-15 (n = 2), blaTEM-new or blaTEM-116 (n = 1 each). ESBL producers were frequently resistant to sulphonamides (88%), streptomycin (82%), ciprofloxacin (79%), tobramycin (78%), kanamycin (78%), and gentamicin (77%).

**Conclusion:** We report a shift in ESBL-types in Portuguese hospitals since 2002–2004 (CTX-M-15/SHV-12 vs. TEM-type), linked to the amplification of three *K. pneumoniae* epidemic clones (ST336 and the widespread ST15 and ST147). The recent emergence of ST336-*K. pneumoniae* clone producing different ESBL types should be monitored due to the risk of further expansion.

**P1865** In the era of polymyxins use: emergence of colistin resistance in *Klebsiella pneumoniae*

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**Background:** The fear of infections with carbapenemase-producing Enterobacteriaceae has resulted in excessive empirical use of colistin. The aim of this retrospective observational study was to record and present the parallel increase in hospital use of colistin and the emergence of colistin-resistant *Klebsiella pneumoniae* (CRKP).

**Methods:** In a 280-bed tertiary hospital the administration of colistin was recorded by WHO-recommended methods. Identification and susceptibility testing of isolates were performed by using a Vitek 2 system (bioMérieux, France). Colistin and tigecycline MICs were additionally determined by E-test. For tigecycline, the FDA interpretation was used ( $\leq 2 \mu\text{g/mL}$ , susceptible;  $\geq 8 \mu\text{g/mL}$ , resistant), and for colistin the EUCAST clinical breakpoints for Enterobacteriaceae were applied ( $\leq 2 \mu\text{g/mL}$ , susceptible;  $> 2 \mu\text{g/mL}$ , resistant).

**Results:** The use of colistin grew from 3.49 DDDs per 100 bed days (November 2009–October 2010) to 8.03 (November 2010–October 2011) which represented an incline of 230%. The striking increase of colistin use over the last year prompted the recording of antimicrobial resistance of clinical isolates. Overall 569 *Klebsiella pneumoniae* strains were isolated over a 12 month period, with 201 (35.3%) of them being resistant to colistin. Carbapenemase-production was identified in 175 of the CRKP and in 308 (54.1%) of the total isolates. CRKP strains were implicated as pathogens in 25.9% of *Klebsiella pneumoniae* bacteraemias. Susceptibility to tigecycline was noticed to be 85%. During the study period, 300 *Acinetobacter baumannii* strains and 600 *Pseudomonas aeruginosa* isolates were found with a resistance rate to colistin of 10% and 7.6% respectively.

**Conclusions:** Selective pressure due to inadequate and/or inappropriate colistin use may lead to the emergence of CRKP strains capable of causing serious infections for which there are few or no treatment choices.

**P1866** Five-year screening and phenotypic classification of extended-spectrum beta-lactamases producing *Escherichia coli* and *Klebsiella pneumoniae* at a tertiary care university hospital in Riyadh, Saudi Arabia

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**Objective:** ESBL detection and classification is essential for clinical laboratories for epidemiological and infection control purposes. This study was performed to determine the prevalence of ESBLs producing

*Escherichia coli* and *Klebsiella pneumoniae* in patient samples and to classify the isolates on basis of type of enzymes production.

**Methods:** During the study period between 1 January 2006 to 30 December 2010 a total of 11 231 isolates from various body sites were screened for the presence of ESBLs using Microscan, WalkAway 96 System (Siemens Healthcare Diagnostics, Inc., CA, USA). The positive strains were confirmed by Microscan ESBL plus panel, ESBL E-test (AB bioMérieux, Solna, Sweden). Phenotypic classification was done by combination disks method using 20/10 mg amoxicillin-clavulanate disk in the center surrounded by 30 mg ceftazidime, ceftriaxone, cefotaxime, aztreonam, ceftoxitin and 10 mg ceftopodoxime disks.

**Results:** Out of the total 1160 isolates were confirmed as ESBLs among these 863 (74.4%) were *E. coli* and 297 (25.6%) were *K. pneumoniae*. The yearly percentages of ESBLs for both isolates remained stable and were (8–10%) and (6–9%) for *E. coli* and *K. pneumoniae* respectively. Among them the percentage of Class A TEM type ESBLs was 60%. A steady increase in the percentage of Class A CTX-M type was observed from 5.1% for *E. coli* to 25.3% whereas a modest change from 6.4% to 7.4% was observed in the percentage of Class A CTX-M type for *K. pneumoniae* at the end of the study period. The other class types namely class C, non A non C were <10% of the total isolates. The majority of the *K. pneumoniae* class A TEM type (73.5%) were isolated from urine samples. The highest percentage of *E. coli* CTX-M type (18.5%) recorded in the study was also found in the urine samples. Blood culture on the other hand yielded 68% of *E. coli* class A TEM type and 12% class A CTX-M type. No discordance was observed between the results obtained by Microscan ESBL plus and ESBL E-test for ESBL identification.

**Conclusion:** TEM-type remains the most common phenotype among the ESBLs. The increase in CTX-M type in *E. coli* from urine samples observed in this study is in agreement with the findings of the others and combination disks test appears to be useful test for classification of ESBLs in resource limited laboratories for epidemiological and infection control purposes.

**P1867** Presence of *Klebsiella pneumoniae* sequence type 512 harbouring blaKPC-3 gene in Italy

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**Objectives:** Multilocus sequence typing reveals that few clones of KPC carbapenemase-positive *Klebsiella pneumoniae* are widespread. The study aimed to investigate phenotypic/molecular features and clonal relatedness of carbapenem-resistant *K. pneumoniae* isolates from 17 Italian hospitals.

**Methods:** Seventy-two carbapenem-resistant *K. pneumoniae* isolates were collected at 17 acute care hospitals over a 2-year period (2009–2010). All of them were suspected for KPC production based on modified Hodge test and synergistic activity with amino-phenylboronic acid. Species identification and antimicrobial susceptibilities were obtained by Vitek2 System (bioMérieux). Imipenem, meropenem and ertapenem MICs were also evaluated by Etest. PCR for blaKPC-like genes and sequencing were performed. PFGE and multilocus sequence typing (MLST) were used to investigate clonal relatedness.

**Results:** All isolates resulted KPC-positive and multidrug-resistant. We focused the attention on 15/72 isolates, then fully characterized. Of them, 5/15 collected from 3/17 hospitals, belonged to ST 512 and harboured the blaKPC-3 gene. The above isolates were mainly from medicine wards; 40% were from rectal swabs and 20% from urine, blood and low respiratory tract specimens. Carbapenem MICs ranged from 2 mg/L to more than 32 mg/L. Isolates retained susceptibility only for gentamicin, tigecycline and, in 4/5 cases, colistin. PFGE showed three different profiles (A, B and C), marker of each hospital. The strains belonging to B and C clones showed the presence of blaSHV- and blaCTX-M type genes while blaSHV- and blaTEM- determinants were characteristic of clone A.

**Conclusion:** Population analyses of KPC-producing *K. pneumoniae* isolates by MLST have revealed the successful international spread of a sequence type (ST) 258 clone. Here we report on the first detection of KPC-3-producing ST 512 *K. pneumoniae* in Italy. The inter-hospital dissemination of the ST 512 underscores that the epidemiology of KPC-producing strains in Italy is changing.

**P1868** A nosocomial outbreak of a carbapenem-resistant *Klebsiella pneumoniae* ST-663 producing OXA-48 and CTX-M-15 related to a duodenoscope contamination

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**Objectives:** To characterize a nosocomial outbreak due to a carbapenem-resistant *Klebsiella pneumoniae* (KPCR) expressing OXA-48 and CTX-M-15 beta-lactamases linked to a duodenoscope contamination.

**Methods:** (i) Microbiology procedures: Antibiotic susceptibility testing was done by the microdilution method (MicroScan®; Siemens) and interpreted according to EUCAST breakpoints. Beta-lactamase encoding genes were characterized by PCR and DNA sequencing. The genetic relationship between KPCR isolates was determined by pulsed-field gel electrophoresis (PFGE) and Multilocus Sequence Typing (MLST). Rectal swabs screening for carbapenemase-producing bacteria was done on ESBL plates (bioMérieux). Duodenoscope cultures were performed following standard procedures. (ii) Epidemiological investigations: A case was defined as a patient subjected to an endoscopic retrograde cholangiography (ERC) from whom a KPCR was identified. (iii) Infection control measures: limiting transfers of case patients, flagging the cases, reinforcing of hand hygiene and contact precautions and double-cleaning the case rooms. Outpatients who underwent an ERC were flagged for rectal swab screening at a new admission.

**Results:** On February 2011 two consecutively KPCR cases were detected and the infection control unit traced these cases back to identify their possible source. In total, 12 patients were identified as carrying a KPCR from December 2010 to July 2011. All patients had been subjected to ERC. A KPCR strain was isolated from the duodenoscope culture, in nine patients positive KPCR cultures were obtained from clinical samples (four blood, two bile, one wound, one urine, and one catheter), and three of them presented intestinal colonization. None of the patients with systemic infection died. After implementing the infection control measures, no more KPCR cases related to the duodenoscope have been identified.

KPCR isolates were resistant to cefotaxime, ceftazidime and ertapenem, and positive for the Hodge test; in all of them, an OXA-48 carbapenemase and a CTX-M-15 ESBL were identified. All isolates, including that from duodenoscope, showed the same PFGE pattern and belonged to the new ST663.

**Conclusions:** The rapid identification of the outbreak cases and their source followed by implementation of strict infection control measures stopped the appearance of new cases related to the duodenoscope procedure. Emergence of OXA-48- and CTX-M-15-producing KPCR strains is of great clinical and public health concern.

**P1869** KPC producing *Klebsiella pneumoniae* in Uruguay: first two clinical cases and isolates' characteristics report

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**Objective:** Description of the first clinical cases of KPC-producing *Klebsiella pneumoniae* (KPC-Kp) infections in Uruguay, phenotype and genotype isolates characterization, and the adopted infection control measures.

**Methods:** Four *K. pneumoniae* isolates, (A2, B1, B2, B6), recovered in March and April 2011 from two intensive care unit (ICU) patients in a

General hospital located in a seaside resort of Uruguay, were analysed. Bacterial identification and susceptibility testing were performed using Vitek 2 GN and AST-N082-card and E-test (bioMérieux®). Phenotypic confirmation for KPCs and Metallo-β-Lactamases were performed with the KPC plus MBL confirm ID Kit (ROSCO®). CDC protocol for rectal cultures. The presence of blaKPC, blaIMP and blaVIM, was determined by PCR and further sequencing. Isoelectrofocusing was performed using the PhastSystem and commercial PhastaGels IEF 3-9. The genetic relatedness was evaluated by PFGE using XbaI and MLST. Population analysis and investigation of the stability of meropenem (MEM) heteroresistance were done on isolates B1 and B6, recovered from pre and post antibiotic treatment, respectively. A set of infection control measures and root cause analysis were done.

**Results and discussion:** All isolates showed a multiresistant phenotype including to colistin but susceptibility to tigecycline. B6 exhibited MEM MICs higher (>32 µg/mL) than the initial isolate B1 (8 µg/mL) and also higher MICs to other antibiotic classes. All isolates produced a KPC-2 enzyme with an isoelectric point of 6.7. Their macro-restriction pattern were indistinguishable and A2 isolate belonged to the internationally spread ST258 KPC-Kp clone (Table 1). B1 and B6 isolates represented a heterogeneous and homogeneous MEM resistant population, respectively. Health authorities were notified. Control measures were extended contact isolation; screening of contacts and pre-emptive isolation; close the ICU to new patients; reinforce environmental hygiene. No new cases were detected up to September 2011.

Table - Characteristics of the Patients and *Klebsiella pneumoniae* carbapenem and colistin resistant isolates data

Patient Age	Sex	Hospitalization date	Diagnosis	Antimicrobial Treatment <sup>1</sup>	K. pn isolate <sup>2</sup>	Specimen	MR <sub>MEM</sub>	Sequence type	PFGE (kbst)	Outcome	
30	M	20/10	SLE <sup>3</sup>								
		20/12	Enteric Salmonella Sepsis	CIP							
		20/03	VAP <sup>4</sup>	BMP	A1	Urine	nd	nd	nd		
		20/09			A2	Urine	positive	ST258	A		
		11/03									
		06/04	Salmonella Sepsis shock	BMP + CIP	A3	Catheter	nd	nd	nd		
		15/06									
		16/03									
		20/03	Chest trauma, respiratory failure, diabetic								Dead
		07/04	Mechanical ventilation reinitiated	BMP	B1	TA	positive	nd	A		
67	M	07/04			B2	Catheter	positive	nd	A		
		06/04			B3	TA	nd	nd	nd		
		14/04	VAP, septic shock	DOX + RIF + MEM	B4	Urine	nd	nd	nd		
		20/04			B5	Rectal	nd	nd	nd		
		20/04									
		20/04									
		20/04									
		20/04									
		20/04									
		20/04					B6	TA	positive	nd	A

Notes: <sup>1</sup> Antimicrobial treatments: CIP: Ciprofloxacin; TSP: Tobramycin-Piperacillin; BMP: Imipenem; BMP + CIP: Imipenem plus Ciprofloxacin; TSP + CIP: Piperacillin-Tazobactam plus Ciprofloxacin; DOX + RIF + MEM: Doxycycline plus Rifampicin plus Meropenem; TIG + RIF + MEM: Tigecycline plus Rifampicin plus Meropenem; b-Kp: K. pneumoniae isolates A, B1, K. pneumoniae and P. aeruginosa; A: 10<sup>7</sup> cfu/ml of K. pneumoniae and P. aeruginosa; A: 10<sup>8</sup> cfu/ml of K. pneumoniae on the tip of central line catheter and blood cultures negative; B: 10<sup>6</sup> cfu of K. pneumoniae; e: SLE: Systemic Lupus Erythematosus; VAP: ventilator associated pneumonia; nd: not determined; TA: Treated Ascaris.

**Conclusions:** The first KPC-Kp outbreak was detected in Uruguay. A novel *K. pneumoniae* resistance behaviour was observed after antibiotic therapy, suggesting the selection of not only a MEM resistant homogeneous subpopulation with higher b-lactams MICs but also exhibiting resistance to other antibiotic classes. This finding deserves a close surveillance during an infection treatment caused by KPC-Kp and is of concern since the therapeutic strategies are severely limited.

**P1870** Retrospective study of multidrug-resistant *Klebsiella pneumoniae* isolated from renal transplanted patients: epidemiology, genetic basis of resistance and virulence markers

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**Objective:** The main goal of the study was to assess whether *Klebsiella pneumoniae* isolates infecting/colonizing renal transplanted patients belong to a strain disseminated at the hospital in order to implement more efficient infection control measures.

**Methods:** Between May and July of 2011, 26 *K. pneumoniae* isolates were collected from 22 inpatients submitted to a renal transplant at a tertiary care hospital (Renal Transplant, Urology, Nephrology, Emergency ward and post-transplant consults). Antimicrobial susceptibility was determined by Vitek2AES system (BioMérieux, Portugal). Phenotypic screening of extended-spectrum beta-lactamases

(ESBL) and AmpC was performed by E-test. MICs were determined for ertapenem by E-test. The presence of ESBLs (blaTEM, blaSHV, blaCTX-M) were confirmed by PCR and sequencing of amplicons. Multiplex PCRs were used to screen for Pathogenicity Islands (PAIs), allS and rmpA genes and K1, K2, K5, K54, K57 and K20 capsules antigens. Plasmids were identified by replicon-typing technique. Pulse field electrophoresis was used for typing the isolates.

**Results:** Six different clones were identified among the isolates. The majority was resistant to ciprofloxacin, levofloxacin, gentamicin. All showed resistance to cefotaxime and ceftazidime, and susceptibility to amikacin and ertapenem. Vitek and E-test results suggested the production of ESBLs. blaSHV was detected in all isolates, being the unique ESBL in clone A. Clones B, C, D and F produced also blaTEM and blaCTX-M, while clone E carried blaSHV and blaCTX-M. PAI III 536 and PAI IICFT073 were detected in clones A and F, while the others only had PAI IV536. AllS gene was detected in clones A and E. Neither rmpA or capsular antigens genes tested were found. FIIIs plasmid replicon type was detected in clone A.

**Conclusion:** The results showed a diversity of *K. pneumoniae* clones characterized for DNA fingerprinting, different ESBLs, virulence markers, and plasmids type, what suggest different phylogenies. This may indicate that patients can be already colonized before admission, since they do hemodialysis elsewhere, or are transferred from regional hospitals. The screen for colonization and the knowledge of clinical history before admission would be helpful to prevent further infections. This raises a discussion whether antibiotherapy should be implemented before the transplant, as well as in post-transplanted patients with asymptomatic urinary infections with these MDR *K. pneumoniae* clones.

**P1871** **Detection of blaKPC gene in *Klebsiella pneumoniae* isolates by a real-time- NASBA based method**

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**Objectives:** The dissemination of KPC-producing *Klebsiella pneumoniae* is of great concern to public health services worldwide. The aim of this study was to evaluate the usefulness of the EasyQ method (bioMérieux) to detect the blaKPC gene in *K. pneumoniae* isolates.

**Methods:** Sixty well characterized *K. pneumoniae* strains collected from 10 Italian hospitals between 2009 and 2011 were analyzed. Imipenem (IPM), meropenem (MP) and ertapenem (ET) MIC determinations were performed using Etest (bioMérieux). Confirmatory modified Hodge and synergistic activity tests with amino-phenylboronic acid and EDTA were performed. All isolates were valued for the presence of blaKPC genes using home made PCR and the EasyQ RealTime-NASBA method. The RealTime results were compared with both phenotypic and molecular data previously obtained.

**Results:** 16/60 strains were KPC-negative: 10/16 and 3/16 were carbapenem intermediate/resistant (2011 EUCAST Guidelines) due to a VIM-1 enzyme production and/or a decreased outer-membrane permeability; 2/13 were also aminoglycosides resistant for the presence of armA gene. The remaining 3/16 KPC-negative strains were resistant to third and fourth generation cephalosporins, CTX-M-15 positive and carbapenem susceptible.

Of 44/60 strains from seven hospitals, only 41/60 showed a positive synergistic result using amino-phenylboronic acid; IPM, MP and ET MICs ( $\mu\text{g/mL}$ ) ranged from 1, 1.5 and 3 to higher than 32 respectively. All the above strains resulted KPC-positive by home made PCR. Direct sequencing performed on 15/44 strains identified in 10 cases a blaKPC-2 and in the remaining 5/44 a blaKPC-3 gene variant. Results in agreement were obtained for all the 16/60 KPC negative strains using both phenotypic and RealTime-NASBA molecular tests. EasyQ assay showed 100% sensitivity and specificity.

**Conclusion:** In KPC-producing *K. pneumoniae* infections, antibiotic options are dramatically restricted. EasyQ-KPC assay, allowing a rapid and reliable detection of blaKPC genes, limits the spread of such strains in healthcare settings.

**P1872** **Complex molecular epidemiology of ESBL-producing *Klebsiella pneumoniae*: long-term persistence and spread of epidemic clones among Bulgarian hospitals**

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**Objectives:** *Klebsiella pneumoniae* is still an important ESBL producer associated with severe hospital-acquired infections and within and between hospital outbreaks. The aims of this study were to investigate the prevalence, epidemiology and virulence potential of ESBL-producing *K. pneumoniae*, collected from Bulgarian hospitals.

**Methods:** One hundred and fifty ESBL-positive *K. pneumoniae* isolates collected from four hospitals of Bulgaria during 2000–2010 were selected for study. ESBLs were characterized by IEF, PCR and sequencing. Plasmid analysis included determination of the incompatibility group by PCR replicon typing. Virulence potential was determined by detection of five virulence factors genes and serum resistance assay. Clonal relatedness of the isolates was investigated using antibiotic resistance, RAPD and ARDRA.

**Results:** Among 150 ESBL-producing *K. pneumoniae* isolates, 58 (38.8%) produced SHV-12 and 1 harbored TEM-139. blaCTX-M genes such as blaCTX-M-3 and blaCTX-M-15 were detected in 44.3% and both blaSHV-12 and blaCTX-M-15 genes were determined in 23 (15.4%) isolates. Replicon typing revealed association between specific plasmids and particular ESBLs. The vast majority of genes encoding CTX-M-ESBLs were carried by IncF replicons and TEM-139 was linked by IncA/C replicon. Close relation among number of the determined virulence factors genes, serum resistance and type of ESBLs was found. The genes encoding iron uptake system (yersiniabactin and aerobactin receptor), serum survival factor and mucoviscosity were significant more frequent among CTX-M-15-producers. Sixty percent of the CTX-M-producing *K. pneumoniae* isolates were serum resistant. A polyclonal structure, including epidemic clones with specific ESBLs (SHV-12, CTX-M-3 and CTX-M-15) was observed by RAPD and ARDRA. One hospital had a single CTX-M-15 and three different SHV-12-producing avirulent *K. pneumoniae* clones that were predominant from 2000 to 2005. The remaining three hospitals each had two different virulent CTX-M-producing clones that have been disseminated during 2008–2010.

**Conclusion:** The main features of the current epidemiology of ESBL-producing *K. pneumoniae* in Bulgarian hospitals were long-term persistence and spread of epidemic clones, and transmission of plasmids carrying antibiotic resistance and virulence genes that probably conferred the selective advantages. These results should be taken in account for the implementation of epidemiological containment measures.

**P1873** **Characterisation of extended-spectrum and plasmid-mediated ampC beta-lactamase in *Klebsiella* spp. from urinary tract infections in hospital and community settings in Bosnia and Herzegovina**

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**Objectives:** To characterize extended-spectrum (ESBL) and plasmid-mediated AmpC beta-lactamase producing *Klebsiella* spp. isolates from urinary tract infections (UTIs) in hospital and community settings during December 2009–May 2010.

**Methods:** Double-disk synergy test was used to detect ESBLs. Minimum inhibitory concentrations (MICs) were determined by broth microdilution method according to CLSI guidelines. The transferability of ceftazidime resistance was tested by conjugation

(broth mating method). PCR was used to detect blaESBL and blaampC genes.

**Results:** Twenty-five ESBL-producing *Klebsiella* spp. were isolated from hospital (13 isolates were from paediatric department) and 18 from outpatient urine samples. *K. pneumoniae* was isolated from 16 hospital and 13 outpatient, and *K. oxytoca* from 13 hospital and five outpatient urine samples. All cephalosporins were the least potent antibiotics. Resistance rates to gentamicin and ciprofloxacin in hospital and outpatient isolates were 79% and 67%, and 89% and 44%, respectively. Conjugation frequency was in range  $10^{-1}$  to  $10^{-7}$  in 21 (84%) hospital, and  $10^{-4}$  to  $10^{-8}$  in six (33%) outpatient isolates. All outpatient isolates were cotransferred resistance to gentamicin, sulphamethoxazole and trimetoprim. Nineteen hospital and eight outpatient isolates yielded amplicons with primers specific for TEM, whereas 12 hospital and eight outpatient isolates were positive for CTX-M ESBL. Multiplex PCR revealed group 1 of CTX-M beta-lactamase. PCR reactions with SHV-specific primers were positive indicating the presence of intrinsic SHV-1 beta lactamase, except one *K. oxytoca* hospital isolate which was possessed SHV-5 without any additional beta-lactamases. Nineteen hospital and five outpatient strains harboured CTX-M beta-lactamase combined with TEM-1. AmpC beta-lactamase were detected by phenylboronic acid phenotypic test in eight (32%) hospital and five (28%) outpatient specimens. One hospital strain were positive for CMY and two for DHA group of plasmid-mediated AmpC beta-lactamases, whereas two outpatient strains were positive for CTT and FOX.

**Conclusion:** The study demonstrated high prevalence of CTX-M group 1 beta-lactamase in *Klebsiella* spp. caused hospital UTIs, associated with high level of resistance to cefuroxime, cefotaxime and ceftriaxone. High resistance rates observed for gentamicin and ciprofloxacin are probably due to the fact that plasmids encoding ESBLs also contain resistance genes for non beta-lactam antibiotics.

#### P1874 Frequency of extended-spectrum beta-lactamase in *Escherichia coli* and *Klebsiella* spp. in neonates

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**Objectives:** Nosocomial infections caused by multidrug-resistant gram-negative bacilli that produce extended-spectrum beta-lactamase (ESBL) enzymes have been reported with increasing frequency and are responsible for significant mortality and later morbidity among neonatal patients. The aim of this study is to know the rate of colonization by extended-spectrum beta-lactamase-producing (ESBL) *Escherichia coli*, *Klebsiella pneumoniae* or *K. oxytoca* isolates during the period 2008–2011 in patient of the Intensive Care Unit (ICU) of our hospital.

**Methods:** A retrospective study of ESBL-producing *E. coli*, *K. pneumoniae* and *K. oxytoca* isolated from rectal swabs was performed. Subjects were neonates admitted to the neonatal intensive and non-intensive care units admitted at the Hospital Universitario Miguel Servet of Zaragoza (Spain) from June 2008–2011. One thousand seven hundred and sixty specimens were cultured on McConkey agar supplemented with 1 µg/mL of cefotaxime. Isolates were identified and tested for antibiotic susceptibility by microdilution system (MicroScan Walkaway® Siemens). ESBL production was confirmed by the double-disk diffusion method according to CLSI standards.

**Results:** A total of 1760 specimens from 1027 patients were collected during the study, 321 (31.3%) were from patients admitted at ICU and 706 (68.7%) from non-UCI patients. The percentage of ESBL-positive isolate recovery from the ICU-patients were: 8.4% (27), 2.8% (9) and 0.93% (3) for *K. pneumoniae*, *E. coli* and *K. oxytoca*, respectively. The percentages of isolates from the non-ICU patients were 9.3% (66), 4.6% (33) and 0.42% (3) for *K. pneumoniae*, *E. coli* and *K. oxytoca* respectively.

**Conclusions:** ESBL producing *K. pneumoniae* was the most frequent isolated species from patients of the neonatal units. No important differences were detected in colonization rates among UCI and non-UCI group. The identification in these units is essential for adoption of adequate preventive measures.

#### P1875 Rates of and risk factors for the emergence of tigecycline resistance among multidrug-resistant *Klebsiella pneumoniae* isolates

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**Objectives:** Tigecycline is one the few therapeutic options for the treatment of infections caused by multidrug-resistant *Klebsiella pneumoniae* (MDRKP) strains. Few data exist on the rates of and risk factors for the subsequent emergence of tigecycline resistance (TR) among patients (pts) who initially presented with a tigecycline-susceptible (TS) MDRKP.

**Methods:** We performed a nested case-control study on the emergence of TR among all pts who initially presented with a TS-MDRKP to a tertiary care centre between 1/2008 and 7/2011. MDRKP was defined as the presence of either an extended-spectrum beta-lactamase- (ESBL) or carbapenemase-producing (KPC) isolate, determined as per CLSI guidelines. A case was defined as a pt from whom a TR-MDRKP was isolated between >48 hour and <90 days after the day of TS-MDRKP isolation (=zero time). A control was defined as a pt from whom a TS-MDRKP was isolated during the study period, but no subsequent TR-MDRKP. Controls were matched to cases based on the isolate resistance mechanism (ESBL vs. KPC), but randomly selected in a 1:2 ratio. Cases and controls were compared with regard to a variety of demographic and clinical characteristics including antibiotic exposure. Bivariate and logistic regression analyses were performed to determine risk factors for the subsequent isolation of a TR-MDRKP.

**Results:** TS-MDRKP was isolated from a total of 260 pts during the observation period; 155 (60%) had KPC, and 105 (40%) had ESBL. Among these 260 pts, 24 (9%) had a subsequent clinical specimen culture positive for a TR-MDRKP within our defined follow-up period. These 24 case pts were compared to 48 matched individuals without subsequent TR-MDRKP (N = 72). The median age of the total cohort was 72 years, the median Charlson comorbidity score was 3. MDRKP was most commonly isolated from urine at zero time (57%). 18 (75%) of 24 cases vs. only 16 (33%) of 48 controls were exposed to tigecycline after zero time (p = 0.001 on bivariate analysis). Cases and controls were similar with regard to isolate source, clinical infection vs. colonization, and present hardware. On logistic regression analyses, age (p = 0.04) and tigecycline therapy after zero time (adjusted OR 7.4, 95% CI 2.3–23.8; p = 0.001) were independent predictors of subsequent isolation of a TR strain.

**Conclusion:** The emergence of TR was observed in a significant proportion of pts with initial cultures positive for a TS-MDRKP; exposure to tigecycline was a strong and independent predictor.

#### P1876 A swordless knight: the epidemiology and molecular characteristics of the blaKPC-negative sequence-type 258 *Klebsiella pneumoniae* clone

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**Objectives:** The KPC-producing *Klebsiella pneumoniae* sequence type (ST) 258 shows formidable epidemiological success. This clone has spread rapidly across all Israeli hospital in 2006. In July 2009, a blaKPC-negative, ertapenem-resistant ST-258 *K. pneumoniae* strain was isolated from a patient in the Laniado hospital. Our aims were (i) to

assess whether the blaKPC-negative ST-258 *K. pneumoniae* clone will gain similar spread to that seen by its KPC producing isogenic strain and (ii) to describe its molecular characteristics and resistance mechanisms.

**Methods:** A prospective surveillance of all ertapenem non-susceptible, carbapenemase-negative *K. pneumoniae* (ENSCNKP) isolates was conducted at the Laniado hospital (314 beds) from July 2010 to June 2011, and at the Tel-Aviv Sourasky Medical Center (TASMC) (1200 beds) it was done retrospectively from July 2008 to December 2010. In addition, 50 ertapenem-susceptible and 20 KPC-producing *K. pneumoniae* isolates, collected during 2010–2011 were studied. Molecular typing was done by PFGE and multi-locus sequence typing. Mechanisms of resistance to ertapenem were studied by PCR of beta-lactamase genes and by sequencing of the ompK genes. Plasmid composition was studied by S1-nuclease analysis. The fate of the blaKPC-carrying plasmid, pKpQIL, was determined by PCR of the Tn4401 transposon and its unique genes, repA and the truncated blaOXA-9 gene.

**Results:** During the study periods the ENSCNKP ST-258 clone was found in six of 44 ENSCNKP isolates in Laniado hospital (four of them from a single ward during a 2-months period) and in one of 38 ENSCNKP in TASMC. ENSCNKP ST-258 strains were not associated with invasive infections. In contrast, none of the 50 ertapenem-susceptible *K. pneumoniae* and 17/20 of KPC-producing *K. pneumoniae* isolates belonged to ST-258. The MIC for ertapenem in the seven ENSCNKP ST-258 isolates ranged from 4 to >32 mg/L. The isolates carried either the blaCTX-M-2 or the blaCTX-M-25 genes, and all possessed a frameshift mutation at the ompK35 gene, similar to that identified in the KPC-producing ST-258 strains. Plasmid analysis showed variability in plasmid composition and absence of the pKpQIL plasmid in the ENSCNKP ST-258 isolates.

**Conclusion:** Our results suggest that ENSCNKP ST-258 evolved by loss of the blaKPC carrying plasmid pKpQIL and retained high ertapenem MIC due to porin loss. In contrast with the behavior of the parent strain, ENSCNKP ST-258 appears to have low epidemic and virulence potential.

**P1877** **First outbreak of KPC-2 producing *Klebsiella pneumoniae* in Korea detected by class A carbapenemase multiplex PCR**

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**Objectives:** KPC-producing *Klebsiella pneumoniae* were rare in Korea. Two cases were reported from 2010 and national surveillance for carbapenem resistant Enterobacteriaceae from November 2010 to April 2011 isolated only four KPC-2 producing *K. pneumoniae* from several hospitals. We aimed to explore the presence and incidence of Class A carbapenemase (CAC)-producing *K. pneumoniae* in our hospital.

**Methods:** A total of 104 non-duplicated consecutive clinical isolates of 3rd cephalosporin non-susceptible *K. pneumoniae* were collected during July to October, 2011. CAC multiplex PCR were performed for detection of KPC, SME, IMI, NMC-A, and GES enzymes encoding genes. PCR amplification for other beta-lactamase (TEM, SHV, OXA, CTX-M, and AmpC) genes were done using family-specific primers. Sequence data for KPC genes were obtained using flanking primers and sequence of GES gene determined by partial analysis. To characterize carbapenemase inhibitory phenotype of CAC multiplex PCR positive strains, we did disk combination tests (Rosco, Denmark). Clonal relatedness of three KPC-2 and one GES-5-like beta-lactamase producing *K. pneumoniae* was reviewed by pulsed field gel electrophoresis (PFGE).

**Results:** Three KPC-2 and one GES-5-like CAC-producing *K. pneumoniae* were isolated. All of KPC-producing isolates showed intermediate imipenem susceptibility (MIC, 2 or 4 mg/L), two isolates were also intermediate susceptible to meropenem (MIC, 2 mg/L), but

one isolate high resistant to meropenem (MIC,  $\geq 16$  mg/L). This susceptibility patterns were unlike previous reports (imipenem MIC,  $\geq 32$  mg/L) in Korea. Otherwise, GES-producing isolate showed high susceptibility to carbapenem. Three KPC-producing isolates carried three beta-lactamase genes, blaTEM, blaSHV, and blaKPC-2 genes, and one GES-5-like-producing strain had three beta-lactamase genes, blaSHV, blaOXA, and blaGES-5-like genes. PFGE patterns of three KPC-producing isolates were identical, but we couldn't get a pattern from GES-producing one.

**Conclusion:** Overall presence of KPC in *K. pneumoniae* from Korea is rare. However, in our hospital, three KPC-2-producing strains were isolated during only 4 months. The first clonal outbreak in Korea was suspected from PFGE patterns. To reduce the spread of KPC-producing strains, the rapid detection with CAC multiplex PCR would be helpful.

**P1878** **Virulence and resistance characteristics of *Klebsiella pneumoniae* causing community-acquired urinary tract infection**

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**Objectives:** *Klebsiella pneumoniae* is the second most common species causing urinary tract infections (UTI). The aim of this study was to evaluate the virulence and resistance determinants of *K. pneumoniae* isolates and host factors potentially relevant to community-acquired urinary tract infection.

**Methods:** During 2010, a total of 50 *Klebsiella pneumoniae* isolates causing community-acquired UTI were collected from 10 community-centres in Portugal. Isolates were recovered from patients with <50 years (22%, 11/50), more than 50 years (78%, 39/50), complicated cystitis (18%, 9/50) and recurrent UTI infections (38%, 19/50). Susceptibilities to antimicrobial agents were determined by disk diffusion and interpreted according to CLSI guidelines: amoxicillin/clavulanic acid, cefoxitine, ceftazidime, cefotaxime, imipenem, gentamicin, fosfomicin, ciprofloxacin and levofloxacin. The isolates were screened by PCR amplification with specific primers for bla-CTX-M, bla-TEM and bla-SHV extended-spectrum- $\beta$ -lactamases (ESBL) and six virulence factors genes: k2A (K2 serotype), fimH (fimbrial adhesin type 1), mrkD2 and mrkD3 (fimbrial adhesin type 3), khe (haemolysin) and iucC (aerobactin). A p-value of  $\leq 0.05$  was used to indicate statistical significance.

**Results:** Ten percent (5/50) of the *K. pneumoniae* isolates showed the bla-TEM-type  $\beta$ -lactamase (3/50) and the bla-CTX-M-1 ESBL (2/50), only associated with adults  $\geq 50$  years old. Worrysome quinolone resistance was found to ciprofloxacin (16%) and levofloxacin (16%). The most frequent virulence genes were the mrkD3 (62%), fimH (40%) and khe (46%). 6% of the isolates of *Klebsiella pneumoniae* belong to capsular serotype K2.

**Conclusions:** Fimbrial adhesins type 3 (variety mrkD2) and the serotype K2 was correlated with elderly women UTI while haemolysin and fimbrial adhesins type 1 and 3 (variety mrkD3) may have a role in community-acquired UTI by older adults. These data provide information on the resistance and virulence patterns among *Klebsiella pneumoniae* isolates currently causing community-acquired UTI.

**P1879** **Evaluation of Nuclisens EasyQ KPC for the rapid detection of blaKPC genes in surveillance rectal swabs**

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**Objective:** Carbapenem resistance among Enterobacteriaceae is an emerging problem worldwide. *Klebsiella pneumoniae* carbapenemases (KPC) are very common beta-lactamases described. Since patients colonized with *K. pneumoniae* KPC have been associated with both high mortality rates and risk of invasive infection, screening for

asymptomatic carriers should be considered in settings where this microorganism is endemic. In this study, the performance of the EasyQ KPC assay was evaluated and compared with culture methods for the detection of the blaKPC gene from fecal specimens of patients recovered in ICU of Policlinico Hospital, Bari, Italy.

**Methods:** In the period between September and October 2011, 40 rectal swabs collected from 27 patients were evaluated for the presence of KPC. In particular, the samples were collected at the admission and once weekly. Each sample was inoculated in 2 mL of sterile PBS and divided into two aliquots. One was used for culturing in tryptic soy broth containing a 10 µg disk of imipenem (final concentration 2 mg/L). After overnight incubation the broth was streaked on MacConkey agar. Modified Hodge Test and synergy tests with boronic acid and EDTA were used for screening of carbapenemases and differentiation between KPC and EDTA, respectively. The other aliquot was used for EasyQ KPC assay (bioMérieux, France) that allows the detection of the blaKPC gene by using NucliSens easyMAG extraction and real time NASBA amplification to detect the target KPC RNA present in the total nucleic acids.

**Results:** By cultural method *K. pneumoniae* KPC was isolated from 16/40 (40%) samples corresponding to 14 patients. All fecal isolates resulted sensible to fosfomicin, gentamycin, tigecycline and colistin. EasyQ KPC allowed the detection of blaKPC gene in 17/40 (42.5%) samples, whereas 21 (52.5%) were negative and two invalid. In particular, two patients were KPC positive only by realtime NASBA; one patient was positive for the isolation of *K. pneumoniae* KPC but negative to the molecular test.

**Conclusions:** *Klebsiella pneumoniae* KPC is an important life threatening nosocomial microorganism. The NucliSens EasyQ KPC assay appears to be sensitive and its utilization is less time consuming (48 vs. 4 hours) when compared to the traditional cultural methods. Therefore it might significantly help in both rapidly detecting colonized/infected patients and assigning them to cohorts in order to prevent the further spreading of the microorganism.

## Staphylococcus aureus – antimicrobial activity and resistance to newer agents

### P1880 Activity of ceftaroline tested against methicillin-resistant *Staphylococcus aureus* clones from Australia and New Zealand, 2010

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**Objective:** To evaluate the activity of ceftaroline (CPT) against methicillin-resistant *Staphylococcus aureus* (MRSA) clones isolated from patients in Australia (AUS) and New Zealand (NZ). CPT, the active metabolite of the prodrug ceftaroline fosamil, is a novel cephalosporin exhibiting broad-spectrum in vitro bactericidal activity against Gram-positive organisms, including MRSA. We evaluated the activity of CPT against MRSA clones isolated from patients in AUS and NZ.

**Methods:** Susceptibility testing for CPT and comparator antimicrobials was performed using CLSI broth microdilution methods on 141 isolates obtained from AUS (n = 131) and NZ (n = 10) as part of the SENTRY Programme, Asia Pacific Region (2010). Isolates were assigned to their clonal complex (CC) using a novel HRM SNP typing assay (Minim typing).

**Results:** Hospital-associated clones (CC8 and CC22) accounted for 48% of all MRSA isolates examined. CPT demonstrated good activity against all MRSA CC's. CPT MIC90 values (0.5 mg/L) were lower for MRSA strains with community-associated clonal complexes (CC93, CC1, CC30, CC5, and CC88). Resistance to mupirocin, tetracycline, gentamicin, fusidic acid, erythromycin, or cotrimoxazole did not affect CPT activity against MRSA isolates (overall MIC90, 1 mg/L; range 0.5–2 mg/L by CC). No vancomycin-intermediate or -resistant strains were detected.

Clonal complex (no. tested)	Ceftaroline MIC (mg/L)				MIC <sub>50</sub>	MIC <sub>90</sub>
	0.25	0.5	1	2		
<b>Hospital</b>						
8 (43)		4	34	5	1	2
22 (25)		21	4		0.5	1
<b>Community</b>						
93 (30)	1	29			0.5	0.5
1 (15)		15			0.5	0.5
30 (15)		14	1		0.5	0.5
5 (9)	2	7			0.5	0.5
88 (2)		2			0.5	0.5
45 (1)		1			0.5	0.5
59 (1)		1			0.5	0.5
<b>All (141)</b>	<b>3</b>	<b>94</b>	<b>39</b>	<b>5</b>	<b>0.5</b>	<b>1</b>

**Conclusion:** CPT exhibited potent activity against MRSA isolates and commonly circulating clonal complexes from AUS and NZ, in both community and hospital settings. All community-associated isolates had both MIC50 and MIC90 of 0.5 mg/L. Compared to community-associated MRSA clones, some hospital clones had slightly higher CPT MIC values, especially CC8 (MIC90, 2 mg/L).

### P1881 Antimicrobial activity of ceftaroline and comparator agents against contemporary (2010) *Staphylococcus aureus* isolates from Europe and South Africa

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**Objective:** To determine the activity of ceftaroline (CPT), the active metabolite of the prodrug ceftaroline fosamil, and comparator agents tested against recent (2010) *S. aureus* (SA) isolated in Europe (EU) and South Africa (SAF). CPT is a novel cephalosporin exhibiting broad-spectrum in vitro bactericidal activity against Gram-positive organisms including methicillin-susceptible (MS) and resistant (MR) SA, as well as many common Gram-negative pathogens.

**Methods:** Susceptibility testing for CPT and commonly used antimicrobials was performed by the CLSI broth microdilution methodology on a total of 3598 isolates from the 2010 Assessment Worldwide Antimicrobial Resistance Evaluation (AWARE) Programme. Susceptibility interpretations for the comparators were as published in CLSI and EUCAST guidelines. Isolates were collected from patients in 57 medical centres in 19 EU countries, including Israel and Turkey, and in SAF (1 medical centre).

**Results:** CPT was very active (MIC50/90, 0.25/1 mg/L) and inhibited >99.9% of all 3598 isolates at a MIC of ≤2 mg/L (see Table 1). CPT showed potent activity against MRSA (MIC50/90, 1/2 mg/L overall) but lower than seen against MSSA (MIC50/90, 0.25/0.25 mg/L overall). Only one strain (0.03%) demonstrated a CPT MIC value of >2 mg/L; the single isolate was from Spain with a CPT MIC value of 8 mg/L. Resistance (EUCAST) to several common-use antimicrobial agents was moderate; oxacillin/levofloxacin/erythromycin/clindamycin/tetracycline resistance, respectively, by region was: EU 25.4/24.9/26.4/11.2/9.7%, and SAF 28.3/30.4/30.4/23.9/15.2%.

Region	MR status (n)	MIC (mg/L)								
		0.03	0.06	0.12	0.25	0.5	1	2	4	8
All	MS and MR (3598)	3	10	420	2220	404	414	126	0	1*
EU	MSSA (2649)	3	10	416	2160	59	1			
	MRSA (903)			1	30	345	407	119	0	1
SAF	MSSA (13)									
	MRSA (13)			3	30		6	7		

\* isolate from Spain

**Conclusions:** This study demonstrated the potent in vitro activity of CPT tested against recent (2010) SA isolates, including MRSA strains in EU and SAF. Resistance to many commonly used antimicrobial agents was moderate with variability observed between geographical regions. These data suggest that ceftaroline fosamil could emerge as an important therapy for infections caused by SA, including MRSA, in EU and SAF.

**P1882** Increased MICs of vancomycin compared to those of teicoplanin, linezolid and daptomycin for *S. aureus* clinical isolates from Greece

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**Objectives:** Vancomycin is widely used for the treatment of Methicillin-Resistant *Staphylococcus aureus* (MRSA) infections, and is often chosen as empiric therapy in regions with high MRSA prevalence. Its use is questioned lately because of elevated vancomycin Minimal Inhibitory Concentrations (MICs), associated with treatment failures, observed in many parts of the world. Alternative approaches are explored for the treatment of MRSA. We determined the MICs of vancomycin, teicoplanin, linezolid and daptomycin against *S. aureus* clinical isolates.

**Methods:** We studied 140 *S. aureus* isolates collected from March to October 2011 at the Clinical Microbiology Department of "Attikon" University hospital. Most isolates were collected from pus (43.16%), followed by sterile-site fluids (20%), sputum and bronchial secretions (18%), blood (14.84%), and intravascular catheters (4%). Serial two-fold dilutions of pure antibacterial powders, commercially purchased from Sigma, were in-house prepared in 96-well microplates. MICs were determined by the broth microdilution method, according to the CLSI guidelines. For daptomycin, medium was appropriately modified (50 µg/mL Ca<sup>++</sup>). Cefoxitin MIC was also assessed by the same method. Microplates were read after 20 hours (24 hours for vancomycin) of incubation at 35°C.

**Results:** Forty-three percent of the isolates had a cefoxitin MIC ≥8 µg/mL, and were thus characterized as MRSA. All isolates were susceptible to vancomycin, but notably 12.85% of them had an elevated vancomycin MIC (2 µg/mL). All isolates were susceptible to teicoplanin, linezolid and daptomycin. More specifically, teicoplanin MICs ranged from 0.5 to 2 µg/mL, those of linezolid from 0.5 to 2 µg/mL and those of daptomycin from 0.25 to 1 µg/mL.

**Conclusions:** As previously reported for Greece, a high MRSA rate was observed. Vancomycin MICs, although within the susceptibility range, were found to be elevated. In regions with high MRSA prevalence, vancomycin MICs should be closely watched and when they are increased, teicoplanin, linezolid and daptomycin can be considered as empiric therapy alternatives.

**P1883** Activity of oritavancin against recent clinical isolates of methicillin-resistant staphylococci from Western Europe

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**Objectives:** The in vitro activity of oritavancin (ORI) and comparators was determined against methicillin-resistant (MR) staphylococci collected in 2011 from 42 hospitals in France (FRA), Germany (GER), Italy (ITL), Spain (SPN) and the United Kingdom (UK).

**Methods:** Two hundred and four MR *S. aureus* (MRSA) and 177 MR coagulase-negative staphylococci (MRCNS) – mainly *S. epidermidis* (132, 75%) were submitted to a central reference laboratory and their identification was confirmed by MALDI-ToF mass spectrometry. Of these 38% of MRSA and 67% of MRCNS were from bacteraemia and the remainder from acute bacterial skin and skin structure infections. MIC was determined by CLSI broth microdilution for ORI, vancomycin

Table 1.

		ORI	VAN	TEI	DAP	LZD	TGC	TET	AMP
MRSA	%S	N/A	100	100	98.5	100	99.5	87.7	1.5
(204)	MIC <sub>50/90</sub>	0.03/0.06	1/1	0.5/0.5	0.5/1	2/2	0.12/0.25	0.5/16	16/16
MRCNS	%S	N/A	100	92.7	98.3	98.3	96.6	76.3	0.6
(177)	MIC <sub>50/90</sub>	0.06/0.12	2/4	4/8	1/1	1/2	0.25/0.5	2/32	8/16
		CLI	LEV	SXT	ERY	CHL	KAN	TOB	GEN
MRSA	%S	70.1	9.8	99.0	36.3	97.1	36.8	41.2	94.3
(204)	MIC <sub>50/90</sub>	0.12/16	>8/8	≤0.12/0.25	-	-	-	-	-
MRCNS	%S	55.4	17.5	43.5	14.7	88.8	21.5	29.9	30.5
(177)	MIC <sub>50/90</sub>	0.25/16	8/8	4/4	-	-	-	-	-

N/A, susceptibility breakpoints have not yet been defined

(VAN), teicoplanin (TEI), daptomycin (DAP), linezolid (LZD), tigecycline (TGC), tetracycline (TET), ampicillin (AMP), clindamycin (CLI), levofloxacin (LEV) and trimethoprim-sulphamethoxazole (SXT). Susceptibility to chloramphenicol (CHL), erythromycin (ERY), kanamycin (KAN), tobramycin (TOB) and gentamicin (GEN) was determined by CLSI disk diffusion methodology. CLSI breakpoints were used throughout where available, except for TGC (EUCAST).

**Results:** Summary data for all isolates are given in the Table 1. Susceptibility (%S) for VAN against MRSA and MRCNS was 100% in all countries. Some non-susceptibility was observed for DAP against MRSA (three GER & one ITA) and MRCNS (three GER & one ITA), for TGC against MRSA (one GER) and MRCNS (five GER & one ITA) and for LZD against MRCNS (three GER). The LZD non-susceptible (NS) MRCNS had a MIC of ≥8 mg/L. Overall the MRCNS were more resistant than MRSA. By MIC<sub>50/90</sub>, ORI was at least 16-fold more potent than VAN, DAP, and LZD against MRSA and at least eight-fold more potent than these agents against MRCNS.

**Conclusion:** ORI showed potent activity against all MR staphylococci collected from FRA, GER, ITL, SPN and the UK during the 2011 year. Although not high, there was some evidence of resistance to newer agents such as TGC, DAP or LZD. Interestingly these were restricted to GER and/or ITL only.

**P1884** Oritavancin activity tested against *Staphylococcus aureus* responsible for documented infections in European hospitals

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**Objectives:** To assess oritavancin (ORI) activity, a lipoglycopeptide under late-stage clinical development for the treatment of acute bacterial skin and skin structure infections (ABSSSI). ORI potency has been continuously monitored against Gram-positive clinical organisms from hospitals in the USA and Europe (EU) for four years. The aim of this study was to compare the activity of ORI with that of other marketed ABSSSI agents tested against *S. aureus* (SA) from EU.

**Methods:** SA isolates (9274) were collected (2008–2011) from 38 hospitals in 14 EU countries, including Turkey and Israel, as part of the SENTRY Antimicrobial Surveillance Program. Isolates were submitted to a central laboratory where bacterial identifications were confirmed using standard algorithms and Vitek 2. Isolates were tested for susceptibility (S) against ORI and comparators by CLSI methods (M07-A8, 2009). EUCAST (2011) and CLSI (2011) interpretative criteria were applied, when available. Isolates displaying resistance to oxacillin (OXA),

Organism (No. tested) <sup>a</sup>	MIC (mg/L)									
	Oritavancin		Daptomycin		Vancomycin		Linezolid		Clindamycin	
	MIC <sub>50</sub>	MIC <sub>90</sub>	MIC <sub>50</sub>	MIC <sub>90</sub>	MIC <sub>50</sub>	MIC <sub>90</sub>	MIC <sub>50</sub>	MIC <sub>90</sub>	MIC <sub>50</sub>	MIC <sub>90</sub>
Overall (9,274)	0.03	0.06	0.25	0.5	1	1	1	2	>0.25	>2
Control group (2,588)	0.03	0.06	0.25	0.5	1	1	1	1	>0.25	>0.25
MSSA (6,890)	0.03	0.06	0.25	0.5	1	1	1	2	>0.25	>0.25
MRSA (2,384)	0.03	0.06	0.25	0.5	1	1	1	2	>0.25	>2
MDR (151)	0.03	0.06	0.5	0.5	1	1	1	2	>2	>2

<sup>a</sup> Control group is represented by isolates susceptible to all comparator agents tested: MSSA, methicillin (oxacillin)-susceptible *S. aureus*; MRSA, methicillin-resistant *S. aureus*; MDR, multidrug-resistant (i.e. isolates displaying resistance to oxacillin, erythromycin, clindamycin, levofloxacin and tetracycline).

erythromycin (ERY), clindamycin (CLI), levofloxacin (LEV) and tetracycline were considered multidrug-resistant (MDR; Table 1).

**Results:** Isolates were mostly from SSSI (37.9%) and bloodstream infections (35.7%). The potent activity of ORI (MIC<sub>50/90</sub>, 0.03/0.06 mg/L) was consistent across all subsets analyzed. Moreover, ORI inhibited 99.1% of SA at ≤0.12 mg/L. Vancomycin (VAN; 100% S; EUCAST) and daptomycin (DAP; >99.9% S) also showed stable MIC<sub>50/90</sub> results, except for DAP, which had a MIC<sub>50</sub> value against MDR strains slightly higher (two-fold) than that obtained against the S control group. Overall, comparator agents showed adequate antimicrobial coverage (≥90% S) when tested against all SA. However, ERY (71.6% S), CLI (89.1% S), LEV (73.0% S) and beta-lactams (OXA, 74.3% S) displayed suboptimal coverage when EUCAST criteria were applied. When tested against MRSA and MDR strains, ORI was at least eight-fold more potent than DAP, and at least 16-fold more potent than both VAN and linezolid (LZD). VAN (100% S), teicoplanin (95.4% S), DAP (100% S) and LZD (100% S) were active (EUCAST) against MDR isolates.

**Conclusions:** ORI continues to demonstrate potent in vitro activity when tested against a contemporary (2008–2011) collection of SA recovered from EU hospitals. In addition, ORI exhibited activity greater ( $\geq$ eight-fold) than comparator agents, including when tested against selected MDR strains.

**P1885** Oritavancin retains bactericidal activity in vitro against standard and high inocula of heterogeneous vancomycin-intermediate *Staphylococcus aureus*

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**Objective:** We studied the impact of inoculum density on the growth-inhibitory and killing activities of oritavancin (ORI) and comparators in vitro against hVISA by broth microdilution minimum inhibitory concentration (MIC) and time-kill assays at clinically-relevant concentrations of drugs.

**Methods:** MIC determinations and time-kill assays followed CLSI guidelines. Assays were performed at standard inocula ( $10^5$  CFU/mL) and high inocula ( $10^7$  CFU/mL). Drugs tested were ORI, vancomycin (VAN), daptomycin (DAP) and linezolid (LZD). In duplicate time-kill assays, drugs were tested at static concentrations approximating their free peak ( $fC_{max}$ ) and free trough ( $fC_{min}$ ) in plasma when administered at approved doses for complicated skin and skin structure infections. ORI  $fC_{max}$  was predicted from simulated Phase 2/3 patients receiving a single 1200 mg dose; ORI  $fC_{min}$  was the predicted concentration in plasma 24 hour after the single 1200 mg dose. Antibacterial effects were described as bactericidal (BC,  $>3\log$  kill), bacteriostatic (BS,  $\leq 3$  log kill), or no effect (NE, no difference from growth control). The *S. aureus* (SA) strains used were ATCC 43300 (methicillin-resistant SA[MRSA], non-hVISA), NRS 2, NRS 11 and NRS 28 (all hVISA and MRSA). hVISA phenotype was assessed by the population analysis profile-area under curve procedure.

**Results:** Whereas MICs of comparators were two- to eight-fold higher when tested at the higher inoculum relative to standard inoculum, ORI MICs were 16-fold higher for all strains at the higher inoculum. In time-kill assays, when tested at its  $fC_{max}$  and  $fC_{min}$ , ORI was BC against ATCC 43300 at standard and high inocula. At its  $fC_{max}$ , ORI was BC against the hVISA strains at standard and high inocula. At  $fC_{min}$ , ORI was BC against standard inocula of hVISA but had NE at high inocula. At both  $fC_{max}$  and  $fC_{min}$ , VAN was BC at standard inocula and BS against high inocula of the hVISA strains. LZD was BS at both  $fC_{max}$  and  $fC_{min}$  against standard inoculum of hVISA and had NE against all strains at high inoculum. DAP had NE at  $fC_{min}$  against both inocula of hVISA. At  $fC_{max}$ , DAP was BC against standard inocula of hVISA.

**P1886** In vitro activity of telavancin against staphylococci circulating in Europe during 2010 and 2011

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**Objectives:** The in vitro activity of telavancin [TLV] and comparator agents was evaluated against contemporary *Staphylococcus* spp. [S] from France, Ireland, Italy, Poland, Spain and the UK isolated from patients with complicated skin and soft tissue infections and hospital-acquired pneumonia between October 2010 and May 2011.

**Methods:** One thousand one hundred and twenty-four S were collected including 836 *Staphylococcus aureus* [SA], 386 being methicillin-resistant [MR], 288 coagulase-negative S [CNS], 178 being MR. MICs for ciprofloxacin [CIP], clindamycin [CLI], co-trimoxazole [SXT], daptomycin [DAP], erythromycin [ERY], gentamicin [GEN], linezolid [LZD], oxacillin [OXA], synercid [SYN], teicoplanin [TEI], TLV, tigecycline [TGC] and vancomycin [VAN] were determined by CLSI broth microdilution methodology. CLSI breakpoints were used except for TGC and TLV, where EUCAST and FDA breakpoints were used, respectively.

**Results:** TLV had excellent activity against SA and CNS with MIC90 of 1 and 0.5 mg/L, respectively. TLV inhibited all S at  $\leq 1$  mg/L (including MR) compared to DAP  $\leq 2$  mg/L, TEI  $\leq 8$  mg/L and VAN  $\leq 2$  mg/L against MRSA, and DAP  $\leq 2$  mg/L, TEI  $\leq 32$  mg/L and VAN  $\leq 4$  mg/L against MRCNS. Summary data for TLV and comparators are given in the Table 1. There were six DAP non-susceptible [NS] isolates (0.5% of S); these included three MRSA (one from France and the two from the UK) and three MRCNS (two from France and one from Italy). In addition, two LZD-resistant isolates were found (one MRSA and one MRCNS) both from Spain.

		TLV	VAN	TEI	LZD	DAP	CIP	CLI	SXT	ERY	GEN	SYN	TGC
MRSA (386)	%S	100	100	100	100	99	9	67	98	32	81	98	100
	MIC50/90	0.5/1*	1/1	0.5/1	2/4	0.5/1	$\geq 16$	$\geq 0.5$	$\geq 0.5$	$\geq 32$	$\geq 32$	$\geq 0.5$	0.12*
MRCNS (450)	%S	100	100	100	100	100	90	96	100	80	98	100	100
	MIC50/90	0.5/1	1/1	0.5/1	2/4	0.5/1	$\geq 0.5$	$\geq 0.5$	$\geq 0.5$	$\geq 0.5$	0.5/1	0.5/1	0.12*
MRCNS (178)	%S	100	100	97	99	98	22	67	47	21	41	94	100
	MIC50/90	0.5/1	2/4	4/8	2/4	0.5/1	$\geq 16$	$\geq 0.5$	$\geq 32$	$\geq 32$	$\geq 32$	0.25/1	0.5
MRCNS (110)	%S	100	100	99	100	100	92	90	90	65	92	99	100
	MIC50/90	0.5/0.5	2/2	1/4	1/4	0.5/1	$\geq 0.5$	$\geq 0.5$	$\geq 0.5$	$\geq 0.5$	0.25/1	0.25/1	0.12*

\* 96 MRSA isolates (43 Italy, 31 France, 12 Poland, 7 Spain, 3 UK) had a TLV MIC of 1 mg/L.

**Conclusion:** TLV showed equal or greater activity to the majority of comparators against S including more potent activity than VAN or TEI against CNS.

Table. Activity of oritavancin and comparator agents against *S. aureus* strains in time-kill assays at high ( $\sim 10^7$  CFU/mL) versus standard ( $\sim 10^5$  CFU/mL) inocula\*

Strain <sup>a</sup> (Inoculum)	Oritavancin ( $fC_{max}$ )	Oritavancin ( $fC_{min}$ )	Vancomycin ( $fC_{max}$ )	Vancomycin ( $fC_{min}$ )	Daptomycin ( $fC_{max}$ )	Daptomycin ( $fC_{min}$ )	Linezolid ( $fC_{max}$ )	Linezolid ( $fC_{min}$ )
ATCC 43300 (Standard)	5–10 min	5–10 min	6–24 h	6–24 h	+3.1	1–2 h	+1.0	-1.5
ATCC 43300 (High)	15–30 min	5–10 min	6–24 h	6–24 h	+2.4	2–4 h	+1.5	+1.2
NRS 2 (Standard)	4–6 h	2–4 h	6–24 h	6–24 h	+5.1	4–6 h	-1.0	-1.5
NRS 2 (High)	+2.7	6–24 h	+0.1	-1.1	+2.1	+1.9	+1.9	+1.9
NRS 11 (Standard)	4–6 h	2–4 h	6–24 h	6–24 h	+2.5	4–6 h	-0.9	-1.0
NRS 11 (High)	+3.1	6–24 h	-1.0	-1.5	+1.9	+1.5	+2.1	+1.9
NRS 28 (Standard)	4–6 h	2–4 h	6–24 h	6–24 h	+2.0	2–4 h	-1.5	-1.0
NRS 28 (High)	+2.5	6–24 h	+0.1	-1.1	+1.5	+1.5	+2.0	+1.5

\*Time to efficacy is indicated for drugs that achieved bactericidal activity ( $\geq 3$  log decrease in cell density relative to initial inoculum). Change in cell density (in log CFU/mL) at 24 h relative to starting inoculum is indicated for drugs with bacteriostatic activity (0 to  $< 3$  log kill at 24 h relative to initial inoculum) and for drugs with little to no inhibition of growth relative to growth control at 24 h. <sup>a</sup> ATCC 43300, methicillin-resistant *S. aureus* (MRSA); NRS 2, NRS 11 and NRS 28, vancomycin-intermediate *S. aureus* (hVISA).

**Conclusions:** ORI retained BC activity at its  $fC_{max}$  against MRSA and hVISA strains at both standard and high inoculum, suggesting that it may have potential utility in treatment of infections with high bacterial densities such as bacteraemia, endocarditis or osteomyelitis caused by MRSA and heterogeneous vancomycin-intermediate *Staphylococcus aureus* (hVISA).

**P1887** Telavancin in vitro activity against relevant Gram-positive isolates (TARGET multicenter study) prospectively collected from ICU patients in Spain

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**Background and objective:** Telavancin (TLV) is a semisynthetic lipoglycopeptide that simultaneously inhibits cell-wall synthesis and disrupts bacterial cell-membrane barrier functions of Gram-positives including anaerobes. Published data supports this drug as an option for treating staphylococcal and enterococcal infections including those caused by methicillin resistant strains as well as those due to isolates with decreased susceptibility to vancomycin and/or teicoplanin. TLV in vitro activity was comparatively studied against recent Gram-positive isolates prospectively collected from ICU-patients in nine Spanish hospitals representing different geographical areas.

**Methods:** A total of 426 isolates were studied. Methicillin-resistant *Staphylococcus aureus* (MRSA, n = 252) and coagulase-negative staphylococci (CoNS, n = 88) recovered from bacteriemic onco-haematological patients as well as *Enterococcus faecalis* (n = 54) and *Enterococcus faecium* (n = 32) of various clinical sources were



included. In vitro activities of TLV and comparative antimicrobials including vancomycin (VAN), teicoplanin (TEL), daptomycin (DAP), tigecycline (TIG) and linezolid (LNZ) were determined by broth microdilution (Sensititre® panels). Susceptibilities to comparators were interpreted according to EUCAST criteria. TLV-susceptible breakpoint ( $\leq 1$  mg/L) for both *S. aureus* and vancomycin-susceptible enterococci (VSE) was that approved by the FDA.

**Results:** TLV (MIC 50/90, 0.25/0.5 mg/L) for both *S. aureus* and CoNS showed high activity regardless of methicillin susceptibility. In the case of one *S. aureus* isolate with VAN 4 mg/L, the activity of TLV (MIC 0.25 mg/L) was fully retained. The same occurred in those CoNS isolates with higher teicoplanin MIC results (8–16 mg/L) as well as in those resistant to LNZ (7%). It is worth mentioning that a higher intrinsic activity of TLV against *E. faecium* (MIC90, 0.25 mg/L) when compared with *E. faecalis* (MIC90, 1 mg/L) was observed.

**Conclusion:** Management of infections caused by methicillin-resistant staphylococci and vancomycin-resistant enterococci is a problematic issue. Moreover, a matter of concern is represented by MRSA with decreased susceptibility to glycopeptide compounds (VISA). These in vitro data document the activity of TLV that demonstrated equal or greater potency than the comparators against contemporary Gram-positive isolates and supports its clinical use in the management of infections caused by these isolates.

**P1888** **Telavancin and daptomycin activity against methicillin-resistant *Staphylococcus aureus* strains after vancomycin resistance selection in vitro**

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MRSA infections represent a major threat world-wide. Although vancomycin is the drug of choice, clinical failure in patients with serious gram-positive infections have been increasingly reported. Moreover *S. aureus* (SA) strains with intermediate resistance to vancomycin (VISA) were reported in Europe, US, and Asia. Daptomycin, has good activity against MRSA, VISA and VRSA. However SA strains with a daptomycin MIC at the upper range of susceptibility has been reported during treatment. Moreover, it has been reported an association between reduced susceptibility to daptomycin and to vancomycin in SA. It has been demonstrated that a thickened cell wall is a common characteristic for VISA strains. Telavancin is active against MRSA, VISA and VRSA. Objective of our study is to evaluate the in vitro activity of telavancin and daptomycin against MRSA strains with a vancomycin MIC  $\leq 0.5$   $\mu\text{g/mL}$  and against MRSA strains after

induction of vancomycin MIC  $\geq 2$   $\mu\text{g/mL}$ . Nineteen MRSA strains with a vancomycin MIC  $\leq 0.5$   $\mu\text{g/mL}$  isolated from patients with bloodstream, respiratory tract, skin and soft skin infections, were considered.

After the first evaluation, multistep resistance selection was performed using the broth macrodilution method to generate strains with a vancomycin MIC  $\geq 2$   $\mu\text{g/mL}$ . At this time, all the MRSA strains were tested again for susceptibility to oxacillin, telavancin and daptomycin using Sensititre plates. We demonstrate that in vitro activity of both, telavancin and daptomycin, maintain a MIC range within 0.25 and 1 against MRSA with induced MIC increase to vancomycin. In conclusion, on the basis of our study in agreement with other investigations, daptomycin and telavancin seems to be represented a good alternative for the treatment of MRSA infections with a vancomycin MIC  $\leq 2$   $\mu\text{g/mL}$ .

**P1889** **Comparative activity of telavancin combined with nafcillin, imipenem, and gentamicin against *Staphylococcus aureus***

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**Objectives:** Combination therapy is frequently used to treat infections. Previous investigations with telavancin (TLV) have found synergy (SYN) most commonly with gentamicin (GEN). However, this combination may not see widespread clinical use due to concerns over nephrotoxicity. Previous studies have found synergy between vancomycin, a structurally and mechanistically similar drug to TLV, and various beta lactams. The purpose of this study was to investigate the potential of beta lactam combinations with TLV.

**Methods:** Thirty strains of *S. aureus* including 10 methicillin susceptible *S. aureus* (MSSA), 10 methicillin resistant *S. aureus* (MRSA), and 10 heterogeneously vancomycin intermediate *S. aureus* (hVISA) confirmed by population analysis profile area under the curve ratio (Mu3 as positive control) were evaluated for minimum inhibitory concentrations (MIC) to TLV, nafcillin (NAF), imipenem (IMP), and GEN by broth microdilution in duplicate. Potential for SYN was evaluated by time-kill analysis (TKA) of TLV, NAF, IMP, and GEN alone and TLV combined with NAF, IMP, and GEN at 1/2x MIC. TKA was performed in triplicate. SYN, indifference (IND), and antagonism (ANT) were defined as  $\geq 2$  log 10 CFU/mL kill,  $< 2$  log 10 CFU/mL kill compared to the most active single agent and  $< 1$  log 10 CFU/mL growth, or  $\geq 1$  log 10 CFU/mL growth compared to the least active single agent respectively.

**Results:** MIC50/MIC90 were 0.5/0.5 (range 0.25–0.5), 32/256 (range 0.25–256), 0.5/4 (range 0.125–16), and 8/64 (range 0.015–128)  $\mu\text{g/mL}$  for TLV, NAF, GEN, and IMP respectively. In the TKA, 70% (21/30) of strains displayed SYN between TLV and NAF, 67% (20/30) displayed SYN between TLV and GEN and 60% (18/30) displayed SYN between TLV and IMP with all the remaining strains being IND. For beta lactam combinations the per cent displaying SYN was greater against strains resistant to beta lactams (MRSA and hVISA) with 80% (16/20) of strains showing SYN with both NAF and IMP combinations. This pattern was not observed with TLV + GEN combinations. No ANT was observed.

**Conclusion:** TLV combined with NAF and IMP showed similar rates of SYN to TLV combined with GEN, a known synergistic combination. TLV combined with NAF and IMP may represent an alternative to using GEN, a known nephrotoxic agent.

STRAIN	TELAVANCIN MIC (FIRST PARAGRAPH)		TELAVANCIN MIC (SECOND PARAGRAPH)		DAPTOMYCIN MIC (FIRST PARAGRAPH)		DAPTOMYCIN MIC (SECOND PARAGRAPH)		DAPTOMYCIN MIC (THIRD PARAGRAPH)		GENTAMICIN MIC (FIRST PARAGRAPH)		GENTAMICIN MIC (SECOND PARAGRAPH)		GENTAMICIN MIC (THIRD PARAGRAPH)	
	MIC	TIME	MIC	TIME	MIC	TIME	MIC	TIME	MIC	TIME	MIC	TIME	MIC	TIME	MIC	TIME
1	0.5	1	1	1	0.5	1	1	1	1	1	>4	>4	>4	>4	>4	>4
2	0.5	0.5	0.5	0.5	1	1	1	1	1	1	>4	>4	0.5	0.5	>4	>4
3	0.5	0.5	0.5	0.5	1	1	1	1	1	1	>4	>4	0.5	0.5	>4	>4
4	0.5	0.5	0.5	0.5	1	1	1	1	1	1	>4	>4	>4	>4	>4	>4
5	0.5	0.5	1	1	0.5	1	1	1	1	1	>4	>4	>4	>4	>4	>4
6	0.5	0.5	0.5	0.5	1	1	1	1	1	1	>4	>4	>4	>4	>4	>4
7	0.5	0.5	1	1	0.5	1	1	1	1	1	>4	>4	>4	>4	>4	>4
8	0.5	1	1	0.5	1	1	1	1	1	1	>4	>4	>4	>4	>4	>4
9	0.5	0.5	1	1	1	1	1	1	1	1	>4	>4	>4	>4	>4	>4
10	0.5	0.5	1	1	0.5	1	1	1	1	1	>4	>4	>4	>4	>4	>4
11	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	>4	>4	>4	>4	>4	>4
12	0.5	1	1	1	0.25	1	1	1	1	1	>4	>4	>4	>4	>4	>4
13	0.5	0.5	0.5	0.25	0.25	0.25	0.25	0.25	0.25	0.25	>4	>4	>4	>4	>4	>4
14	1	1	1	1	1	1	1	1	1	1	>4	>4	>4	>4	>4	>4
15	0.25	0.5	0.5	1	1	1	1	1	1	1	>4	>4	0.5	0.5	>4	>4
16	0.5	1	1	1	1	1	1	1	1	1	>4	>4	>4	>4	>4	>4
17	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	>4	>4	>4	>4	>4	>4
18	0.5	1	1	1	1	1	1	1	1	1	>4	>4	0.5	0.5	>4	>4
19	0.25	0.5	0.5	0.25	0.5	0.5	0.5	0.5	0.5	0.5	>4	>4	>4	>4	>4	>4

**P1890** Dalbavancin maintains potent in vitro activity against *S. aureus* resistant to currently utilised anti-MRSA therapeutics

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**Introduction:** Dalbavancin (DAL) is a lipoglycopeptide undergoing clinical development for the treatment of skin and skin structure infections caused by Gram-positive pathogens. Emerging resistance among *S. aureus* to commonly utilized agents including linezolid (LZD), daptomycin (DAP), vancomycin (VAN), and tigecycline (TIG) is troubling, given the clinical prevalence and lack of other agents with activity against MRSA. It is important that newly developed agents are evaluated for their in vitro activity against such problematic isolates. This study was done to analyze the in vitro activity of DAL against non-susceptible (NS) isolates to current anti-staphylococcal therapies.

**Methods:** Thirty-four clinical isolates of *S. aureus* previously characterized NS to linezolid (n = 9), daptomycin (n = 18), and tigecycline (n = 7) were evaluated for susceptibility to DAL and comparators by broth microdilution per CLSI M7 and M100 guidelines.

QC <i>S. aureus</i> (ATCC 29213)				
	Range	Mode	MIC <sub>50</sub>	MIC <sub>90</sub>
Dalbavancin	0.03	NA	NA	NA
Vancomycin	1	NA	NA	NA
Oxacillin	0.25	NA	NA	NA
Daptomycin Non-Susceptible (n=18)				
	Range	Mode	MIC <sub>50</sub>	MIC <sub>90</sub>
Dalbavancin	0.03-0.5	0.06	0.06	0.5
Vancomycin	1-8	2	2	8
Oxacillin	0.12->4	>4	>4	>4
Linezolid Resistant (n=9)				
	Range	Mode	MIC <sub>50</sub>	MIC <sub>90</sub>
Dalbavancin	0.03-0.06	0.03	NA	NA
Vancomycin	0.5-2	1	NA	NA
Oxacillin	2->4	>4	NA	NA
Tigecycline Resistant (n=7)				
	Range	Mode	MIC <sub>50</sub>	MIC <sub>90</sub>
Dalbavancin	0.03-0.06	0.03	NA	NA
Vancomycin	1-1	1	NA	NA
Oxacillin	>4->4	>4	NA	NA

**Conclusions:** DAL had potent in vitro activity against the evaluated *S. aureus* consisting of isolates non-susceptible to currently available and commonly utilized agents, with lower MICs than comparator glycopeptides (vancomycin) and the lipopeptide daptomycin. These data show the potential of dalbavancin for the treatment of *S. aureus* infections, including those where *S. aureus* are resistant to other available therapeutics.

**P1891** Comparative activity of various antibiotics alone and in combination against high inocula methicillin-resistant *Staphylococcus aureus* with reduced susceptibilities to vancomycin

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**Background:** Methicillin-Resistant *Staphylococcus aureus* (MRSA) is endemic worldwide, with increasing vancomycin (V) heteroresistance. Many new antibiotics (abx) developed are active against MRSA but the optimal chemotherapy is not well defined in endocarditis & osteomyelitis (high inocula infections). We aim to examine the utility of various abx alone & in combination (combi) at high inocula for potential clinical use against a variety of well characterized MRSA with vancomycin heteroresistance.

**Methods:** Ten well-known MRSA clones (ST239 V-intermediate, UK-EMRSA-15, ST30 community-associated [CA] & ST8 USA300 CA) were selected for the study. Minimum inhibitory concentrations (MIC) were determined according to reference broth-dilution methods. Time-kill studies (TKS) were performed with 8 log CFU/mL at baseline using maximum achievable, clinical, unbound concentrations (mg/L) of linezolid (L) (19), daptomycin (D) (5), rifampicin (R) (2), tigecycline (T) (2) & V (12) alone & in combi against the test strains. Bactericidal ( $\geq 3$  log CFU/mL drop from baseline inocula) activity & synergism ( $\geq 2$  log CFU/mL drop from most active abx) were evaluated at 24 hour.

**Results:** The test strains had T MICs of 0.06 to  $\geq 8$  mg/L, R MICs of  $\leq 0.06$  mg/L, V MICs of 1 to 4 mg/L, D MICs of 0.5 to  $\geq 16$  mg/L & L MICs of 0.06 to  $\geq 16$  mg/L. In single TKS, there were at least 1 abx alone that exhibited bactericidal activity in 4/10 strains; D, L & V was bactericidal against strain 1 (4.29 log), 2 (4.85 log) & 7 (4.48 log) respectively, while T & R individually was bactericidal against strain 6 (4.95 log & 4.74 log respectively). In combi TKS for these four strains, only L + R was bactericidal against strain 1 (5.00 log) & D + T was bactericidal against strain 2 (3.51 log) at 24 hour.

In 6/10 strains (3, 4, 5, 8, 9, 10) where no abx alone exhibited bactericidal activity, the combi TKS showed that only D + T was bactericidal against strain 9 (4.52 log), D + T & T + R were bactericidal against strain 8 (4.42 log & 4.79 log respectively), D + R & D + V were synergistic against strain 3 (5.90 log for both combi).

**Conclusions:** Combi therapy may be useful against MRSA infections with reduced susceptibilities to V based on the encouraging in vitro activity. However, 2-drug abx combi may not be sufficient to totally eradicate the infection. Our approach may be used to identify abx combi that can be used for long-term adjuvant antibiotic therapy in clinical situations like osteomyelitis. Further research is warranted.

**P1893** Susceptibility of clinical *Staphylococcus aureus* isolates to the glycopeptides and comparators at a district general hospital in the UK

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**Objectives:** Recent reports indicate decreasing in vitro susceptibility of *Staphylococcus aureus* to vancomycin and poor clinical outcome when vancomycin is used to treat strains with a minimum inhibitory concentration (MIC) between 1 and 2 mg/L. With the emergence of glycopeptide resistance and routine use of vancomycin in serious staphylococcal infections, continued monitoring of vancomycin MIC is required in order to target antibiotic therapy.

This study compares in vitro susceptibility of *S. aureus* isolates, evaluated via gradient strip, against routine and novel antimicrobials used in treatment of serious gram-positive infections.

**Methods:** Clinically significant *S. aureus* isolates (n = 182), cultured on non-selective media from patient samples at the Queen Elizabeth Hospital, Kings Lynn, were retrieved for MIC testing. MIC values were determined by antibiotic gradient strip (AB biodisk, Sweden and Oxoid, UK) for daptomycin, tigecycline, linezolid and the glycopeptides, following BSAC methodology.

**Results:** Using BSAC susceptibility breakpoints, all isolates were susceptible to vancomycin, teicoplanin and linezolid. However, 29% of isolates exhibited an MIC of 2 mg/L for vancomycin. Non-susceptibility was observed for daptomycin (2%) and tigecycline (6%).

**Table 1.** MIC frequency distribution for five antimicrobials against *S. aureus* clinical isolates

MIC (mg/L)	Daptomycin (%)	Teicoplanin (%)	Vancomycin (%)	Linezolid (%)	Tigecycline (%)
0.125	1	1	0	0	5
0.25	14	2	0	0	53
0.5	37	22	13	0	36
1	46	60	58	5	5
2	2	15	29	91	1
4	0	0	0	4	0

**Conclusions:** Continued surveillance of *S. aureus* MIC is required at a local level to monitor emerging resistance to vancomycin and comparator antibiotics.

**P1894 Update on daptomycin activity and spectrum tested against Gram-positive organisms collected in 2011 from European medical centres**

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**Objectives:** To evaluate the in vitro activity and spectrum of daptomycin (DAP) by surveillance testing against clinical isolates collected in European (EU) hospitals in 2011 and to compare DAP activity against isolates from two time periods, in 2005 (i.e. before approval for clinical use by the European Medicines Agency [EMA]) and 2011. DAP is a cyclic lipopeptide approved in the United States (2003) and EU countries (2006) for the treatment of complicated skin and skin structure infections and *S. aureus* (SA) associated bacteremia and right-sided endocarditis.

**Methods:** Two thousand nine hundred and seventy-seven and 5307 consecutive strains were collected in 2011 and 2005, respectively, from 25 medical centers in 11 EU countries and Israel. The strains were tested for susceptibility against DAP and comparators by CLSI broth microdilution. Cation-adjusted Mueller-Hinton broth supplemented to 50 mg/L of calcium was used for DAP tests. MIC results were interpreted according to EUCAST and CLSI breakpoint criteria.

**Results:** DAP remains very active against SA (100.0% S) and coagulase-negative staphylococci (CoNS; 99.9% S, only one non-S strain from 2011) with MIC<sub>50/90</sub> of 0.25/0.5 mg/L for both organisms (see Table 1). In 2011, MRSA rates ranged from 1.0% in Sweden to 61.5% in Portugal, and seven countries had an MRSA rate >25.0%. DAP was highly active against MRSA (MIC<sub>50/90</sub>, 0.25/0.5 mg/L) as was linezolid (LZD; MIC<sub>50/90</sub>, 1/1 mg/L; 100% S), tigecycline (TIG; MIC<sub>50/90</sub>, 0.06/0.12 mg/L; 100.0% S) and vancomycin (VAN; MIC<sub>50/90</sub>, 1/1 mg/L; 100.0% S). All enterococcal strains were S to DAP (MIC<sub>50/90</sub>, 1/2 mg/L) and TIG (MIC<sub>50/90</sub>, 0.06/0.06 mg/L). LZD and ampicillin were active against 99.3% and 65.0% of

Organisms (no. tested 2005/2011)	Cumulative % (2005/2011) inhibited at daptomycin MIC (mg/L) of:					
	≤0.12	0.25	0.5	1	2	4
<i>S. aureus</i> (2746/1572)	3.4/2.6	68.9/83.9	99.3/99.8	100/100*		
MSSA (1946/1140)	4.0/2.9	74.7/86.8	99.6/99.9	100/100*		
MRSA (800/432)	2.0/1.9	54.8/76.4	98.5/99.5	100/100*		
CoNS (941/344)	9.1/13.1	56.6/52.9	97.1/96.5	100/99.7	100/100	
<i>E. faecalis</i> (648/271)	0.6/2.2	5.9/4.4	52.8/30.3	97.8/92.3	100/100	
<i>E. faecium</i> (307/156)	0.3/0.6	1.3/1.9	7.5/10.9	31.0/40.4	80.1/93.0	100/100
VAN-S (252/119)	0.4/0.8	1.6/2.5	8.7/11.8	32.5/41.2	80.2/92.4	100/100
VAN-non-S (55/37)			1.8/6.1	23.6/37.8	80.0/94.6	100/100
BHS (439/245)	63.8/76.3	100/97.6	100/100			
VGS (195/132)	34.4/22.0	72.3/54.6	93.3/84.9	100/99.2	100/100	

\* Susceptible breakpoint (EUCAST and CLSI)

enterococci, respectively. The overall VAN resistance (R) among *E. faecalis* and *E. faecium* were 0.7% and 23.7% in 2011. VAN-R did not adversely influence DAP activity against enterococci. DAP was also active against beta-haemolytic (BHS; MIC<sub>90</sub>, 0.25 mg/L) and viridans group streptococci (VGS; MIC<sub>90</sub>, 1 mg/L) strains from 2011. No significant variation was observed in the overall MRSA rate between time period; in contrast, VAN-R increased from 17.9% in 2005 to 23.7% in 2011 among *E. faecium*.

**Conclusions:** DAP showed sustained potency against recent (2011) clinical Gram-positive organisms isolated in EU medical centers. A decrease in DAP potency has not been observed since EMEA approval for clinical use.

**P1895 Reliable activity of daptomycin on *Staphylococcus epidermidis* from bone and joint infections**

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**Objectives:** Glycopeptides resistant *Staphylococcus epidermidis* (GRSE) strains are of increasing concern in bone and joint infections (BJIs). The use of Daptomycin appears to be a useful alternative but only few clinical strains of *S. epidermidis* representative of BJIs ecology have been tested to date. We report the MICs of vancomycin and daptomycin on a collection of 86 *S. epidermidis* isolates from BJIs, as well as the minimal concentration inhibiting biofilm formation (MCIB) and the minimal concentration bactericidal on mature biofilm (MCBB) for daptomycin.

**Methods:** A collection of 86 *S. epidermidis* isolates cultured from surgical specimen of MSIs from Hopital Raymond Poincaré (Garches, France) are tested for MIC according to EUCAST criteriae using the broth microdilution method (BMD) with Resazurin-Resafurin vitality marker. The minimal biofilm inhibitory concentrations (MBICs) was determined by evidencing inhibition of regrowth after a thorough wash of the plates. Bactericidal activity on mature biofilm was established by evaluating vitality after exposure of 48 hours old biofilm to the antibiotic.

**Results:** Susceptibility to daptomycin was observed in 85/86 isolates (99%) with a median MIC of 0.5 mg/L whereas vancomycin resistance was observed in 18/86 isolates (21%). These results were confirmed by agar dilution (19/86, 22%). Etest ellipsometry determined as much as 74/86 (86%) of isolates to be resistant to vancomycin. Further studying daptomycin, the MIC and MCIB were identical in 48/86 isolates (56%), within one dilution in 34/86 isolates (40%) and MCIB four times greater than MIC in four isolates (4%). The MCBB was ≤1 mg/L in 10/86 isolates (12%), ≤4 mg/L in 32/86 (37%), ≤8 mg/L in 47/86 (55%) and ≤32 mg/L in 74/86 (86%).

**Conclusion:** Daptomycin susceptibility was observed in all but one isolate whereas vancomycin resistance by BMD occurred in (21%) isolates. Bone concentrations of daptomycin have been determined to be >4 mg/L using a 6 mg/kg/day scheme and serum levels are reported to be greater than 32 mg/L. Based on our in vitro data, biofilm formation should be inhibited by daptomycin in most *S. epidermidis* isolates. Using the commonly reported 12 mg/kg/day dosage, bactericidal activity on mature biofilm would be observed in as much as 55% of isolates in a cancellous bone environment. Contrary to vancomycin, daptomycin appears to be reliably active in vitro on isolates of *S. epidermidis* from BJIs.

**P1896 In vitro susceptibility of *Staphylococcus aureus* in Africa-Middle East**

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**Background:** *Staphylococcus aureus* is the most common cause of skin and soft tissue infections followed by bloodstream infection and pneumonia. Patients with methicillin-resistant *S. aureus* (MRSA)

infections have increased and changed the treatment for *S. aureus* infections. Tigecycline (TIG) has been shown to have potent activity against community and hospital acquired staphylococcal pathogens. The Tigecycline Evaluation Surveillance Trial (TEST) determined the in vitro activity against methicillin-susceptible *S. aureus* (MSSA) as well as MRSA of TIG and other antimicrobials commonly prescribed for *S. aureus* infections.

**Methods:** A total of 1111 clinical isolates (312 MRSA; 799 MSSA) from Africa-Middle East throughout 2006–2010 were evaluated. Isolates were identified to the species level at each participating site and confirmed by a central laboratory. Minimum inhibitory concentrations (MICs) were determined by the local laboratory using supplied broth microdilution panels, and interpreted according to CLSI guidelines.

\*Penicillin, cepheems, and carbapenem susceptibilities are based on cefoxitin susceptibility for MRSA.

**Results:** 28.1% of *S. aureus* were resistant to cefoxitin (MRSA). Tigecycline and Vancomycin inhibited 100% of all MRSA.

Drug	MSSA N=799			MRSA N= 312		
	MIC90	%S	%R	MIC90	%S*	%R*
Ampicillin	>16	13	87	>16	0	100
Ceftriaxone	4	98.4	0.1	>64	0	100
Levofloxacin	0.5	95.5	2.3	32	13.8	85.6
Meropenem	0.3	100	0	>16	0	100
Minocycline	0.5	98.9	0.3	8	81.1	5.8
Tigecycline	0.5	100	0	0.5	100	0
Vancomycin	1	100	0	2	100	0

**Conclusions:** Tigecycline and Vancomycin retained potent activity against *S. aureus* inhibiting 100% of all MRSA. Since the prevalence of MRSA is increasing worldwide, antimicrobial surveillance is useful in monitoring the performance of different antimicrobials.

#### P1897 Activity of JNJ-Q2 against *Staphylococcus aureus* isolated from patients with acute bacterial skin and skin structure infection obtained during a phase II clinical trial

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**Objective:** To determine the activity of JNJ-Q2 against *S. aureus* isolated from patients with clinically diagnosed acute bacterial skin and skin structure infection (ABSSSI) in the United States (USA) during a phase II clinical trial and to determine the mechanisms of fluoroquinolone (FQ) resistance (R) in FQ-R strains. JNJ-Q2 is a broad-spectrum bactericidal 4-fluoroquinolone with potent activity against Gram-positive and -negative pathogens.

**Methods:** Of 280 pathogens isolated, *S. aureus* (n = 248; 88.6%) was the predominant pathogen isolated (including 45.2% methicillin-susceptible [MSSA and 54.8% methicillin-resistant MRSA]). Susceptibility testing was performed by the CLSI broth microdilution. Type II topoisomerase quinolone-resistant determinant regions (QRDR) were amplified by PCR and sequenced for FQ-R strains.

**Results:** JNJ-Q2 demonstrated good activity against all *S. aureus* and was very active against both MSSA (MIC50/90, 0.008/0.12 mg/L) and MRSA (MIC50/90, 0.12/0.12 mg/L). One hundred and seven strains had moxifloxacin (MOX) MIC values of  $\geq 1$  mg/L (non-S); 102/107 had only gyrA S84L with 4/5 of the remaining strains also having parC E84G (two strains), E84I (1), parE T461I (1). No QRDR mutations were found in the remaining strain. 101/107 strains had a JNJ-Q2 MIC of 0.12 mg/L (range, 0.06–0.25 mg/L). All isolates were susceptible to linezolid (LZD) and vancomycin (VAN). JNJ-Q2 was the most active agent tested with a MIC90 16-, 64-, 16-, and eight-fold lower than MOX, levofloxacin (LEV), LZD and VAN, respectively.

Species (no. tested)	No. (cum. %) of isolates inhibited at JNJ-Q2 MIC (mg/L)						
	$\leq 0.004$	0.008	0.015	0.03	0.06	0.12	0.25
<i>S. aureus</i> (248)	70 (28.2)	56 (50.8)	12 (55.6)	3 (56.9)	2 (57.7)	101 (98.4)	4 (100.0)
MSSA (112)	48 (42.9)	33 (72.3)	10 (81.3)	2 (83.0)	0 (83.0)	18 (99.1)	1 (100.0)
MRSA (136)	22 (16.2)	23 (33.1)	2 (34.6)	1 (35.3)	2 (36.8)	83 (97.8)	3 (100.0)

**Conclusions:** JNJ-Q2 demonstrated very potent activity against contemporary *S. aureus* isolated from patients in the USA with clinically diagnosed and microbiologically confirmed ABSSSI's. JNJ-Q2 exhibited greater activity compared to LEV and MOX, including strains R to currently utilized FQs. These encouraging results support the further clinical development of JNJ-Q2 for ABSSSIs.

## Diagnosis of tuberculosis

### P1898 Evaluation of automated BACTEC MGIT 960 system for testing susceptibility of *Mycobacterium tuberculosis* strains to first-line drugs: comparison with the radiometric BACTEC 460TB system

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**Objective:** The reliability of nonradiometric, fully automated BACTEC MGIT 960 system for drug susceptibility testing (DST) of *Mycobacterium tuberculosis* to first-line drugs isoniazid (INH), rifampin (RMP), streptomycin (STR) and ethambutol (EMB) was compared to that of radiometric BACTEC 460TB system. Detection of resistance conferring mutations in appropriate regions of *M. tuberculosis* genome by DNA sequencing was used to resolve discrepant results.

**Methods:** *Mycobacterium tuberculosis* strains (n = 38) with various susceptibilities to first-line drugs were tested. DST by BACTEC 460TB and MGIT 960 systems were performed according to protocols supplied by the manufacturer (Becton Dickinson). DNA sequencing of three regions of rpoB, katG codon 315 and inhA regulatory region, embB codons 306, 406 and 497, rpsL codons 43 and 88 and 500 and 1400 regions of rrs gene was performed to resolve discrepant results. False resistance and false susceptibility results were defined as major errors (ME) and very major errors (VME), respectively.

**Results:** Overall level of agreement between BACTEC 460TB results and those of BACTEC MGIT 960 method was 93.4%. All strains yielded identical results by both methods for INH and STR. Rifampin results agreed for 36 strains (95% agreement) while EMB results agreed for 30 strains (79% agreement). Ten strains yielded discrepant results of which eight and two strains were resistant to EMB and RMP, respectively, by BACTEC 460TB but susceptible by MGIT 960 system. DNA sequencing studies resolved all discrepant results in favor of BACTEC 460TB system.

**Conclusions:** Our data demonstrate that MGIT 960 system is an accurate method for rapid DST of *M. tuberculosis* against INH and STR while two VME for RMP were caused due to a very rare (I572F) rpoB mutation. However, eight VME associated with DST to EMB by MGIT 960 system are in line with previous reports showing EMB with least concordant results. More studies are needed to solve the problem of DST for EMB by MGIT 960 system.

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### P1899 How to screen health care workers for latent tuberculosis? Tuberculin skin test or Quantiferon-TB gold test?

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**Objectives:** Tuberculin skin test (TST) is used for many years for early diagnosis of latent tuberculosis infection (LTBI), but it is not sufficiently specific and sensitive. Quantiferon-TB gold test is an indirect test that measure interferon-gamma which is released after stimulation of mycobacterial proteins including ESAT-6, CFP-10, and TB 7.7. BCG strains and the majority of other non-tuberculosis mycobacteria do not harbor ESAT-6, CFP-10, and TB 7.7 proteins; thus, in this patients test is negative. In this report, we aimed to compare TST and Quantiferon-TB gold test for screening LTBI in health care workers (HCWs) in our hospital.

**Methods:** Age, sex, occupation, duration of work, tuberculosis history, contact history with tuberculosis patient, number of BCG vaccine were

recorded. TST, chest radiogram and Quantiferon-TB gold test (Cellestis, Carnegie, Australia) was performed in all HCWs.

**Results:** Totally 137 HCWs were enrolled into the study. Of them, 55 were male (40.1%), 82 female (59.9%) and mean age was  $32.6 \pm 6.4$  years. Sixty-six of HCWs were doctors (48.2%), 40 nurses (29.2%) and 31 cleaning and maintenance staff (22.6%). Mean duration of work was  $7.6 \pm 6.2$  years, 34 HCWs (24.8%) were working for more than 10 years. There was history of tuberculosis in four HCWs themselves (2.9%) and in 12 in their families (8.8%); contact history with tuberculosis patient was found in 78 HCWs (56.9%). Of HCWs, 129 (94.2%) had one or more BCG vaccine scar. TST was found to be positive in 59 HCWs (43.1%) and Quantiferon-TB gold test in 20 (14.6%). Chest radiogram was normal in all HCWs. Sex, duration of work, tuberculosis history, contact history with tuberculosis or BCG vaccination were not significantly different between Quantiferon test positive and negative patients ( $p > 0.05$ ). In HCWs aged 40–50 years Quantiferon-TB test was significantly more positive than HCWs aged 20–29 and 30–39 years ( $p < 0.05$ ). The test was more positive in cleaning and maintenance staff than doctors ( $p < 0.05$ ). Quantiferon-TB test results were not different in TST positive and negative patients ( $p > 0.05$ ). TST was not significantly different in all of the compare groups, there was also no difference in BCG vaccinated and not vaccinated HCWs ( $p > 0.05$ ).

**Conclusion:** Despite the little differences, we think that Quantiferon-TB gold test and TST results were compatible and each can be used for screening the LTBI in HCWs.

**P1900** Rapid identification of clinical mycobacterial strains by matrix-assisted laser desorption/ionisation-time of flight mass spectrometry (MALDI-TOF MS)

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**Objectives:** Mycobacterial identification is based on several methods: conventional biochemical tests that require several weeks for accurate identification, and molecular tools that are now routinely used. These techniques are expensive and time-consuming. Over the last few years matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI TOF MS) has been employed as a primary identification method for the identification of microorganisms in many clinical diagnostic laboratories. Here we seek to optimize the use of MALDI-TOF MS in rapid identification of clinical mycobacterial strains including *Mycobacterium tuberculosis* in a clinical laboratory setting.

**Material and methods:** Clinical strains from 39 mycobacterial species and species complexes were included in the study. The mycobacterial strains were grown on Middlebrook 7H10 medium and/or liquid mycobacterium growth indicator tube [MGIT] medium. Starting with five different inactivation methods we established an optimized inactivation protocol for mycobacteria as well as an optimized protein extraction protocol for MALDI TOF MS by the Microflex mass spectrometer (Bruker Daltonics, Germany). The protocol included two steps of bead-beating using the MagNA Lyser instrument (Roche) with ceramic or silica beads.

The spectrum generated was analyzed by MALDI Biotyper 3.0 software (Bruker Daltonics) using both the Bruker and a supplementary home-made library.

**Results:** Correct identification was obtained for nearly all of the mycobacterial strains. There were some difficulties in distinguishing a few very closely related mycobacterial species, but whether or not the strains were misidentified by 16S rRNA sequencing or by MALDI TOF MS remains unclear.

**Conclusions:** Based on our optimized protocols, we suggest that the use of MALDI-TOF MS represents a rapid, feasible and inexpensive system for identification of mycobacterial species in clinical diagnostics.

**P1901** Evaluation of a point-of-care molecular test for *M. tuberculosis* detection in an emergency setting

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**Objective:** Tuberculosis incidence in Portugal has decreased to an half during the last decade, but still remains at the intermediate level (22 new cases per 100 000 inhabitants) emphasizing the need to improve control measures, with special focus on rapid and accurate diagnosis and resistance tests. We evaluated the prospective use of Xpert® MTB/RIF in the emergency laboratory, and compared the results with AFB smear, culture and two other molecular tests.

**Methods:** Seventy four clinical samples (64 respiratory and 10 non-respiratory) were studied. AFB smear microscopy, MGIT® and Lowenstein culture were performed. Molecular methods included Xpert® MTB/RIF, Cepheid, performed on arrival at the Emergency Microbiology Laboratory. MTD® Test, Gen-Probe and *M. tuberculosis* PCR Kit®, Abbott, performed at the Molecular Biology Unit, using decontaminated samples, after automated nucleic acid extraction.

**Results:** *Mycobacterium tuberculosis* complex was identified by culture in 32% of samples ( $n = 24$ ), 79% respiratory ( $n = 19$ ) and 21% non-respiratory ( $n = 5$ ). AFB smear was positive in 84% of respiratory samples ( $n = 16$ ) and 80% of non-respiratory samples ( $n = 5$ ). Positive AFB smear without *M. tuberculosis* detection, was found in two samples, identified as *M. avium*. Compared with culture, the overall results showed sensitivity, specificity, PPV and NPP as follows: AFB smear – 83%, 96%, 90%, 92%; Xpert® MTB/RIF – 91%, 100%, 100%, 96%; MTD® Test – 100% for all; *M. tuberculosis* PCR Kit® – 96%, 100%, 100%, 98%. No rifampicin resistance was detected by Xpert® MTB/RIF, which was in full agreement with both phenotypic susceptibility test and molecular detection of rpoB mutations in house real time PCR.

**Conclusion:** The high AFB smear sensitivity, points towards a high bacterial load, which explains the overall elevated sensitivity values amongst all methods. Xpert® MTB/RIF failed to detect 2 AFB negative-culture positive respiratory samples, both detected by MTD® Test, and only one detected by *M. tuberculosis* PCR Kit®. The recent inclusion in our Hospital Centre of an Infectious Diseases Unit is shifting our population to a high HIV prevalence setting, reinforcing the need to rapid results. Xpert® MTB/RIF seems a good alternative to be used in the emergency laboratory as a point of care test given its short turnaround time. However further tests are needed in order to assure a better sensitivity, particularly in respiratory samples.

**P1902** Improved detection of positive AFB smears in labs without cultural facilities

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**Objective:** A cross sectional study to compare two methods of detection of AFB smears among suspected Pulmonary tuberculosis patients in a Hospital in Northern Nigeria.

**Method:** All sputa samples of suspected Tb patients sent to the Medical microbiology laboratory over a consecutive period of six (6) months were used for the comparison analysis. Direct smears were prepared from all samples and smears also made after concentrating the sputa using the Sodium hypochlorite concentration method. All smears were stained by the Zeihl Neelson technique for the detection of Acid fast bacilli. In house controls of known positive and negative smears were also stained concurrently.

**Results:** A total of 1232 samples from 496 patients were analyzed during this period. Total number from males were 295 (59.5%) and females 201 (40.5%). Using the direct smear method, positivity rate was 4.2% while positivity rate using the concentration method was 9.3% which was statistically significant ( $p < 0.05$ ). There was no statistically significant difference between both methods in the detection of AFB from smears of patients <10 years of age. However in patients from the HIV clinic, detection of AFB was better using the concentration method (26.7%) when compared with the direct smear method (16.7%).

**Conclusion:** The sodium hypochlorite method is a method which has been reported to improve the detection of AFB in smears from tuberculosis patients, while at the same time, rendering the smears less infective to its handlers. The major drawback is that once treated with sodium hypochlorite, the sample is no longer useful for culture. In resource poor setting where cultures are rarely done, the use of sodium hypochlorite concentration method is likely to improve detection of AFBs particularly in patients also positive for HIV while at the same time protecting the handlers of the specimens as the tubercle bacilli are killed.

**P1904 Performance of a commercially available IFN-gamma release assay in body specimens other than blood**

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**Objectives:** The diagnosis of extra-pulmonary tuberculosis (e-TB) is often very difficult because of poor sensitivity of traditional microbiological methods. Commercially interferon-gamma (IFN-gamma) release assays (IGRA) hold promise to provide a more accurate diagnosis of latent tuberculosis infection (LTBI) and active tuberculosis (TB).

**Methods:** In a prospective study, a commercially available IFN-gamma assay (QuantiferON-TB Gold In-Tube [QFT-IT]; Cellestis Ltd., Victoria, Australia) was evaluated in 31 specimens other than whole blood from patients with a high suspicion of active e-TB from May 2010 to May 2011.

**Results:** In our study population: 26/31 (84%) were born in Italy and 5 (16%) were foreign-born (2 [6.5%] coming from Ghana; two from India and 1 [3.2%] from Philippines); mean age was  $64.4 \pm 20.3$  years D.S.; four (12.9%) subjects were BCG-vaccinated; three (9.7%) had an active extrapulmonary-TB (e-TB) and 28 (90.3%) had an alternative diagnosis other than e-TB. About 31 specimens: 25 (80.6%) were pleural fluid (PE), three (9.7%) ascites and three (9.7%) BronchoAlveolar Lavage (BAL).

QFT-IT Blood	QFT-IT Body Specimen	Total	EXTRAPULMONARY TB	PULMONARY DISEASE OTHER THAN TUBERCULOSIS
		n	n	n
Positive	Positive	2	0	2
Positive	Negative	1	0	1
Positive	Indeterminate	3	1	2
Negative	Positive	3	1	2
Negative	Negative	16	0	16
Negative	Indeterminate	2	1	1
Indeterminate	Negative	4	0	4

Only five (16.1%) specimens other than blood were QFT-IT indeterminate (four were pleural fluid and one BAL): three (9.7%) due a low response to positive control (Mitogen) and two (6.4%) for high background. Four (12.9%) patients tested indeterminate with QFT-IT on blood; of those, all were QFT-IT-negative. Among three patients with e-TB (all pleural TB): two (6.4%) patients showed an indeterminate QFT-IT result on PE and one of them tested positive with QFT-IT on blood. Interestingly, the third patient with e-TB was positive on PE and negative on blood. Among patients with disease other than e-TB, four (12.9%) subjects showed a positive QFT-IT results on specimens from the site of infection and five (16.1%) tested positive on blood. In our study, the sensitivity and specificity of QFT-IT on specimens other than blood from the site of infection was 33.3% and 85.7%, respectively; on blood, the sensitivity and specificity of QFT-IT was 33.3% and 82.1%, respectively.

**P1905 Comparison of on-demand PCR testing for *Mycobacterium tuberculosis* with culture and batched PCR**

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**Objectives:** The "on demand" Cepheid GeneXpert® MTB/RIF PCR (Xpert) assay simultaneously detects *Mycobacterium tuberculosis* complex (MTBC) and markers of rifampicin resistance from samples. The assay does not require specialist molecular facilities. We compared the sensitivity and specificity of the Xpert assay with culture and batched PCR.

**Methods:** Following auramine microscopy for acid fast bacilli (AFB) and inoculation of MGIT® tubes (BD), the residual deposit from 96 samples was divided into two aliquots, assigned a random study number and frozen for later analysis. Samples were selected from three groups: (1) AFB seen (2) AFB not seen but clinical risk of tuberculosis (TB); (3) AFB not seen, TB unlikely. Samples included sputa (67), BAL (4), pleural fluid (6), tissue (4), pus (6), CSF (2) and urine (7). One aliquot of each pair was analysed using the Xpert assay and the other was processed by manual DNA extraction and PCR using the QIAmp DNA Mini kit (Qiagen) and MTB Q-PCR Alert Kit (Nanogen Advanced Diagnostics) ("PCR"). Aliquots were processed in batches. All results were analysed at the end of the study.

**Results:** Full datasets were available for 93 samples: (i) From 32 AFB seen samples, 24 grew MTBC with MTBC detected in 25 samples by Xpert and 23 samples by "PCR"; (ii) From 30 clinical risk of TB, AFB not seen samples, two samples grew MTBC; Xpert detected MTBC in one sample whereas five were positive by "PCR"; (iii) MTBC was not grown or detected by the Xpert assay in 31 samples where TB was unlikely although "PCR" was positive for one sample. All isolates were sensitive to rifampicin and resistance markers were not detected by Xpert. One AFB positive culture negative sample was positive for MTBC DNA in both molecular assays. This patient had previously grown MTBC and was on anti-TB therapy. Samples that were positive by "PCR" alone all had high C<sub>t</sub> values suggesting contamination by other samples processed in the batch.

**Conclusions:** When compared with culture, the sensitivity and specificity for the Xpert MTB/RIF assay was 96.2% and 98.5% and for the "PCR" assay 92.3% and 92.5%. When adjusted to include the patient on treatment, the specificities of the assays were 100% and 94% respectively. Contamination between samples was not observed using the Xpert assay unlike the batched PCR. The Xpert assay is highly suited to a TB laboratory where samples likely to grow MTBC can be rapidly identified and the results utilised by clinicians within hours of laboratory receipt.

**P1906 Cryopreserved human PBMC maintain full functionality in the T-SPOT.TB assay for up to 3 years**

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**Objective:** The T-SPOT.TB test detects both latent Tuberculosis infection (LTBI) and active TB disease by measuring the response of T-cells that have been specifically activated by *Mycobacterium tuberculosis* (MTB) antigens. T-Spot assay performed on freshly isolated peripheral blood mononuclear cells (PBMC) is an established routine tool providing diagnostic information about a patient's LTBI/TB status. However, performing T-SPOT assays only with freshly isolated cells is not always feasible in the context of large clinical trials and is inefficient from an economic point of view. Obtaining valid T-SPOT data from cryopreserved samples would both increase TB monitoring capabilities and permit retrospective studies.

**Method:** We compared the antigen-specific IFN- $\gamma$  T-cell response using the T-SPOT.TB assay in 30 T-SPOT.TB positive (>6 spots) patients in freshly isolated PBMC and once again from frozen PBMC at intervals representing from 1 month up to a maximum of 3 years of cryopreservation. PBMC were cryopreserved at  $-150$  to  $-140^\circ\text{C}$  in 90% FCS and 10% DMSO. Viability of thawed PBMC was determined by trypan-blue staining.

**Results:** All 30 patients showed consistently positive qualitative T-SPOT.TB results from cryopreserved PBMC. Further analysis showed that also quantitative results (number of spots) from fresh and frozen PBMC were comparable; no significant difference was observed between the number of spots detected using fresh and frozen samples.

**Conclusion:** Our results demonstrate that the use of fresh PBMC is not an absolute requirement for obtaining valid T-SPOT.TB results. Using frozen aliquots of blood samples provides qualitatively correct results when compared to fresh samples. Therefore, frozen PBMC samples may be used in retrospective studies; this in turn offers significant savings with regard to analytical costs and enables specimens to be transported to centralized laboratories for testing under standardized conditions in the context of multi-center clinical studies.

**P1907 Analysis of the results of SEIMC External Quality Control Programme in the diagnosis of mycobacteria, 1998–2010**

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**Objective:** To evaluate the results obtained by the External Quality Control SEIMC in identifying mycobacteria.

**Material and methods:** From 1998 to 2010 two mycobacterial controls per year were sent to an average of 95 centers, comparing their results with those obtained by a reference laboratory. Different mycobacterial species were sent: *M. tuberculosis*, *M. kansasii*, *M. avium*, *M. fortuitum*, *M. smegmatis*, *M. gordonae*, *M. chelonae*, *M. thermoresistibile*, *M. bovis*, *M. gastri*, *M. intracellulare*, *M. szulgai*, *M. marinum*, *M. abscessus*.

**Results:** The mean of participation rate in controls was 78.1%. The percentage of success in identifying varies by species: in controls MB-1/98, MB-2/02 y MB-2/06 (*M. tuberculosis*), the percentage of centers that identify the species varies from 62.3% in the first two, to 50.6% in the last, in which there was greater correlation with the identification method used. In shipment MB-2/03, only 18.9% of centers achieved a correct identification of species (*M. bovis*), while shipment MB-1/10, 55.8% of participants identified correctly the strain as *M. bovis* BCG. In controls MB-1/99, MB-1/03, MB-2/05, MB-1/08 (*M. kansasii*), the percentage of success in identifying rose from 79.6% to 91.1%, improving the ability of participants to identify the genotype; in control MB-1/04, a strain phenotypic and genotypically similar species (*M. gastri*) was sent and the success rate in identify was only 47.9%, and amounted to 75.5% on shipping MB-1/09. When a strain of the *M. avium* complex was sent, the percentage of laboratories that came to a diagnosis of species increased from 35.7% (MB-2/99), to 54.1% (MB-2/04) and 81.7% (MB-2/10). When unusual strains in clinical practice were sent, identification rates were lower: MB-1/02 (*M. thermoresistibile*, 27.6%) and MB-2/00 (*M. smegmatis*, 38.1%), percentage that rises to 57.1% (MB-1/05) by the introduction of hybridization techniques. In the case of *M. fortuitum*, *M. chelonae* and *M. gordonae*, successfully identification rates ranged between 70% and 93.8%.

**Conclusions:** There was no clear correlation between the participation rate and a higher percentage in success identifying. There is progressive improvement in the identification by the introduction of molecular methods (hybridization, PCR-RFLP, sequencing), so that a greater number of species can be identified routinely in the laboratory.

**P1908 Evaluation of the use of Quantiferon TB-Gold in the routine setting at a university hospital, 2007–2011**

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**Objectives:** To study retrospectively the experience with the use of Quantiferon TB-Gold (QFT) for the diagnosis of latent tuberculosis in a routine clinical setting.

**Methods:** A retrospective review of the records from the Microbiology department was performed. Patients with both Mantoux and QFT tests performed with a difference of <7 days were selected for review. Healthcare workers were excluded from the study. Demographics and data about BCG vaccination, immunosuppression, tuberculosis and treatment of latent tuberculosis infection were analysed. Statistics include kappa correlation analysis, Fisher's exact test and Student's *t*-test.

**Results:** During the study period, QFT was performed in 389 patients. Three hundred and eight were selected for the study. The mean age of the population was 42.44 (range 0–93 years). The kappa analysis showed a moderate correlation between QFT and Mantoux test ( $k = 0.509$ ). Two hundred and eleven patients had identical results in both tests (156 negative and 55 positive), but 45 patients had discordant results (21 QFT positive-Mantoux <5 mm; 24 QFT negative-Mantoux  $\geq 5$  mm). Twelve patients had indeterminate results due to negative results of the positive control (1). Forty-two patients did not come to the lecture of their Mantoux test (one with an indeterminate result).

The department with the highest values of requested QFT was Internal Medicine (N = 168, including Infectious Diseases). BCG status was registered only in 40 clinical charts (only six were vaccinated), HIV status in 270 (103 positive), and presence of immunosuppression in 307 (including 56 HIV negative patients). Twenty-nine patients were diagnosed of active tuberculosis, and five of past tuberculosis. Only 41 patients received treatment for latent infection, including two patients QFT positive-Mantoux <5 mm and three patients QFT negative-Mantoux  $\geq 5$  mm (two from Rheumatology and one from Paediatrics). Statistical analysis showed that there was a correlation between QFT positive result and VIH positive ( $p = 0.0266$ ), immunosuppression ( $p = 0.0319$ ), and diagnosis of tuberculosis ( $p < 0.0001$ ). Student's *t*-test showed that the mean values of Mantoux test when  $\geq 5$  mm were higher when QFT had positive values ( $p = 0.0347$ ).

**Conclusions:** QFT test is included in some departments as part of the protocols of initial evaluation of the patients. However, QFT results are not used to decide to treat patients with latent tuberculosis, so it is necessary to improve the knowledge of the significance of QFT among clinicians to improve its correct use.

**P1909 Loop-mediated isothermal amplification for rapid diagnosis of pulmonary tuberculosis and its comparison with IS6110 polymerase chain reaction and conventional techniques in resource-poor setting**

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**Objectives:** To evaluate LAMP assay based on the amplification of the rimM (encoding 16S rRNA-processing protein) gene of *Mycobacterium tuberculosis* complex and its comparison with IS6110 PCR and conventional techniques in TB endemic country

**Methods:** Sputum samples were collected from 133 patients which were divided into following three groups (i) confirmed cases of tuberculosis (83) (ii) Suspected tuberculosis (20). (ii) Controls (30) in whom tuberculosis was excluded on the basis of clinical, radiological and microbiological test. The samples were subjected to microscopy, culture, IS 6110 and LAMP assay which used set of six specific primers targeting 16s rRNA gene of *M. tuberculosis*.

**Results:** Of these 103 patients infected with tuberculosis, Acid Fast Bacilli (AFB) was positive in 70 (67.9%) and culture in 78 (75.7%) patients. Overall, LAMP positivity was observed in 84.5% (87/103) and IS6110 could detect TB in 74 (71.8%) patients. The positive and negative predictive values of LAMP was 100% and 65.2% respectively. Both IS6110 and LAMP were negative in all the 30 Non TB patients giving the specificity of 100%. The proportion of agreement among IS6110 and LAMP by using kappa was approximately significant i.e. 0.6

**Conclusions:** The study showed that the LAMP assay is a rapid, sensitive and specific method to detect pulmonary tuberculosis and

that it is superior to the conventional PCR assay. LAMP is very simple and it can be performed in any laboratory and in resource poor settings.

#### **P1910** Role of PCR for diagnosing male genital tuberculosis

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**Objectives:** Conventional methods like smear and culture for *Mycobacterium tuberculosis* are of limited sensitivity and specificity. Histopathological examination (HPE) for the tissues, in the absence of caseous necrosis or stained acid-fast bacilli, is often reported as granulomatous infection. This study was conducted to determine the utility of PCR for diagnosing tuberculosis of the male genital tract and comparative evaluation of PCR for tissue samples with HPE.

**Methods:** A prospective study was conducted from January 2007 to October 2011 with 38 tissues (prostate, epididymis and periurethral fistulous tract tissue) and 11 semen samples from patients with clinically suspected male genital tuberculosis. All the tissue samples were processed for both PCR and histopathology.

**Results:** In 34 tissue (89.5%) samples, results for both PCR and HPE were coinciding. False positivity and false negativity was observed in 2.6% (1/38) and 7.9% (3/38) samples, respectively. With HPE as the gold standard, PCR has shown sensitivity of 86.4% (95% CI 72.1; 90.7) and specificity of 93.7% (95% CI 74.1; 99.7) and positive agreement between two tests was observed as significant (0.8). PCR results were obtained within a mean period of 3.4 days while those of HPE were obtained in 7.2 days. PCR was positive for 4/11(36.4%) semen samples.

**Conclusions:** Tissue PCR is a sensitive and specific method for obtaining early and timely diagnosis of male genital tuberculosis. Application of tissue PCR results can augment the diagnostic accuracy in histopathologically reported granulomatous inflammations. Semen PCR adds qualitative benefit for diagnosing such cases.

#### **P1911** Use of a new qualitative molecular dipstick assay for the rapid detection of *Mycobacterium tuberculosis* in smear-positive and smear-negative clinical specimens

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**Objectives:** The Genoquick® MTB (Hain Lifescience, Nehren, Germany) is a qualitative molecular assay recently developed for the rapid detection and identification of *M. tuberculosis* complex (MTBC) from decontaminated clinical specimens. The aim of the study was to evaluate the clinical accuracy of Genoquick® MTB compared to conventional culture.

**Methods:** This prospective study was performed at the National Reference Laboratory for Mycobacteria (Athens, Greece). The molecular assay was performed according to the manufacturer's instructions, and results were compared to culture performed in both Löwenstein-Jensen (LJ) and the automated BACTECTM MGIT 960 system (Becton Dickinson, USA). Statistical analysis included the determination of sensitivity, specificity, positive (PPV) and negative predictive value (NPV) ratios. The significance of the difference in accuracy between smear-positive and smear-negative specimens was assessed by the chi-square test.

**Results:** In total, 60 samples were examined. Thirty-three were smear-positive and 27 smear-negative; 48 samples were of pulmonary (41 sputum; five washings; one gastric fluid; one bronchial secretion) and 12 of extra-pulmonary origin (four CNS; four pus; two urine; one pleural fluid; one pericardial fluid). Genoquick® MTB was positive in 39 specimens (24 smear-positive and 15 smear-negative). The molecular assay yielded a sensitivity of 91.2% and a specificity of 69.2% for the direct identification of MTBC (91.3% and 70% for

smear-positive and 90.9% and 68.75% respectively). The positive and negative predictive values were 79.5% and 85.7%, respectively (87.5% and 77.8% for smear-positive and 66.7% and 91.7%, respectively). The difference in PPV and NPV between smear-positive and smear-negative samples was not statistically significant ( $p > 0.05$ ).

**Conclusions:** Our preliminary results showed that Genoquick® MTB assay performance was compatible with other commercially available molecular methods certified for direct application to smear-positive and smear-negative pulmonary and extra-pulmonary samples. In addition, the Genoquick® MTB leads to considerably faster results compared to conventional hybridization methods.

#### **P1912** Evaluation of Genotype® MTBDRplus for the rapid detection of *Mycobacterium tuberculosis* resistance to rifampicin and isoniazid in clinical samples

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**Objectives:** The Genotype® MTBDRplus (Hain Lifescience, Nehren, Germany) is a DNA strip hybridization assay approved for the rapid detection of rifampicin (RMP) resistance associated with *rpoB* mutations and isoniazid (INH) resistance caused by *katG* and/or *inhA* mutations. The assay is validated for application on culture isolates and smear-positive (AFB+) clinical specimens as well, precluding the need to wait cultures to grow. The aim of the study was to assess the performance of the Genotype® MTBDRplus as a routine diagnostic assay directly on clinical specimens.

**Methods:** The study was prospectively carried out at the National Reference Laboratory for Mycobacteria (Athens, Greece). We performed the assay on consecutive AFB (+) specimens. A number of AFB (-) specimens, from patients at increased risk for drug resistance, were also tested. The results were compared to those obtained using conventional phenotypic drug susceptibility testing (DST). The proportion method on Löwenstein-Jensen medium (critical concentrations for RMP and INH 40 µg/mL and 0.2 µg/mL, respectively) or the automated Bactec MGIT960 system (critical concentrations 1.0 and 0.1 µg/mL, accordingly), were used for DST on cultures derived from the clinical specimens. For estimating the time reduction, by the application of the test directly on clinical specimens, we calculated the days elapsed before patient's first positive culture was available for molecular testing (from any specimen).

**Results:** In total, 426 clinical specimens were analyzed. Interpretable Genotype® MTBDRplus results were obtained for 295/362 (81.5%) smear-positive and 27/64 (42.2%) smear-negative. A substantial reduction in the time for drug susceptibility diagnosis was recorded (14.7 ± 6.4 days). The test correctly detected 11/12 RIF resistant strains (the one missed was due to a polymorphism at nucleotide 1341 of the *rpoB* gene) and 23/25 INH resistant strains. The sensitivity, specificity, PPV and NPV values of the assay for the detection of RMP and INH was 100%, 99.8%, 92%, 100% and 92%, 100%, 100%, 99.5%, respectively.

**Conclusions:** The MTBDRplus assay was informative for the great majority of AFB (+) clinical samples. This assay was highly accurate for the screening of the RMP and INH *M. tuberculosis* susceptibility and its application achieved a substantial reduction in diagnostic delay.

#### **P1913** QuantiFERON-TB Gold In-Tube and active tuberculosis treatment: evaluation of IFN-gamma production and immunological asset in pulmonary and extrapulmonary cases

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**Objectives:** Successful tuberculosis (TB) treatment is not only important for the individual patient but it is also crucial in controlling



the disease spread: however, monitoring its efficacy may be difficult. Interferon-gamma (IFN- $\gamma$ ) release assays, implemented mainly for latent TB diagnosis, have also been proposed as surrogate markers of mycobacterial burden.

The objective of this study was to assess the utility of QuantiFERON-TB Gold In-Tube (QFT-IT) in evaluating treatment response in active TB patients, analysing IFN- $\gamma$  production and lymphocyte subpopulations.

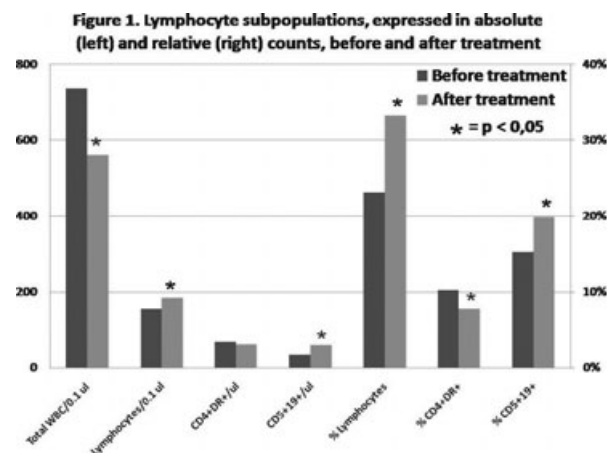
**Methods:** We evaluated 54 patients affected by active TB, all confirmed by cultural exam and treated with positive outcome. Subjects affected by immunosuppressive pathologies were excluded. All patients performed QFT-IT (<sup>®</sup>Cellestis Ltd) and lymphocyte subpopulations measurement before the beginning and at the end of anti-TB treatment. Lymphocyte subpopulations were studied by using monoclonal antibodies and flow cytometry (<sup>®</sup>Becton-Dickinson).

Pulmonary TB (PTB) cases underwent thoracic CT scan to evaluate the disease extension.

**Results:** QFT-IT results were 83% positive, 9% indeterminate and 7% negative before the therapy and 56% positive and 44% negative after. Following the treatment, we recorded a statistically significant increase in mean mitogen-stimulated IFN- $\gamma$  production.

Mean leukocytes decreased significantly after the therapy, with an increase in total and relative lymphocyte counts and proportional reduction of activated (DR+) CD4+ cells. Relative and absolute counts of CD5+ CD19+ cells were also increased.

Smear-positive PTB patients with extended (>2 lobes) and cavitary disease showed a significant decrease in IFN- $\gamma$  response to TB antigens after the treatment, reverting in 55% of subjects from positive to negative QFT-IT results as opposed to just 21% in extrapulmonary cases.



**Conclusion:** Following treatment we found an expected reduction in total leukocytes and activated CD4+ cells, associated with total lymphocyte and CD5+ CD19+ cells increase. This finding is probably related to the recovery of immune system functionality, as confirmed by the increased response to mitogen, with a compensatory switch towards B-cell immunity.

Our data show a considerable reduction in QFT-IT positive results after TB therapy, in particular in patients affected by advanced PTB. QFT-IT may thus be considered a useful tool for monitoring TB treatment outcome, with the possible exception of extrapulmonary cases.

**P1914** Semi-liquid culture media for rapid drug susceptibility testing of *Mycobacterium tuberculosis* and quantitative estimation of growth in dilute suspensions

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**Objectives:** To estimate suitability of semi-liquid media for rapid drug susceptibility testing (DST) and identification of separated Mtb colonies.

**Methods:** Thirty-eight panel and 47 clinical Mtb strains have undergone DST with first-line drugs: (i) using BACTEC MGIT 960 (SIRE Kit; BD) system, (ii) Middlebrook semi-liquid medium (Msl, broth 7H9, 0,125% agar, 10% supplement OADC, BD), (iii) Sauton semi-liquid medium (Ssl, Sauton medium with 0,3% nutrient agar and 25% horse serum), (iv) Löwenstein-Jensen (LJ) solid medium. The suspensions, density 1.5 MacFarland units, were diluted 10 (S:10) and 1000 (S:1000, control) folds and 0.5 mL of the solution was introduced into liquid Middlebrook medium and Msl, 0.2 mL to Ssl and LJ. Mtb was applied to semi-liquid media superficially, by layers without agitation and stirring. An opportunity of reception of separated Mtb colonies at inoculation of dilute suspensions (100 bacteria/mL) into semi-liquid media was studied. 2 g/L glucose was added to Ssl additionally and after addition Msl and Ssl suspensions were carefully mixed.

**Results:** *Mycobacterium tuberculosis* (Mtb) growth on Msl and Ssl was registered visually. Growth period S/1000 for all investigated strains in BACTEC system was  $7.9 \pm 0.2$  days, for Msl  $7.5 \pm 0.4$ , for Ssl  $8.3 \pm 0.6$  days. S:10 growth on semi-liquid media was identified in 3.5–4 days. Sensitivity and specificity of all media (except for BACTEC MGIT 960 at ethambutol testing) at DST of panel strains concerning first-line preparations has made 94.1–100.0%. DST of Mtb clinical strains to isoniazid has made 100%, to streptomycin and rifampicin – 91.4–100.0%, to ethambutol for Msl – 83.3–95.2%, for Ssl – 76.2–92.3%. For BACTEC MGIT 960 system average period of strain identification with multidrug resistance has made  $8.4 \pm 0.4$  days. Growth of all MDR strains on Msl and Ssl was observed within the time noticed for the control one, inoculated S:10. For Msl it was  $3.8 \pm 0.3$  days, for Ssl  $4.2 \pm 0.4$  days. Separated Mtb colonies was identified visually on 14th – 21st day of incubation on Msl and Ssl.

**Conclusion:** Middlebrook and Sauton semi-liquid media are suitable for rapid (4–6 days) cultural identification of MDR strains and for formation of separated Mtb colonies.

**P1915** Construction of a membrane array for detection of rifampin and isoniazid resistance in *Mycobacterium tuberculosis* complex

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**Objectives:** Rapid detection of rifampin (RIF) and isoniazid resistance (INH) in *Mycobacterium tuberculosis* complex (MTBC) is important for the control of tuberculosis. More than 95% of rifampin-resistant MTBC have mutations in rpoB, while Isoniazid resistance is related to mutations in katG and the mabA promoter. Oligonucleotide probes have the potential to detect point mutations in rpoB, katG, and mabA promoter to rapidly predict antibiotic resistance of MTBC to RIF and INH. This study aimed to develop a membrane oligonucleotide array to rapidly analyze point mutations in genes conferring RIF and INH resistance to MTBC.

**Methods:** Oligonucleotide probes (20–30 nucleotides) were designed from the rpoB, katG, and mabA promoter region to detect single nucleotide mutations in these genes. The detection method consisted of multiplex PCR amplification of these genes, followed by hybridization of the digoxigenin-labeled PCR products to oligonucleotide probes immobilized on a nylon membrane. A collection of 195 clinical MTBC strains with known susceptibility patterns were tested. Among these strains, 50 and 70 were resistant to RIF and INH, respectively. After hybridization, alkaline phosphatase-conjugated anti-digoxigenin antibodies were used to produce the hybridization signals.

**Results:** Single nucleotide mutations in genes of rpoB, katG, and the mabA promoter were successfully detected in MTBC strains by the array. The sensitivities of the array for detecting antibiotic resistance were 98% (RIF) and 89% (INH), respectively, while the specificities were 97% (RIF) and 98% (INH), respectively. The positive predictive values were 93% (RIF) and 97% (INH), respectively, and the negative predictive values were 99% (RIF) and 93% (INH), respectively.

**Conclusion:** The array can effectively detected point mutations in gene mutations associated with RIF and INH resistance in MTBC. In addition to this, the hybridization results also could reveal the exact mutations occurred in a strain of MTBC and the information had important epidemiological value. The test could be completed within 6 hour.

**P1916** Comparison of two nucleic acid amplification assays, the ProbeTec ET assay and Xpert MTB/RIF assay, for detection of *Mycobacterium tuberculosis* in pulmonary and extrapulmonary specimens

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**Methods:** Smear microscopy for acid-fast bacilli, culture by automated BACTEC MGIT 960 system and direct detection of *M. tuberculosis* by ProbTec ET and Xpert MTB/RIF systems were performed on 989 pulmonary and 454 extrapulmonary specimens obtained from suspected tuberculosis patients during January 2010 to August, 2011. Drug susceptibility testing for first-line drugs was performed using BACTEC MGIT 960 system and results of susceptibility to rifampicin were compared with Xpert.

**Results:** Of 989 pulmonary specimens, 90, 66 and 833 were; smear positive – culture positive, smear negative – culture positive and smear negative – culture negative, respectively. Both molecular tests detected *M. tuberculosis* in 81 of 90 smear positive – culture positive samples, one sample was negative by both molecular tests while eight samples yielded non-tuberculous mycobacteria (NTM). The ProbeTec and Xpert detected *M. tuberculosis* in 46 (70%) and 43 (65%) of 66 smear negative – culture positive samples, respectively. All 833 smear negative, culture negative samples were also negative by both molecular tests. Of 454 extrapulmonary specimens, 25, 1, 58 and 370 were; smear positive – culture positive, smear positive – culture negative, smear negative – culture positive and smear negative – culture negative, respectively. Both molecular tests detected *M. tuberculosis* in 23 of 25 smear positive – culture positive samples while two samples yielded NTM. One smear positive – culture negative sample was from a leprosy patient. The ProbeTec and Xpert detected *M. tuberculosis* in 33 (57%) and 50 (86%) of 58 smear negative – culture positive samples, respectively. All 370 smear negative – culture negative samples were also negative by both molecular tests. Xpert also detected rifampicin resistance in 13 of 14 rifampicin-resistant *M. tuberculosis* isolates.

**Conclusions:** Both, ProbTec and Xpert offer rapid and sensitive detection of *M. tuberculosis* in smear positive – culture positive pulmonary and extrapulmonary specimens and smear negative – culture positive pulmonary samples. However, the performance of Xpert was markedly better than ProbeTec for smear negative – culture positive extrapulmonary specimens. Rapid simultaneous detection of rifampicin resistance is another advantage offered by GeneXpert for proper patient care.

**P1917** Comparative analysis of various diagnostic techniques for tubercular lymphadenitis – a pilot study from a resource poor country

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**Objectives:** (i) To compare the various diagnostic methods available for diagnosis of tubercular lymphadenitis (the most common presentation of extra pulmonary tuberculosis) i.e. cytological and microbiological diagnostic tests namely the fine needle aspiration cytology (FNAC), microscopic smear examination & culture. Although FNAC & smear examination were rapid, but none of these techniques were able to differentiate between M.tb and other members of *Mycobacterium* spp., which is highly essential for planning anti-microbial therapy programme.

**Methodology:** In the present study, smear, FNAC, culture and hupB gene (Rv2986c) based PCR, were applied and each method was

analyzed in terms of sensitivity, specificity, along with reliability and cost effectiveness.

**Results:** Considering culture as a gold standard, all other diagnostic methods were compared. Direct PCR, showed the sensitivity & specificity of 47% and 75% whereas when performed on culture isolates, the sensitivity rose to 76%. The sensitivity & specificity of FNAC were 60% and 49% respectively whereas of direct smear examination was 50% and 70% respectively.

**Conclusion:** We conclude that smear and FNAC are rapid, cost effective, easily available, but has lower specificity and may not be able to differentiate tubercular lymphadenitis from non tubercular lymphadenitis. PCR(hupB gene based) being a singular target for M.tb showed reliability and potential to rapidly detect & identify causative agent of LNTB, can help clinician to initiate correct and timely treatment.

**P1918** Evaluation of the new HyBeacon-based PCR assay, FluoroType MTB, for the direct detection of *Mycobacterium tuberculosis* in respiratory and non-respiratory specimens

U. Eigner\*, A. Veldenzler, M. Weizenegger, M. Holfelder (Heidelberg, DE)

**Objectives:** The performance of the new FluoroType (FT) MTB assay (Hain Lifescience, Nehren, Germany) for the direct detection of *Mycobacterium tuberculosis* in respiratory and nonrespiratory specimens was evaluated. Results were compared to conventional liquid and solid culture media. In addition a subgroup of the specimens were also tested with the Cobas TaqMan (CT) MTB test (Roche, Mannheim, Germany).

**Methods:** In total 261 NALC-decontaminated specimens were investigated with the FT MTB assay. Microscopy was performed directly from the patient specimens. After decontamination 500 µL of the phosphate-buffered suspension were taken for inoculation of BACTEC MGIT medium and 100 µL each for solid media (Löwenstein-Jensen, Stonebrink). For DNA extraction 700 µL of the suspension was incubated at 70°C for 15 minute. DNA purification was performed fully automated on the GenoXtract system and the GXT DNA/RNA extraction kit (Hain Lifescience). Identification of cultured acid fast bacteria was performed with the GenoType MTBC and GenoType Mycobacteria CM/AS strip assays (Hain Lifescience). The new FT MTB test is based on HyBeacon fluorescence-technology and is performed on the FluoroCycler (Hain Lifescience). After PCR amplification melting curves are created with HyBeacon probes at probe specific temperatures. The FT MTB and the CT MTB assay were performed according to manufacturer's instructions.

**Results:** Eighty of 261 specimens were culture-positive for *M. tuberculosis*. FT MTB correctly identified 39 of 40 smear-positive, 30 of 31 smear-negative and nine of nine smear-scanty specimens. Two specimens (one abscess-swab and one sputum) were negative with the FT MTB assay. In 181 culture negative specimens, FT MTB showed 179 correct negative results. 82 specimens were utilized for CT MTB assay, 26 were culture-positive for *M. tuberculosis*. CT MTB correctly identified six of seven smear-positive, 25 of 26 smear-negative and seven of seven smear-scanty specimens. In 42 culture-negative specimens, CT MTB showed 42 correct negative results. Overall sensitivity, specificity, NPV and PPV were 97.5%, 98.8%, 98.9% and 97.5% with the FT MTB assay and 95.0%, 100%, 95.4% and 100% with the CT MTB-PCR respectively. Sensitivity in smear-negative specimens was 96.7% with the FT MTB test and 96.1% with the CT MTB test.

**Conclusion:** Both PCR-assays, the new FluoroType MTB and the Cobas TaqMan evaluated for the direct detection of *Mycobacterium tuberculosis* provide sensitive and specific results in about 3 hours.

**P1919** A novel single-tube PCR assay for rapid identification of MDR-TB

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**Objective:** To evaluate a highly-informative, rapid, single-tube PCR assay that can simultaneously detect and distinguish *Mycobacterium tuberculosis* complex species and identify mutations conferring isoniazid and rifampicin resistance.

**Methods:** PCR-ready sterile lysates were prepared by heating mycobacterial suspensions in the presence of chloroform. Amplification using LATE-PCR™ technology generated four abundant single-stranded products which were simultaneously analysed at endpoint by hybridization to Thermalight™ probes in a standard four-colour fluorescent thermocycler. The multiplexed assay amplified gyrB, to identify and discriminate members of the *M. tuberculosis* complex; the rifampicin resistance-determining region (RRDR) of rpoB; and katG plus the mabA promoter to determine isoniazid resistance. Inclusion of PrimeSafe™ eliminated mispriming.

**Results:** A panel of 42 rifampicin-resistant *M. tuberculosis* isolates harbouring different mutations within the rpoB RRDR, plus seven sensitive isolates with neutral mutations, was assembled. Each rpoB amplicon reliably generated a unique reproducible fluorescent signature that was easily distinguished from wild-type. In two further colour channels, three polymorphisms in katG codon 315 and mabA promoter mutations at positions -15 and -8 also gave fluorescent signatures distinct from wild-type. GyrB was amplified from all *M. tuberculosis* complex isolates but none of the 20 species of non-tuberculous mycobacteria tested. The gyrB amplicon generated unique fluorescent signatures for *M. tuberculosis*, *M. africanum*, and *M. bovis* in the fourth colour channel.

**Conclusion:** The novel LATE-PCR/Thermalight assay is highly specific for the *M. tuberculosis* complex and reliably predicts rifampicin and isoniazid resistance.

**P1920** Discordant QuantiFERON®-TB Gold In-Tube and tuberculin skin test results in various high-risk groups

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**Objective:** To retrospectively evaluate QuantiFERON®-TB Gold In-Tube (QFT-GIT) for the diagnosis of latent tuberculosis infection (LTBI) comparing with tuberculin skin test (TST) in a low endemicity tuberculosis area.

**Methods:** To determine QFT-GIT, 1751 whole blood samples were collected from 778 men (44.4%) and 973 women (55.6%), mean age 45.7 ± 17.7 years (range, 1–94), belonging to following categories: (i) Patients with active microbiology tuberculosis (TB) (2% of total); (ii) Close contacts (11.4%); (iii) Patients with suspicious clinical and/or chest X ray (15.2%); (iv) Human immunodeficiency virus (VIH) patients or those receiving immunosuppressive therapy (49.3%); (v) Healthcare workers (22%). QFT-GIT was performed according to the manufacturer specifications. TST was performed in 458 patients and positive PPD was defined as indurations larger than 5 mm. Agreement between QFT-GIT and TST was assessed by the kappa index in the same samples (N = 458).

**Results:** Globally, QFT positive results were 141/458 (30.8%) cases, being 75%, 45.2%, 41.4%, 29.3% and 18.2% by category (A, B, C, D and E, respectively). TST were positive in 151/458 (32.7%), being 50% (A), 34.4% (B), 41.4% (C), 25.7% (D) and 38% (E). In a logistic regression model, the following variables were independently associated with a QFT-GIT positive result: Age ≥51 years (OR 2.0, 95%CI 1.2–3.4, p = 0.001), Group A (OR 22.7, 95%CI 3.4–150.0, p = 0.001), Group B (OR 6.6, 95%CI 3.0–14.1, p < 0.001), Group C (OR 3.0, 95%CI 1.1–8.9, p = 0.037), Group D (OR 3.0, 95%CI 1.5–6.0, p = 0.002) and TST positive result (OR 11.0, 95%CI 6.4–19.0, p < 0.001).

Table 1 shows the concordance by categories.

Category	N	Q+ T-	Q- T+	kappa	IC 95%	Sig
Global	460	49 (10.7%)	56 (12.2%)	0.47	0.39 – 0.56	<0.001
▪ A	8	2 (25.0%)	0 (0.0%)	0.50	-0.02 – 1.00	NS
▪ B	96	20 (22.0%)	9 (9.9%)	0.35	0.16 – 0.53	0.001
▪ C	29	1 (3.4%)	1 (3.4%)	0.86	0.67 – 1.00	<0.001
▪ D	191	21 (11.0%)	14 (7.3%)	0.54	0.41 – 0.67	<0.001
▪ E	137	5 (3.6%)	32 (23.4%)	0.36	0.21 – 0.51	<0.001

Table 1

**Conclusions:** QFT-GIT is a highly useful method for diagnosing TB in contrast to TST. With our data, the higher agreement between methods was observed in clinical or X-chest tuberculosis suspicious patients, being moderate in HIV-infected persons and those receiving immunosuppressive therapy. In contrast, a poor agreement was observed in health workers. Thus, QFT-GIT reduces over diagnosis of LTBI avoiding unnecessary chemoprophylaxis.

**P1921** Tuberculosis infection in young children: a screening based on skin and blood testing

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**Objectives:** Progression from latent tuberculosis infection (LTBI) to tuberculosis (TB) is higher in children younger than 5 years because of anergy of their immune system. Accurate diagnosis of LTBI is a desirable goal in achieving effective TB control particularly in young children, because the preventive chemotherapy is effective and relatively safe. Aim of this study was to evaluate the “performance” of a commercially available Interferon-gamma (IFN-gamma) assay (QuantiFERON-TB Gold In-Tube [QFT-IT]; Cellestis Ltd., Victoria, Australia) in this vulnerable population.

**Methods:** In a retrospective study, QFT-IT test was performed in 145 young children ≤5 years of age, recent close contact of active pulmonary TB during the period April 2007–December 2010. Tuberculin skin test (TST; 5UI Biocine Test PPD; Chiron, Siena) was available only in 112 (77.2%) children.

**Results:** In our pediatric population: mean age was 2.5 ± 1.5 years, 74 (51%) subjects were born in Italy from immigrant families, nine (6.2%) were BCG-vaccinated. QFT-IT was positive in 24 (16.6%) children, negative in 112 (77.2%) and indeterminate in nine (6.2%). TST was positive (cut-off >10 mm) in 28 (25%) of 112 evaluated patients. By statistical analysis, TST and QFT-IT showed a fair agreement: 82.9%, k = 0.52. Among the discordant results (n = 19): 12 (63.2%) children were TST-positive/QFT-IT-negative, of them seven (58.3%) were BCG-vaccinated. Three (42.9%) of seven patients TST-negative/QFT-IT-positive had TB and four (57.1%) had LTBI.

Among 16 children TST-positive/QFT-IT-positive, 10 had clinical diagnosis of active TB and six of LTBI. In this group the analysis of IFN-gamma level as continuous variable revealed: 7/10 (70%) children with active pulmonary TB showed high level of IFN-gamma (>10 UI/mL) and a TST size ranging from 10 to 15 mm; 3/6 (50%) children with a diagnosed LTBI had high IFN-gamma level >10 UI/mL and a TST size of 11–20 mm.

**Conclusion:** These preliminary data suggest and support the potential advantages of using blood test, e.g. QFT-IT, in young children ≤5 years for diagnosing LTBI.

**P1922** Antimicrobial susceptibility pattern of clinically isolated rapidly growing mycobacteria by broth microdilution

B. Buabut\*, P. Hongmanee, P. Santanirand (Bangkok, TH)

Follow the increase incidence of the rapidly growing mycobacteria (RGM) infection in Thailand, requirement of antimicrobial susceptibility test for clinical isolates RGM has become larger. In order to

retrieve information of the susceptibility patterns as well as the trend of antimicrobial resistance among RGM isolated in the country, 50 clinical isolates of non-duplicated RGM were tested. The antimicrobial susceptibility pattern of 36 and 24 clinical isolates of *M. abscessus* and *M. fortuitum*, respectively, were tested against 15 antibiotics by microbroth dilution method using Sensititre RAPMYCO plates (Trek Diagnostic Systems Limited, UK). The endpoints were read after 72 hour of incubation. Both *M. abscessus* and *M. fortuitum* appeared resistant to various antibiotics including all beta-lactam drugs (amoxicillin/clavulanic acid, cefoxitin, ceftriaxone, cefipime, imipenem and doripenem), aminoglycosides, trimethoprim-sulfamethoxazole, clarithromycin, linezolid, and minocycline. However, the MIC90 of tigecycline of both species remained low at 2 µg/mL. These two organisms showed similar resistant pattern in nearly all tested antibiotics except fluoroquinolones. The MIC90 of ciprofloxacin and moxifloxacin of *M. fortuitum* were 1 and 0.5 µg/mL while *M. abscessus* were >4 and >8, respectively. Regarding to the above results, there are very limited antibiotics which could be used against RGM infection. Nevertheless, further clinical studies are required to determine how well in vitro results correlated with therapeutic in vivo outcome.

## Tuberculosis; clinical and molecular epidemiology and treatment

### P1923 Factors associated with diabetes mellitus among tuberculosis patients in a Western European city

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**Objectives:** According to WHO, diabetes mellitus (DM) increases by three-fold the risk of a person developing tuberculosis (TB) and could hinder TB control worldwide. Countries with the highest TB burden and mortality in the world like, China and India, are experiencing the fastest increase in the prevalence of DM. The increasing DM epidemic could be influencing TB incidences in some settings, especially in big cities. The objectives were to study the evolution of DM prevalence and identify factors associated with DM among TB patients in Barcelona.

**Methods:** A population-based retrospective study was performed in Barcelona, Spain. Patients with TB notified between 2000 and 2010 were studied by the TB Control Program. Previous diagnostic of DM or two fasting glucose measurements above 126 mg/dL (7.0 mM) were considered DM. Socio-demographic, clinical and treatment variables were examined. Prevalence of DM among TB patients was calculated. Differences between DM and non DM on bivariate and multivariate levels were analysed using a logistic regression model and odds ratio (OR) with a 95% confidence interval (CI).

**Results:** Among the 5146 patients, 287 (5.6%) were cases with DM. Among the patients with DM, median age was 63 years (interquartile

range: 51–74), 198 (69%) were male, 238 (81.5%) foreign born, 21 (7.3%) homeless, seven (2.4%) HIV-infected, 13 (4.5%) injecting drug users (IDU), 69 (24%) alcohol abusers, 211 (73.56%) pulmonary or mixed TB, 33 (11.5%) had TB treatment side effects, and 267 (93%) had TB symptoms. DM prevalence among TB patients was 5.8% in year 2000 and 5.6% in 2010 (Fig. 1). In the multivariate analysis the factors associated with DM were: age >39 years (OR: 6.5; CI:4.5–9.4), Spanish born (OR: 1.6; CI:1.2–2.2), IDU (OR: 3; CI:1.4–6.3), TB treatment side effects (OR: 1.9; CI:1.3–2.8), and TB symptoms (OR: 1.7; CI:1.1–2.7). TB HIV-infected patients had less risk of DM (OR: 0.1; CI:0.04–0.26).

**Conclusion:** The prevalence of DM among TB patients in Barcelona remained stable in the last 10 years. DM was associated with age greater than 39 years, being Spanish born and IDU. Diabetics had more side effects and TB symptoms compared to non DM patients, and were less HIV-infected. Following the clinical evolution and the treatment adherence could improve the outcomes and TB control in this population. Doctors and health providers must be aware and increase the level of suspicion and screening when seeing a patient with either diabetes or TB.

### P1924 An epidemiological, clinical and diagnostic study of female genital tuberculosis

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**Objective:** This study was done to study epidemiological, clinical and diagnostic parameters of female genital tuberculosis. The findings of the diagnostic modalities were compared to evaluate their efficacies.

**Methods:** Thirty clinically suspected cases of female genital tuberculosis were included in this study. Clinical history was taken. Laparoscopy was done in each of the patient. Endometrial biopsy (EB) was collected in each patient and sent to Microbiology and Pathology laboratory. Direct microscopy, culture on LJ medium and BACTEC were done for tuberculosis. PCR was done using two primers MPB64 and HupB for *Mycobacterium tuberculosis*. Histopathology was done in each sample to look out for granuloma and other features of chronic inflammation.

**Results:** Majority of patients were in age group 30–34 years (36.7%). The mean age was 28 years. Large number of patients were of low educational status (86.6%). Forty percent patients gave past history of active pulmonary tuberculosis. Only 10% had been immunized with BCG. Fifty-seven percent patients were reactive for tuberculin test. Most common symptom was menstrual problems (56%). Seven (23.3%) samples were positive by PCR using MPB64 and Hup B primers. Positivity in other diagnostic modalities were one (3.3%), two (6.7%) and four (13.3%) for direct microscopy, culture on LJ medium and BACTEC medium respectively. None of the samples were positive on either LJ medium or BACTEC culture and negative by PCR. Three out of three cases (100%) showing suggestive findings of tuberculosis in laparoscopy were positive by PCR. Five out of five cases (100%) showing granuloma in histopathology were positive by PCR.

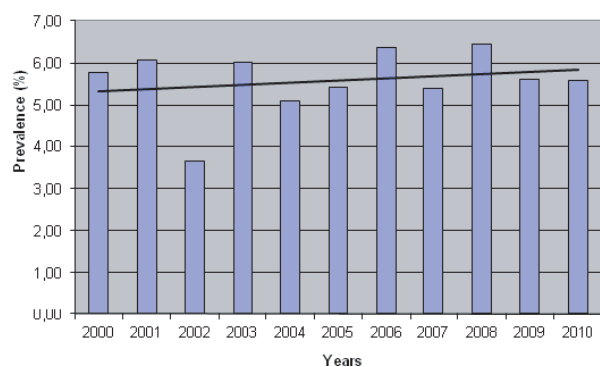
**Conclusions:** Female genital tuberculosis is a common problem especially in reproductive age group. Most lesions are secondary. Strong clinical suspicion is required for proper diagnosis. Polymerase Chain Reaction (PCR) show higher sensitivity and specificity than conventional techniques and show good correlation with laparoscopic findings in diagnosis of female genital tuberculosis.

### P1925 Tuberculosis in native residents and immigrants in an area of northern Italy: a 10-year survey

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**Objectives:** Tuberculosis (TB) has re-emerged as a public health concern in high-income countries in the last few decades and the

Diabetes mellitus prevalence among tuberculosis patients in Barcelona 2000-2010



immigration produced significant changes on the epidemiology of tuberculosis in our region. We sought to identify the main disease features and epidemiological findings in foreign-born individuals who were diagnosed with tuberculosis in our institution in the last decade.

**Methods:** Consecutive cases of TB admitted from January 2000 through December 2010 were reviewed and epidemiological, clinical and microbiological data of immigrant patients were compared with those of native residents.

**Results:** During the study period 236 consecutive patients with TB were recorded: Italians were 104 (44%) and immigrants were 132 (56%). The incidence of TB cases among foreign-born rose sharply in the period 2006–2010 if compared with the period 2000–2005 (72.1% vs. 36.2%;  $p < 0.0001$ ). Among immigrants the median time from arrival in Italy to TB diagnosis was 36 months (interquartile range, IQR: 10–96). Immigrants were significantly younger (mean age 30.5 vs. 62.2;  $p < 0.0001$ ) and were more commonly affected by social risk factors such as low-income and homelessness (25% vs. 2%;  $p < 0.0001$ ), whereas italians were more frequently affected by concomitant chronic illnesses (17.4% vs. 8%). Sputum-positive pulmonary TB on direct microscopy were more frequently detected among immigrants than in native residents (81.1% vs. 64.4%;  $p < 0.0001$ ) as well as radiological signs of cavitory lesions (54.5% vs. 33.8%;  $p = 0.0004$ ). Mean delay from onset of symptoms until initiation of TB treatment was independent of ethnicity (2.4 vs. 2.3 months;  $p = 0.7769$ ) but the number of patients lost to follow-up during outpatient visits was higher among foreigners (22.6% vs. 1.7%;  $p = 0.0002$ ). Resistance to any drug was detected in 39 out of 159 *Mycobacterium tuberculosis* isolates (25.7%) and multidrug-resistant *M. tuberculosis* isolates were 3.9%. The distribution of any resistances did not show difference between local and immigrants patients (29.2% vs. 19.6%;  $p = 0.1384$ ).

**Conclusions:** In our survey immigrants with TB diagnosis are represented by otherwise healthy younger people, they develop more commonly smear-positive pulmonary TB and interruption of treatment is more frequent among this group. Updated local surveillance of population specific risk factors in an immigrant society is mandatory in order to optimize preventive strategies of screening, diagnosis and compliance to drug treatment.

#### **P1926** Molecular epidemiology and prevalence of mutations in *Mycobacterium tuberculosis* strains from an Italian northeastern area

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**Objectives:** Evaluate multiplex allele-specific polymerase chain reaction (MAS-PCR) to detect mutations associated with rifampicin (RIF), isoniazid (INH) and ethambutol (EMB) resistance.

**Methods:** We set up a MAS-PCR targeting codon 315 of the katG gene, the mabA-inhA promoter region (–15), codons 516, 526, and 531 of the rpoB gene and codon 306 of the embB gene. Results obtained with MAS-PCR were confirmed by DNA sequencing.

**Results:** We tested 89 resistant isolates (based on phenotypic drug susceptibility test [DST]); for 75% (64/89) of them results were concordant with those obtained by DST. Discordances were confirmed using MTBDRplus, suggesting that in discordant strains resistance was due to rare or still unknown mutations. Mutated alleles were then sequenced and the frequency of the various mutations in our sample was determined. We detected two isolates with rare mutations (a TTC insertion in rpoB, and a mutation at –17 bp in the mabA-inhA promoter region) and two isolates with not documented mutations (a triple mutation in rpoB [codons 516,522, and 541] and a single point mutation at +11 bp in the inhA gene). Finally, among the EMB resistant isolates, we found eight cases that shown a point mutation at codon 306.

**Conclusions:** The MAS-PCR method is more accessible, poor expensive for resource-limited countries and efficient in terms of the

ability to detect the commonly seen genetic mutations in INH and RIF resistance. A major limitation of molecular genetic detection of drug resistance by MAS-PCR is that the test detects only known mutations. As not all mutation conferring resistance to anti-TB drug are known, and prevalence of mutations may change through geographic area, the aim of future studies will be to gain a more complete understanding of the genetic mechanisms of *Mycobacterium tuberculosis* (MTB) drug resistance with the aim of incorporating additional targets in the molecular tests improving sensitivity and specificity of molecular detection.

#### **P1927** Incidence and characteristics of tuberculosis in the elderly

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**Objectives:** To research the incidence and characteristics of tuberculosis (TB) in older patients.

**Methods:** An active search of all TB cases diagnosed in our sanitary area from 1991 to 2010 was performed. In 1991: population 218 749 inhabitants (16.3%  $\geq 65$  year), 0.29% immigrants; in 2010: 204 063 (23.5%  $\geq 65$  year), 2.6% immigrants. Of all TB cases, we performed an analysis in HIV negative patients followed prospectively up in TB unit, comparing the characteristics of  $\geq 65$  year group with those of  $< 65$  year. Data were gathered for each patient: socio-demographic and clinical characteristics, drug resistance, TB site, diagnostic delay, treatment adherence, tolerance and outcome.

**Results:** Out of 2014 TB cases; 304 (15.1%)  $\geq 65$  year (all HIV negative). TB incidence rate remained stable in elderly and decreased in  $< 65$  year group. In 1991: 10 cases  $\geq 65$  year, incidence 28.1/100 000; 138 cases  $< 65$  year, incidence 75.3; in 2010: 33 cases  $\geq 65$  year, incidence 27.1; 13  $< 65$  year, incidence 21.1. Of the 2014 cases, 1255 HIV negative patients were prospectively followed, 205  $\geq 65$  year old. Older patients group included more frequently than that of  $< 65$  year: females (49.8%/41.7%), in-hospital management (80%/68.9%), diabetes mellitus (16.1%/3.4%), neoplasia (5.4%/1%), immunosuppression (9.3%/1.6%) and chronic renal failure (5.4%/0.3%); and less frequently alcoholism (9.3%/15.5%), smoking habit (12.7%/44.2%), injection drug users (0%/1.8%), BCG vaccination (0.5%/17.6%) and contact with TB (19.3%/32.8%). The elderly showed higher diagnostic delay, more frequent ganglionic (27.8%/10.1%), osteoarticular (8.3%/2%) and miliary TB (5.4%/2.7%), and lower frequency of pulmonary (32.7%/58.1%, bacilliferous 16.6%/33.6%), pleural TB sites (10.7%/18.9%), fever (24.2%/38.2%) and positive PPD (72.9%/85.8%). There was not difference in the global frequency of adverse effects (36.4%/32.7%), but elderly more often received simultaneously other drugs (54.1%/15.1%), developed severe hepatotoxicity (10.8%/6.5%), digestive intolerance (17.6%/10.6%), and needed to change therapy (8.3%/3.7%). Supervised treatment (13.8%/4.9%) and TB mortality were more frequent in older group (5.8%/0.7%) and there were no differences between isoniazid resistance (0%/2.6%), treatment default (0.9%/2.2%) and relapses (0.5%/1.4%).

**Conclusions:** TB incidence rate in elderly has not decreased and its proportion increased. The elderly have a higher frequency of extrapulmonary TB, diagnostic delay, severe hepatotoxicity and TB mortality than youngest ones.

#### **P1928** Epidemiology and clinical significance of non-tuberculous mycobacterial isolates

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**Objectives:** Non-tuberculous mycobacteria (NTM) are considered emerging pathogens implicated in lung, lymphnode, skin/soft tissue or disseminated infection. This retrospective study assessed the microbiological characteristics, clinical relevance and risk factors of NTM isolates recovered from patients in Crete, Greece over the decade 1/2001–12/2010.

**Materials and methods:** Clinical specimens were processed by standard methodology and inoculated into L-J slants, and BacT/Alert 3D (bioMérieux, Durham, NC). NTM were identified with Genotype Mycobacterium CM and AS (Hain-Lifescience), while 16S rRNA and hsp65 gene sequencing were applied when necessary. Established bacteriological criteria for NTM lung disease by the American Thoracic Society (ATS) were used to determine the clinical relevance of pulmonary isolates.

**Results:** During the study period, 291 NTM isolates were recovered from 202 patients, which belonged to 24 species. For 51 patients (mean age 44, range 2–84 years), the recovered NTM considered as clinically significant. Men were significantly older than women (mean age 52 vs. 37 years,  $p = 0.02$ ). Risk factors in patients with NTM were underlying lung disease, mainly chronic obstructive pulmonary disease and asthma, smoking rheumatoid arthritis, AIDS, alcohol or drug abuse, and malignancies. Only 15/51 (29%) of the patients with NTM disease had positive smears for acid-fast bacilli. Among patients with pulmonary disease, the following NTM were recovered: *M. avium* complex (MAC) (n = 15), *M. kansasii* (n = 10), *M. abscessus* (n = 4), *M. parascrofulaceum* (n = 2), *M. fortuitum* (n = 1), *M. chelonae* (n = 2), *M. gordonae* (n = 2), *M. peregrinum* (n = 1), and *M. xenopi* (n = 1). Four patients suffered from skin and soft tissue infection, two AIDS patients from disseminated disease and six girls (mean age 4 years) from cervical lymphadenitis. The recovered NTM were *M. avium* (n = 5) and *M. marseillense* (n = 1). For the remaining 151 patients the most prevalent recovered NTM were *M. gordonae* (49/151, 32.5%) and *M. lentiflavum* (41/151, 27.2%) probably representing contamination.

**Conclusions:** Among the 24 NTM species recovered, almost only half of them 11/24, (46%), were linked to human disease. The most common clinically significant isolates are MAC and *M. kansasii* responsible for 70% of NTM disease. Only a minority of patients with pulmonary NTM isolates met the ATS criteria, mainly because of inadequate sampling of a large number of individuals.

#### P1929 Description and molecular epidemiology of 100 tuberculosis cases

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**Objectives:** To investigate for the first time, the molecular epidemiology of tuberculosis in Greece by a hospital-based study.

**Methods:** Demographic and laboratory characteristics of 100 consecutive tuberculosis patients, who were diagnosed in the Laboratory of Clinical Microbiology (2007–2011) of “Attikon” Hospital in Athens, were recorded. The strains were genotyped using the Mycobacterial Interspersed Repetitive Units-Variable Number of Tandem Repeats (MIRU-VNTR) method by analyzing 24 polymorphic loci.

**Results:** The majority of the patients (86%) originated from Attica. Most patients were Greek (72%) with a higher mean age (62.6 years, range 1–97) compared to the foreigners (28.8 years, range 0.5–42). However, 38% of Greeks were aged <60 years, and five patients were children (three Albanians, two Greeks). Isolates were identified as *Mycobacterium bovis* (1 strain), *Mycobacterium bovis* BCG (1) and *Mycobacterium tuberculosis* (98). Resistance rates were isoniazid 6%, rifampin 1%, streptomycin 5%, ethambutol 1% and pyrazinamide 3%; only one strain was multidrug-resistant. MIRU-VNTR classified 55 isolates in 10 *Mycobacterium tuberculosis* families (most frequently Haarlem [18 strains], S [12], Delhi/CAS [9] and Beijing [8]), whereas the remaining 43 strains did not belong to a known family. The distribution of Greeks in these two subgroups was 45% and 55% respectively, whereas the distribution of the foreigners was 81% and 19% respectively. The genotypic analysis confirmed two suspected cases of intrafamilial transmission (six patients in total, of which four were children). The remaining strains showed a unique genotype.

**Conclusion:** Tuberculosis is still more frequently diagnosed in Greeks, and transmission within the community seems to continue. Commonly encountered *M. tuberculosis* families were identified, but Greek patients may be infected by unclassified strains, possibly “domestic” strains.

#### P1930 First detection of Beijing and Beijing-like *M. tuberculosis* genotypes among MDR strains from Bulgaria

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In 1995, IS6110 DNA fingerprinting identified in *Mycobacterium tuberculosis* isolates from China, a genetically closely related group of bacteria – the Beijing genotype family. Strains of this genotype family dominate in Southeast Asia and are globally spread. This genotype is often associated with multi-drug resistance among *M. tuberculosis* isolates. Beijing genotype was not identified in Bulgaria during our previous nation-wide study performed in 2005. This is the first nation-wide study for the genotyping of MDR *M. tuberculosis* strains.

**Objectives:** To identify possible presence and spread of Beijing genotype among isolates of *M. tuberculosis* in Bulgaria.

**Methods:** Spoligo- and 24 MIRU-VNTR reference typing methods were applied for typing of *M. tuberculosis* sensitive and multi-drug (MDR) resistant strains collected during the last 7 years. One hundred and ninety-five MDR *M. tuberculosis* strains were isolated and typed during 2008–2011.

**Results:** For 2009 and 2010 and till November 2011, 100% of the MDR strains identified across the country are included in this study. Moreover results from our previous study performed in 2005 did not identified presence of *M. tuberculosis* Beijing genotype in Bulgaria. We identified three strains of Beijing genotype with spoligotype pattern 000 000 000 003 771. Two strains with Beijing-like pattern 000000000003371 (ST265) were identified.

**Conclusions:** In our previous study performed in 2005 among 225 drug sensitive and drug resistant *M. tuberculosis* strains collected from all over the country we did not identified presence of the Beijing genotype in Bulgaria. In this study Beijing genotype was identified among MDR strains of *M. tuberculosis*. For one strain we have evidences that it is imported from Moldova. The patient is Moldovan of Bulgarian origins. The origins (epidemiological link) of the other two strains are not clear. One of them is of Armenian origins where the Beijing genotype is widely spread. The patient is living since many years in Bulgaria and denied visiting Armenia. There is no epidemiological link among the cases with the Beijing *M. tuberculosis* genotype. The two patients with Beijing-like strains have epidemiological link. Both patients are apartment neighbors. The importation origin of the genotype was not identified. We suppose recent introduction of the Beijing genotype in Bulgaria. The road of introduction of this genotype in the country was not identified.

#### P1931 Primary resistance to anti-tuberculosis drugs in Valladolid, Spain over 30 years

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**Objective:** The global control of tuberculosis remains a challenge from the standpoint of diagnosis, detection of drug resistance and treatment. Surveillance in anti-tuberculosis drug resistance to *M. tuberculosis* (Mt) isolates in newly treated patients from a tertiary general hospital serving 240.000 inhabitants.

**Method:** Prospective analysis included all drug susceptibility test (DST) performed on 1097 initial isolates of Mt between 1981 and December 2010, from newly treated human immunodeficiency virus (HIV) negative patients, devised in five periods. DST was conducted by a National Reference Laboratory (Dra. Marisol Jiménez, Instituto de Salud Carlos III, Madrid, Spain) using the standardised proportion method, according to the guidelines of the external quality assurance programme. Resistance to streptomycin (SM), isoniazid (INH),

rifampicin (RMP) or ethambutol (EMB) was evaluated. Primary drug resistance (PDR) was defined as resistance to strains of Mt in patients without a history or other evidence of previous treatment. Mono-resistance was defined as resistance to only one drug. Multi-drug resistance (MDR) was defined as resistance to at least isoniazid and rifampicin.

**Results:** Resistance to first-line drugs in *Mycobacterium tuberculosis* are showing in Table 1.

	1981-1990 (N=301)	1991-1995 (N=338)	1996-2000 (N=185)	2001-2005 (N=170)	2006-2010 (N=103)
Primary drug resistance	6.3	4.7	3.24	1.76	6.8
Mono-resistance	5	3.8	2.7	1.2	5.8
> 2 drugs resistance	1.3	0.9	0.54	0.5	1
Multi-drug resistance	0	0	0	0	0
SM- resistance	3	2.3	1	1.2	1.9
INH- resistance	4.3	1.8	2.1	1.2	5.8
RMP- resistance	0.3	0	0	0	0
EMB- resistance	0	1.5	0.5	0	0
PYR- resistance					4

Table 1. Resistance to first-line drugs in *Mycobacterium tuberculosis* (%)

**Conclusion:** Periodic information about susceptibility patterns of Mt isolates against antituberculosis drugs is an important aspect of tuberculosis control. In the last 5 years resistance to first line anti TB drugs shows an ascending trend, comparing to former results of ours previous studies, being INH the less active drug. It is necessary study the epidemiological impact of immigrant people in this new field.

#### P1932 Occurrence of eis gene mutations among extensively drug-resistant tuberculosis strains

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**Objectives:** The emergence of resistance to second-line drugs severely limits the treatment options for multidrug-resistant tuberculosis (MDR-TB) cases and may ultimately lead to extensive drug-resistant TB (XDR-TB). Injectable second-line drugs have a fundamental role in MDR-TB treatment regimens. However, the molecular basis of resistance is not widely known, in particular, the molecular basis of cross-resistance between kanamycin and amikacin. Recent studies associate the overexpression of an aminoglycoside acetyltransferase encoding gene (eis) to kanamycin resistance but not with amikacin resistance.

**Methods:** In the present study we have studied 21 XDR-TB isolates previously characterized by mutational analysis of genes inhA, rpoB, gyrA, rrs and tlyA. All isolates have also been genotyped by 12-loci MIRU-VNTR. The promoter region of eis gene was amplified in all isolates and characterized by sequencing analysis.

**Results:** All MDR-TB isolates analyzed belonged to Lisboa3 and Q1 clusters, two highly prevalent clusters responsible for the great majority of MDR-TB cases in Lisbon Health Region. Thirteen of the 21 isolates presented mutation G-10A in eis gene. All isolates bearing the eis gene mutation belonged to Lisboa3 cluster and had tlyA mutations. None of the eight eis wild-type isolates had tlyA mutations, including two Lisboa3 isolates. Moreover, among the 13 eis mutant isolates, four were resistant to both kanamycin and amikacin, seven were resistant to kanamycin only, and two were resistant to neither of these two second-line antibiatic drugs.

**Conclusions:** Our data suggests that acquisition of eis promoter mutations occurred in an ancestral Lisboa3 isolate as a resistance mechanism against second-line aminoglycosides, which underwent later clonal expansion in the community. The acquisition of tlyA mutations and subsequent capreomycin-resistance development would have occurred at a later stage. Regarding cross-resistance between amikacin and kanamycin, our results suggest that the recommended amikacin critical concentration used in the drug susceptibility testing may not always distinguish between amikacin low-level resistance and susceptibility, hampering the association between molecular and phenotypic data.

#### P1933 MIRU-VNTR genotyping of *Mycobacterium tuberculosis* MDR strains in Tunisia

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**Introduction:** The emergence of multidrug resistance TB (MDR-TB) is one of the greatest threats to tuberculosis control. Tunisia is considered as a middle-income country and has an estimated incidence of MDR-TB <5%. Several genotyping methods are used worldwide to complete epidemiological studies. The Mycobacterial interspersed repetitive units-variable number of tandem repeats (MIRU-VNTR) genotyping method is adapted for routine laboratories.

**Objective:** The aim of this study is genotyping 23 MDR *Mycobacterium tuberculosis* strains.

**Materials and methods:** Twenty-three MDR *Mycobacterium tuberculosis* strains, isolated between 2008 and 2011 at the Laboratory of Microbiology of the Pneumology Hospital of Tunis, were genotyped using the 15 MIRU-VNTR set. PCR products were analyzed by gel electrophoresis. The allelic diversity (h) was calculated.

**Results:** Molecular evidence demonstrated that there is no active transmission of MDR-TB. Only two patients without epidemiological links were clustered. MDR-TB could be a consequence of treatment failure.

Allelic diversity allowed us to determine a minimum panel of seven loci. Mtub4, ETR-C, Qub11b, Mtub30, Qub26, Qub4156, Miru31 are distinctive enough for initial epidemiological studies. This minimum panel could save time and minimize expenses when used as a first line genotyping set.

These genotypes are particularly useful for both intra and inter-laboratory comparison studies.

**Conclusion:** MIRU-VNTR genotyping has the advantage of being fast and suitable for *M. tuberculosis* strains. It could be considered as an appropriate tool for genotyping in real time which could improve MDR-TB control.

#### P1934 Mutations in the rpoB gene of rifampin-resistant *Mycobacterium tuberculosis* clinical isolates from Sichuan, China

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**Objectives:** Rifampin is a key component of standard short-course first-line therapy against *M. tuberculosis*, and the resistance to rifampin has been reported to connect to the mutations of the rpoB gene. The objective of this study was to detect the rpoB gene mutations in rifampin-resistant *M. tuberculosis* isolates circulated in Sichuan, as well as the possible correlation between molecular typing of TB population and rpoB mutation patterns that confer rifampin resistance in *M. tuberculosis*.

**Methods:** Antimicrobial drug susceptibility test of *M. tuberculosis* isolates from 3645 pulmonary tuberculosis patients from different regions of Sichuan province from January 2008 to May 2010 was carried out by standard conventional proportion method. Spoligotyping and sequencing of the rpoB gene were performed for 268 randomly selected isolates, including 213 rifampin-resistant and 55 rifampin-susceptible isolates.

**Results:** Mutations in the rpoB gene were detected in 207 (97.2%) of 213 rifampin-resistant isolates and one (1.8%) of 55 rifampin-susceptible isolates, respectively. The most common mutations were in codon 531 (55.9%), 526 (16.4%), 516 (10.3%), together accounting for 82.6% of rifampin-resistant isolates. New mutations were also identified in codon 144 (Val->Gly), 149 (Leu->Pro), 401 (Val->Phe) and 616 (Ser->Leu) of rpoB gene in rifampin-resistant isolates. Among the 213 rifampin-resistant isolates, 150 (70.4%) belonged to the Beijing family and mutation at codon 531 (TCG->TTG) was associated with Beijing genotype (Fisher exact test, p = 0.0025).

**Conclusion:** The new mutations identified in this study illustrate the need to disclose the unknown rifampin-resistance-associated mutations in *M. tuberculosis* isolates for an improved identification of rifampin-resistant isolates. There appeared to be a correlation between Beijing genotype and mutation at codon 531 (TCG->TTG) in rifampin-resistant isolates from Sichuan.

**P1935** **Bacteriological and molecular study of multi-drug resistant *Mycobacterium tuberculosis* in Egypt**

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Tuberculous meningitis (TBM) is the most devastating manifestation of tuberculosis (TB).

**Objective:** Of the study was to describe the characteristics of TBM in HIV negative adults focusing on cases with severe presentation.

**Methods:** This was a retrospective study of TBM cases observed in our department during the last 20 years in HIV negative adults. TBM was defined by clinical signs of meningitis and characteristic cerebrospinal fluid (CSF) findings coupled with microbiological, radiographic or epidemiologic evidences. Patients presentation was defined according to Medical Research Council (MRC) staging. Cure was defined by the absence of sequelae assessed 12 months after the end of therapy. Quantitative data are expressed as median and interquartile range (IQR). Statistical analysis was performed by two-tailed Chi square test, Fisher's exact test, and Mann-Whitney *U*-test, as appropriate.

**Results:** Ninety-one cases were included (median [IQR] age 38 [24–52] years, 57% males). Risk factors were reported in 53 (58%) cases: immigration from areas with high incidence of TB in 21 (23%) cases, history of TB in 20 (22%), immunodepression in 14 (15%), close contact with active TB case in six (7%). Chest X-rays were suggestive of TB in 42 (46%) cases. Median (IQR) duration of general and neurological symptoms prior to admission was, respectively, 15 (7–30) days and 2 (1–5) days. Severe disease presentation (MRC stage III) was reported in 34 (37%) cases. Low CSF cells (median (IQR) cells/ $\mu$ L 135 [90–185] vs 190 [130–400],  $p = 0.04$ ) and longer duration of neurologic symptoms prior to diagnosis (median hours [IQR] 76 [50–196] vs 44 [24–70],  $p = 0.03$ ) were associated with severe disease. No difference in TBM presentation was reported in the other epidemiologic, laboratory, and clinical variables evaluated. Forty (44%) cases reported an unfavourable outcome. Outcome was unfavourable mainly in the cases presenting in MRC stage 3 [26/34 vs 14/57,  $p < 0.0001$ , RR 3.11, 95% CI 1.9–5.1]. Mortality was 4% for cases with less severe presentation and 38% for cases presenting in MRC stage 3.

**Discussion:** In our area, about 40% of TBM cases did not report risk factors for TB suggesting that a high level of circulation of *Mycobacterium tuberculosis* outside target adult population makes difficult appropriate prevention strategies. Long duration of neurological symptoms prior to diagnosis and lower CSF cells are associated to severe TBM presentation and, consequently, to poor outcome.

**P1936** **Eight years of persistence of a *Mycobacterium fortuitum* outbreak strain**

W. Schiavo, J.L.M. Sampaio\* (Sao Paulo, BR)

**Background:** *Mycobacterium fortuitum* is a rapidly growing mycobacteria that causes infections following trauma or surgical procedures. In Brazil *M. fortuitum* is the most frequent species causing surgical site infection following mammary prosthetic surgery. We have previously reported the occurrence of an outbreak at the city of Campinas, São Paulo, Brazil that occurred in 2003 and 2004. Since recently we had an increase in the number of cases of surgical site infection caused by *M. fortuitum*, we aimed at evaluating the clonal relationship among these isolates.

**Methods:** A total of 76 *M. fortuitum* isolates cultivated from patients with surgical site infection following augmentation mammaplasty were analyzed. Identification to the species level was achieved by partial sequencing of the rpoB gene as previously described by Adékambi *et al.* PFGE profiles were obtained as previously described by Sampaio *et al.* with minor modifications and were analyzed using the Bionumerics software with 1% optimization and tolerance. In brief isolates were cultivated on sheep blood agar and checked for purity before they were cultivated in Mueller-Hinton broth with 0.1% Tween 80 at 30°C in an orbital shaker. Bacterial suspensions were centrifuged, the supernatant was removed and the pellet was frozen at –80°C for 2 hour, thawed and included in agarose before treatment with lysozyme and than with proteinase K. Genomic DNA was digested with Fast XbaI before fragments were separated by PFGE.

**Results and discussion:** In 2003 two cases of infection caused by the outbreak strain MFBR100 were detected in the city of Campinas. In 2004 three cases were detected but no cases were detected in 2005 and 2006. In 2007 there was one case, in 2008 there were seven cases all in the city of Campinas, southeast of Brazil. In 2010 the outbreak strain was detected in a patient from a city located at the center west region. Isolates from other cities from Brazil have a low similarity index when compared to the outbreak strain profile. Since mammary prosthetic devices are distributed country wide, the predominance and persistence of a single clone in the city of Campinas may indicate contamination of surgical instruments used during surgery.

**Conclusion:** Our findings indicate the persistence of a single clone during 8 years in the same city in Brazil and depict an urgent need for nosocomial infection control improvement.

**P1938** **Tuberculosis: molecular susceptibility testing reveals mixed infections with three strains**

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**Objectives:** Mixed pulmonary infections, usually involving two strains of *M. tuberculosis* (MTB), have been reported to occur, mostly in settings of high disease incidence.

**Methods:** A 55 year-old male patient from Kazakhstan was admitted for possible pulmonary tuberculosis. During 45 days of hospitalization five samples of bronchial secretions, all Ziehl-Neelsen (+) were obtained for culture and susceptibility testing (MGIT960, Becton-Dickinson). The following assays were applied on both clinical samples and (+) MGIT vials: MTBDRplus, Genotype *Mycobacterium* CM and AS, MTBDRsl (Hain-Lifesciences), a Real Time PCR identifying nontuberculous mycobacteria (NTM) and a PCR detecting the characteristic deletion in the Region of Difference 105 (RD105) of Beijing MTB strains. The MIRU-VNTR method in 24 polymorphic loci was used for genotyping.

**Results:** MTBDRplus showed that both clinical samples and (+) MGIT vials were MuTauBetapositive, however, results of simultaneous resistance and susceptibility were detected for rifampin (rpoB: hybridization with both S531L mutation and wild type probes) and isoniazid (katG: hybridization with both S315T mutation and wild type probes). Coinfections with a NTM strain and laboratory contamination were excluded. The MIRU-VNTR method showed the presence of three distinct MTB strains in MGIT culture (double genotype in 12 loci, triple genotype in 4). Only one of them (strain RIF-R) was isolated by sub culturing in MGIT vials with rifampin (1  $\mu$ g/mL) and was found to be molecularly and phenotypically resistant to rifampin/isoniazid, and phenotypically resistant to streptomycin, ethambutol and pyrazinamide. The same phenotype was obtained by testing the mixed culture, while MTBDRsl showed susceptibility to aminoglycosides, quinolones and ethambutol. The genetic fingerprint of strain RIF-R and the detection of the RD105 deletion demonstrated that it belongs to the Beijing family. After 40 days of treatment (second line drugs), the time to positivity of MGIT cultures increased from 5 to 20 days. The strain that prevailed was one of the two more susceptible strains. The patient died, while it



was found that 4 years ago, he was diagnosed with multidrug resistant tuberculosis in two other hospitals in Athens.

**Conclusion:** Molecular direct susceptibility testing enabled the detection of mixed infection, which apparently may be encountered even in a low incidence setting.

**P1939** Optimal 24 loci MIRU-VNTR typing is highly discriminatory when applied to *M. tuberculosis* strains in the Midlands region of the UK

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**Objectives:** DNA fingerprinting of all *M. tuberculosis* strains in the Midlands region of the UK using the recommended set of 24 MIRU-VNTR (Mycobacterial Interspersed Repetitive Units containing Variable Numbers of Tandem Repeats) loci has been carried out since 1st January 2010. Prior to 2010, a "classical" set of 15 loci were used with the addition of nine loci to comprise 24 loci. We examined the technical performance of all loci combined and individually in all strains and within the six global lineages by calculation of the discriminatory power and strain diversity obtained.

**Methods:** The 24 MIRU-VNTR loci were analysed using PCR and a Transgenomic WAVE System. Strains were assigned to a major global lineage (East African Indian, East Asian, Euro-American, Indo-Oceanic, West African-1, and West African-2) using the MIRU-VNTRplus database. Discriminatory power was calculated using the Hunter-Gaston Discrimination Index (HGDI) and the HPA V-DICE website. Individual MIRU-VNTR loci were assigned as highly (HGDI > 0.6), moderately (0.3–0.6), or poorly (<0.3) discriminatory.

**Results:** Between 2010 and 2011, 1783 strains were typed by 24 loci as part of The HPA National TB Strain Typing Project which was funded by a central HPA Service Development Fund Bid. Using MIRU-VNTRplus, 1602/1783 (90%) strains were assigned to a global lineage with a matching distance <0.3. The two most common lineages were East African Indian (642/1783, 36%) and Euro-American (626/1783, 35%). Across all lineages, a very high level of diversity was obtained (HGDI 0.999, 95% CI 0.999–0.999). Most of the lineages (5/6) exhibited very high levels of diversity (HGDI > 0.996) with the East Asian lineage (n = 108) exhibiting reduced diversity (0.987, 95% CI 0.980–0.994). Across all lineages, 13/24 loci were highly discriminatory (HGDI > 0.6). Within each of the six lineages, at least five VNTR loci were highly discriminatory except for the East Asian lineage where only one locus was highly discriminatory (2163b). Of the extra loci added in 2010, 6/9 (66%) were highly discriminatory whereas 7/15 (47%) of the classical 15 loci were highly discriminatory.

**Conclusion:** The internationally recommended optimal set of 24 MIRU-VNTR loci provided a high level of discrimination for *M. tuberculosis* strains in the Midlands region of the UK. Data from the Midlands confirms that the selection and addition of the nine individual additional loci to increase the discriminatory power of MIRU-VNTR typing is justified.

**P1940** Clinical and molecular analysis of the distribution of the genotypic lineages of *Mycobacterium tuberculosis* clinical isolates in the immigrant population and in Italians in the urban area of Milan, 1994–2009

F. Zanini\*, M. Carugati, C. Schirolli, N. Vanoni, A. Lombardi, L. Codecasa, A. Gori, F. Franzetti (Milan, Monza, IT)

**Objectives:** Geographical distribution of the major *Mycobacterium tuberculosis* (*M. tuberculosis*) lineages is well known worldwide. The intensification of the migratory fluxes could contribute to modify the representativeness of the genotypic families in the time in a specific area. Clinical features of tuberculosis (TB) could, moreover, change

based on infective lineage. The aim of the study was to describe the distribution of mycobacterial lineages into the urban area of Milan evaluating the role of immigrants and to analyze the relation between major genotypes and clinical presentation of TB.

**Methods:** All consecutive cultured confirmed TB cases from 1994 to 2009 were included in the study. Epidemiological, microbiological and clinical data were assessed. The genotypic profile of the clinical isolates was obtained by Spoligotyping method, the association to the correspondent lineages was based on SPOLDB4.0 database. The chi-square test was used to identify predictors significantly associated with the prevalent mycobacterial families.

**Results:** Of the 4356 culture positive TB cases, 1662 were genotyped. The rate of clusterization was similar among immigrants (52.4%) and Italians (53.5%). 903/1662 clinical isolates belonged to a defined lineage (54.3%) and the major genotypes were T (31.2%), Haarlem (H 22.0%), Latin and Mediterranean (LAM 15.4%), Beijing (10.1%). H and LAM were equally distributed among Italians and foreigners while Beijing (84.6%), East African Indian (EAI 83.8%), Central Asian (CAS 95.7%) and African families were clearly prevalent in foreign born population. Beijing family prevailed in Asian (47.0%) ex-USSR (66.7%) and Indian (36.0%). The univariate analysis, respect to genotype, displayed a significant difference for age ( $p < 0.0001$ ), microscopic examination ( $p < 0.03$ ) and drug resistance ( $p < 0.02$ ).

**Conclusion:** Seventy percent of the clinical isolates belonged to the three most common lineages worldwide: T, H and LAM. The growing diffusion of Beijing family in the whole population suggested a sustained transmission between immigrants and Italians. Age, bacterial spreading and drug resistance differed significantly in relation to the mycobacterial lineage.

**P1941** The role of cholesterol oxidase in the pathogenicity of *Mycobacterium tuberculosis*

M. Brzezinska\*, M. Klink, I. Szulc, A. Brzostek, M. Kielbik, Z. Sulowska, J. Dziadek (Lodz, PL)

**Objectives:** Cholesterol oxidase (ChoD) of *Mycobacterium tuberculosis* (Mtb) participates in the degradation of host cholesterol. It appears in a secreted and cell surface-associated forms. The contribution of ChoD in the pathogenicity of Mtb is unknown. The bactericidal activities of human macrophages against Mtb lacking a functional copy of gene encoding ChoD in comparison with MtbH37Rv wild type were investigated.

**Methods:** Using the technique of gene replacement based on the process of homologous recombination we obtained the MtbchoD mutant (Mtbmut) without functional copy of choD gene. Control strain carries the choD gene complemented with an intact choD. The intracellular growth of bacteria, production of nitric oxide (NO), reactive oxygen species (ROS) and tumour necrosis factor-alpha (TNF-alpha) by macrophages activated or not with interferon gamma were tested. In experiments determining the role of TLR2-induced signaling pathway, the macrophages were treated with inhibitor IRAK1/4 or with antibodies anti-TLR2.

**Results:** The intracellular replication of MtbH37Rv and Mtbmut in activated macrophages was similar. In contrast, in non-activated macrophages, the growth of non-opsonised Mtbmut was significantly impaired in comparison with wild type and control strains. Inhibition of TLR2-induced signaling pathway in non-activated macrophages caused increase in the survival of non-opsonised Mtbmut. The production of NO in activated macrophages and infected with MtbH37Rv and Mtbmut was similar. Only non-opsonised Mtbmut induced NO production in non-activated macrophages. In contrast, non-activated macrophages with inhibited TLR2-induced signaling pathway did not produce NO in the response to Mtbmut infection. Although, all Mtb strains inhibited the ROS production by macrophages, the non-opsonised Mtbmut more poorly than wild type and control strains inhibited the ROS production by non-activated macrophages. The production of TNF-alpha by activated macrophages infected with Mtb strains was similar. However, Mtbmut was a weaker stimulator of TNF-

alpha production by non-activated macrophages than wild type and control strains.

**Conclusion:** Cholesterol oxidase facilitates the survival of Mtb in macrophages during the initial phase of infection and participates in the inhibition of bactericidal activity of macrophages.

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**P1942 Ketosteroid dehydrogenase of *Mycobacterium tuberculosis* is involved in the infection**

M. Klink\*, A. Brzostek, M. Brzezinska, I. Szulc, M. Kielbik, Z. Sulowska, J. Dziadek (Lodz, PL)

**Objectives:** Ketosteroid dehydrogenase (KsdD) is an essential enzyme of *Mycobacterium tuberculosis* (Mtb) that is responsible for the host cholesterol degradation. We ask whether KsdD is used by Mtb for the infection of human macrophages or whether it is used for host cholesterol degradation as the source of carbon and energy. We compared the bactericidal activities of human macrophages infected with Mtb lacking a functional copy of gene encoding KsdD or with MtbH37Rv wild type.

**Methods:** MtbH37Rv strain with inactivated ksdD gene (mutant) was prepared by using the technique of gene replacement based on the process of homologous recombination. We also obtained control strain carries the ksdD gene complemented with an intact ksdD. The intracellular growth of bacteria, the production of nitric oxide (NO), reactive oxygen species (ROS) and tumour necrosis factor-alpha (TNF-alpha) by macrophages activated or not with interferon gamma were tested. In experiments determining the role of TLR2-induced signaling pathway, the macrophages were treated with inhibitor IRAK1/4 or with antibodies anti-TLR2.

**Results:** We observed similar intracellular replication of Mtb strains in activated macrophages. In contrast, growth of non-opsonised Mtb mutant was impaired in non-activated macrophages in comparison to wild type and complemented strains. However, in non-activated macrophages with inhibited TLR2-induced signaling pathway, non-opsonised Mtb mutant replicated similarly to MtbH37Rv. Activated macrophages infected with Mtb strains produced similar amount of NO. Mtb mutant but not wild type and control strains stimulated non-activated macrophages to produce NO. However, non-activated macrophages with inhibited TLR2-induced signaling pathway did not produce NO in response to mutant. All Mtb strains inhibited the ROS production by activated macrophages similarly. However, Mtb mutant (but not control strain) was less potent inhibitor of ROS production in non-activated macrophages than MtbH37Rv. We did not find any differences in the production of TNF-alpha by macrophages infected with Mtb strains.

**Conclusion:** Mtb with lacking the functional KsdD is more sensitive for bactericidal activity of human macrophages before being activated with IFN-gamma. It suggests that KsdD can partially participate in the pathogenicity of mycobacterium.

Research co-financed by the European Regional Development Fund under the Operational Programme Innovative Economy, grant POIG.01.01.02-10-107/09.

**P1943 The role of DNA double-strand breaks repair systems in the resistance of *Mycobacterium tuberculosis* against antimicrobial activity of macrophages**

I. Szulc\*, M. Klink, A. Brzostek, M. Brzezinska, M. Kielbik, Z. Sulowska, J. Dziadek (Lodz, PL)

**Objectives:** In *Mycobacterium tuberculosis* (Mtb) the non-homologous end-joining (NHEJ) and homologous recombination (HR) pathways are the most important DNA double-strand breaks (DSBs) repair systems. HR pathway uses RecA as a major protein while NHEJ uses Ku and DNA ligase D proteins. The aim of this study was to

determine the effect of inactivation of genes ku, ligD and recA on the ability of Mtb to survive in human macrophages. The role of the NHEJ and HR systems in protection of Mtb from the bactericidal activity of macrophages was also determined.

**Methods:** MtbH37Rv strain with inactivated ku, ligD and recA genes (MtbkuDrecA mutant) and MtbH37Rv strain with inactivated ku and ligD genes (MtbkuD mutant) were obtained by using the technique of gene replacement based on the process of homologous recombination. The monocyte-macrophages cell line (THP1) activated or not with interferon gamma was infected with Mtb strains (Mtb). Intracellular growth of bacteria as well as antimicrobial activities of macrophages such as production of nitric oxide (NO), reactive oxygen species (ROS), tumour necrosis factor-alpha (TNF-alpha).

**Results:** All tested Mtb strains replicated similarly in the activated macrophages. However, the intracellular growth of MtbkuDrecA mutant but not MtbkuD mutant was significantly inhibited in the non-activated macrophages in comparison to MtbH37Rv. Activated macrophages infected with Mtb strains produced similar amount of NO. Only MtbkuDrecA mutant stimulated the non-activated macrophages to produce NO. We noticed that Mtb strains similarly inhibited the ROS production by activated macrophages. However, non-opsonised mutants: MtbkuDrecA and MtbkuD in the significantly less degree inhibited the ROS production in non-activated macrophages than wild type strain. The production of TNF-alpha by activated macrophages in the response to all tested Mtb strains was similar. We found that non-activated macrophages infected with MtbkuDrecA mutant but not with MtbkuD mutant produced significantly less TNF-alpha than wild type strain.

**Conclusion:** The MtbkuDrecA mutant was more sensitive to bactericidal activity of macrophages than MtbkuD mutant. We suggest that NHEJ repairing pathway can be less important than HR in the survival of mycobacterium in macrophages during initial state of infection.

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**P1944 Isolation of the novel species *Mycobacterium kumamotonense* from a patient with lung disease and latent tuberculosis**

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**Objectives:** The introduction of molecular techniques facilitated the detection and identification of novel non tuberculous mycobacterial (NTM) species, the clinical significance of which is under constant evaluation. *M. kumamotonense* is a novel, slowly growing NTM, most similar to *M. terrae* complex. We describe the isolation of a clinically relevant *M. kumamotonense* strain from the sputum specimens of a patient with latent TB.

**Methods:** A 32-year old immunocompetent English woman, working summertime in Crete, proceeded to the hospital for positive Mantoux test (40 mm). Chest computed tomography (CT) scan revealed a nodular lesion in the right upper lobe and enlarged hilar lymph nodes. Acid-fast bacilli smear was positive for one sputum sample. Acid-fast bacilli were isolated from two sputum samples after 2 weeks incubation in the Bact-Alert 3D system, followed by a positive solid culture (Lowenstein-Jensen; bioMerieux, Marcy l'Etoile, France) 20 days later. Further examinations like hemodiagram, blood count, biochemical tests were in normal range with the only exception of LDH, which was elevated. No other risk factor for particular NTM lung disease was found.

**Results:** The recovered mycobacterium was identified as *M. celatum* by the use of the commercial kits Genotype Mycobacterium CM and AS (Hain, Lifescience, Nehren, Germany). The banding patterns obtained for GenoType AS [1, 2, 3, 6, 12, 14] was specific for *M. celatum* while those obtained for GenoType CM [1, 2, 3] was only genus-specific. Moreover, the sequences for 16S rRNA gene (GeneBank accession: HQ442524) and for the 65-kDa heat shock

protein (hsp65) gene were 99.9% (838/839) and 99.4% (358/360) similar with the corresponding sequences of the type strain *M. kumamotonense* CST7247. According the criteria for NTM lung disease by the American Thoracic Society the recovered *M. kumamotonense* strain was considered as clinically relevant.

**Conclusions:** This report should increase the awareness for the ubiquity to this species and raise the index of suspicion for the detection of the pathogen, particularly in a patient with latent TB. The introduction of more advanced molecular diagnostic methods as sequencing analysis of the 16S rRNA and hsp65 gene improved the ability to identify less common mycobacterial species as *M. kumamotonense* while commercial probes could not provide correct identification of this species.

**P1945 Analysis of diagnostic scorings of Thwaites and Marais in two groups of definite and probable tuberculous meningitis cases**

*K. Kart Yasar, F. Pehlivanoglu, G. Sengoz\* (Istanbul, TR)*

**Objectives:** Laboratory-based diagnostic methods for rapid diagnosis of tuberculous meningitis (TBM) are insufficient. The studies for the diagnostic guidelines based on clinical and laboratory findings are currently progressing. In this study, the diagnostic criteria of Thwaites (TDS) and the case definition criteria of Marais were applied to definite and probable TBM cases to investigate the presence of statistically difference and importance with respect to the data obtained in our country.

**Methods:** The 59 TBM cases with culture positivity and 89 probable TBM cases that clinically diagnosed have been included into this study. The TDS as a scoring system and the "case definition criteria" of Marais were applied to the both groups and the differences between the results were compared statistically.

**Results:** All of 59 definite and 89 clinically diagnosed TBM cases had scores lower than critical TDS scoring for TBM. No statistical difference was found with respect to features of TDS between two groups ( $p = 0.139$ ). According to case definition criteria of Marais, culture positive cases were found to have 68% "probable case" and 32% "possible case" rates respectively. These rates were found 63% and 37% respectively in the clinically diagnosed TBM cases, and no statistically significant difference was found ( $p = 0.543$ ). No statistical difference was found between two groups with respect to the compliance for each criterion of Marais.

**Conclusions:** According to these results obtained in our definite and probable TBM cases, the TDS and the case definition criteria of Marais are seem to be useful methods for early diagnosis. We conclude that both methods may be considered among routine investigations that can be applied safely in probable TBM cases without culture positivity for tuberculosis.

**P1946 Miliary tuberculosis and tuberculous meningitis: review of 25 adult cases**

*K. Kart Yasar, G. Sengoz\*, F. Pehlivanoglu (Istanbul, TR)*

**Objectives:** This study was aimed to evaluate epidemiological, clinical, laboratory and neuroimaging features of 25 adult patients with tuberculous meningitis (TBM) and miliary tuberculosis (TB).

**Methods:** Twenty-five patients with miliary TB of 160 patients with TBM were included into this retrospective study. Diagnosis of TBM was based on clinical, laboratory, neuroimaging signs and Thwaites' Diagnostik Index. Diagnosis of miliary TB was obtained by radiological imaging. Statistical analyses were performed with chi-square test.

**Results:** Of 25 patients with miliary TB, 14 were female and the median age was 35.3. Age range was 20–35 years among males, whereas was 17–78 years among females. Most frequent symptoms on admission were headache (84%), malaise-anorexia (68%) and vomiting (64%); while the most findings were stiffness (84%) and fever (68%).

Symptom duration was 1–3 weeks in 44%, and 3–12 weeks in 44% and longer than 3 months in 12% of cases. *Mycobacterium tuberculosis* was isolated from CSF in 9/22 of patients. Basal meningitis (39%), tuberculoma (50%), hydrocephalus (11%) and ischemia/infarct (11%) were the most signs in neuroimaging. Twenty percent of patients died, and 68% recovered completely, and 12% recovered with neurological sequel at the end of the sixth month. Antituberculous therapy was completed to at least 12 months in all patients. Symptom duration was longer and malaise-anorexia, night sweats, weight loss and higher protein level in CSF were detected in higher rates among patients with miliary TB ( $p < 0.05$ ).

**Conclusions:** TBM is the most severe form of TB and it may reveal secondary to miliary TB in countries with high prevalence of TB. All patients with miliary TB, had similar clinical and radiological findings to other patients with TBM. Distinctively; female dominance, longer symptom duration, higher prevalence of malaise-anorexia, night sweats, weight loss and higher protein level were remarkable in patients with miliary ones.

**P1947 Pancreatic tuberculosis mimicking carcinoma: a case series and review of the imaging and literature**

*T. Fletcher\*, I. Din, S. Aston, A. Miller, N. Beeching, E. Joekes (Liverpool, UK)*

**Introduction:** The incidence of tuberculosis (TB) in developed countries is increasing and pulmonary disease remains the most common presentation. Abdominal tuberculosis is a frequent site of extra-pulmonary infection and has a wide spectrum of clinical presentation. Isolated pancreatic TB is rare and is normally reported in association with immunosuppression. The presentation and imaging often mimic pancreatic carcinoma, with tuberculosis only diagnosed after laparotomy and histological examination.

**Methods:** We present a rare case series of five patients with confirmed pancreatic tuberculosis that have been managed in our regional infectious disease unit in the previous 10 years. Two cases were diagnosed post-operatively following Whipple's procedure for suspected carcinoma. We also discuss the imaging characteristics of pancreatic TB, and the diagnostic role of endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) in pancreatic lesions.

**Discussion:** This case series highlights that the incidence of pancreatic tuberculosis is low and the diagnosis and appropriate treatment opportunity are often missed. It should always be considered in patients with suspected pancreatic malignancy. We present five cases which also demonstrate that pancreatic TB is not always associated with immunosuppression. Patients often have recognised risk factors, particularly their country of origin and it has a wide spectrum of clinical and radiological presentation.

**P1948 Comparative evaluation of severe and mild forms of extrapulmonary tuberculosis: 202 cases**

*E. Kursun\*, T. Turunc, Y.Z. Demiroglu, H. Arslan (Ankara, TR)*

**Objectives:** Tuberculosis can involve any organ system in the body. In this study, we aimed to comparative evaluation of severe and mild forms of the extrapulmonary tuberculosis (EPT) in our clinic.

**Methods:** In this prospective study, 202 HIV seronegative patients of EPT who admitted to our department between January 2001 and October 2011 were included. The patients were evaluated with respect to epidemiological and clinical features, laboratory results and treatment outcome in two groups such as severe and mild forms of EPT.

**Results:** The age range of the patients were 16–84 years old (mean age:  $46.2 \pm 16.6$ ) and 109 of them (54%) patients were female. The most common sites of involvement were lymph nodes (37.1%). There was an underlying disease in 56 (27.2%) of the patients (diabetes mellitus in 12.9%; chronic renal dysfunction in 10.4%, malignancy in 4%), history of passed tuberculosis infection in 21 (10.4%) and history of contact with tuberculosis patient in 41 (20.3%). According to criteria

of World Health Organization (WHO), tuberculosis which is miliary with meningeal, pericardial, pleural effusion that is either bilateral or diffuse, which has spinal, intestinal, or more than one bone and joint involvement or genitourinary involvement is classified as severe clinical form; where as tuberculosis that goes with lymphadenitis, unilateral pleural effusion, involvement of peripheral joints and skin, excluding spine is classified as mild clinical form. Severe and mild forms of EPT were present in 97 (48%) and 105 (52%) of the patients, respectively. In comparison of the patients with severe and mild forms of EPT, the severe form were detected more frequently in males ( $p = 0.01$ ), the positivity rates of culture and acid-fast staining were higher in patients with severe form ( $p < 0.001$  and  $p = 0.02$ , respectively). The mortality rate was also found higher in patients with severe form ( $p = 0.01$ ). Histopathological evaluation was more useful in the mild group.

**Conclusion:** Investigation of epidemiological and clinical characteristics of extrapulmonary tuberculosis on the large series of cases may be essential in early diagnosis and treatment and also prevention of disease especially in endemic countries.

#### **P1949** An assessment of 22 cases of tuberculous meningitis

E. Kursun\*, T. Turunc, Y.Z. Demiroglu, M.A. Habesoglu, H. Arslan (Ankara, TR)

**Objectives:** Tuberculous meningitis is a serious, life-threatening form of tuberculosis. We aimed to evaluate the clinical, laboratory and radiological findings of tuberculous meningitis cases that were followed-up in our clinic.

**Methods:** In the present study, the data of 22 patients with tuberculous meningitis who had been followed-up at our clinic between December 2003 and October 2011 were retrospectively investigated.

**Results:** Out of the total 22 cases, 14 (64%) were women and 8 (36%) were men. The mean age of the patients was 38 years (range 17–63 years). The most frequent complaints at presentation were headache, fever and nausea and vomiting. The cerebrospinal fluid (CSF) pressure was increased in 13 cases and the mean CSF cell count was 268 leukocytes/mm<sup>3</sup>. The mean levels of protein and glucose in the spinal fluid were 148.3 and 37.1 mg/dL, respectively. Smears of CSF were negative for acid-fast bacilli. *M. tuberculosis* was isolated from the CSF of seven cases. Chest X-Ray examinations revealed miliary pattern in three of the cases. Brain computed tomography scans of the patients displayed normal findings in eight cases, while changes related to tuberculosis were detected on the scans of 14 patients. All patients received a four-drug anti-tuberculosis regimen (H + R + E + Z) along with steroids for 2 months, followed by a two-drug anti-tuberculosis treatment (H + R) for 10 months. Three patients (14%) died, while four (18%) of the patients were left with permanent sequelae. The period starting from diagnosis till the beginning of treatment in living cases was determined as 6 days, where this period was determined as 8 days in dead cases.

**Conclusion:** Despite the developments in medicine, tuberculous meningitis remains a disease of high mortality and poor prognosis. For this reason, tuberculosis should be kept in mind in the differential diagnosis of all cases with meningitis. If a patient is suspected to have tuberculous meningitis according to the clinical, CSF and radiological findings, anti-tuberculosis therapy should be commenced without delay without waiting for microbiological confirmation.

#### **P1950** Diagnosis and management of spinal tuberculosis in West London

R.C. Moores\*, E. Wall, M. Harvie, A. Mehta, W. Lynn, F. Sanderson (London, Liverpool, UK)

Spinal tuberculosis (spinal TB) is difficult to diagnose, and even with adequate anti-tuberculous chemotherapy carries significant long term morbidity. Controversies in the management of this condition include the appropriate duration of anti-tuberculous therapy, the use of corticosteroids and the diagnostic value of invasive tests such as spinal biopsy.

**Objectives:** 1 To describe clinical and epidemiological features of patients with spinal TB in the UK

2 To investigate the value of laboratory tests used in the evaluation of suspected cases

3 To assess the role of interval magnetic resonance imaging (MRI) in management of spinal TB

4 To investigate the use of corticosteroids in the treatment of spinal TB

**Methods:** We performed a retrospective review of all adult patients treated for spinal TB at three West London hospitals from January 2000 to September 2008. We collected epidemiological, clinical, radiological and microbiological data from the time of presentation to the latest review.

**Results:** One hundred and nineteen patients were identified, 57% were male, median age 35 years. 95% were born outside the UK and median UK residency at diagnosis was 5 years (range 0–47). Eighty-nine percent presented with back pain and the median symptom duration was 4 months (range <1–48). Significant neurological impairment was present in 30%. CRP was elevated in 83% and ESR in 80%. Ninety-four percent of patients tested were Vitamin D deficient. Sixteen patients underwent neurosurgical intervention.

Needle biopsy or surgical samples from spinal or paraspinal tissue were taken from 84 patients. Sixty-seven percent of those cultured yielded *M. tuberculosis*. Drug-resistant TB was found in seven culture-positive cases. Histology showed granulomatous inflammation in 74% of samples submitted.

Cross sectional imaging was reviewed for 95 patients. Spinal cord compression was present in 58%. Serial imaging was available for 65 patients, at intervals of up to 26 months after diagnosis (median 4.5 months). Radiological abnormalities frequently persisted after completion of TB treatment.

Median duration of TB treatment was 12 months (range 6–26). Fifty-five percent of patients received corticosteroids, for 2.8 months on average.

**Conclusions:** The diagnostic yield from spinal or paraspinal needle biopsy is high, including identification of drug-resistant TB. Treatment duration is highly variable between clinicians and should be standardised. Corticosteroids are widely but inconsistently used. MRI abnormalities may resolve slowly and can persist after completion of TB treatment.

#### **P1951** Severe pneumonia following intravesical BCG therapy in a patient with bladder cancer

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**Objectives:** We describe a man with a severe pneumonia secondary to disseminated *Mycobacterium bovis*-BCG infection after intravesical BCG instillation. This diagnosis was based on positive polymerase chain reaction for mycobacterium tuberculosis complex in bronchoalveolar lavage and on the presence of non necrotizing granulomas in transbronchial biopsy, histopathologically analogous to those found in previous bladder biopsies of the same patient. *M. bovis*-BCG was confirmed by a real time PCR assay in lung and bladder samples.

**Methods:** A 66 years old man treated with periodical intravesical instillations of BCG for a non invasive papillary urothelial bladder cancer, after a traumatic catheterization, complained fever and dysuria; 2 days after he was hospitalized for acute respiratory failure. Chest CT scans were negative for the presence of pulmonary emboli, but showed bilateral interstitial-alveolar areas with ground glass opacities and thickened interlobular septa with crazy paving appearance. Empiric antibiotic therapy and systemic glucocorticoids were started with improvement of respiratory conditions. The patient underwent broncho-alveolar lavage which confirmed giant cells epithelioid microgranulomas and *Mycobacterium tuberculosis* complex was detected by PCR. A standard anti TB treatment with rifampicin, isoniazid, ethambutol and pyrazinamide (then modified for hepatotoxicity) was given for 9 months.

**Results:** Anti TB treatment led to a complete resolution of clinical condition and a CT scan demonstrated totally disappearance of the lung infiltrate. A real time PCR assay later confirmed the presence of *M.*

*bovis*-BCG strain, by amplification of primers detecting the peculiar absence of RD1 on mycobacterial genome found in lung and bladder of our patient but not in lung samples of two controls.

**Conclusions:** Disseminated BCG disease is a rare and severe complication of intravesical BCG immunotherapy for bladder cancer; its diagnosis is often delayed or presumptive. We reported one of the few cases of BCG immunotherapy related pneumonia microbiologically confirmed and the first case with demonstration of *M. bovis*-BCG in involved tissues using a real time PCR. Further studies are necessary to standardize diagnostic methods, to early get a definitive diagnosis and to start a targeted therapy as soon as possible.

#### **P1952 Clinical characteristics and predictive factors of paradoxical response with cervical lymph node tuberculosis in non-HIV-infected adult patients**

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**Objective:** Cervical lymph node tuberculosis (LN TB) is one of the most common extra-pulmonary tuberculosis in Korea. The paradoxical response (PR) of LN TB in non-HIV-infected (non-HIV) patients was frequently reported during anti-TB therapy and remain treatment dilemma. To investigate predictive factors and clinical significance of PR in non-HIV patients with LN TB, a retrospective case-control study was performed.

**Methods:** The data of LN TB in non-HIV patients from January 1995 to August 2006 were retrospectively reviewed in tertiary care hospital. Only patients who had culture-confirmed TB, a positive result of testing for acid-fast bacilli, or histological evidence of necrotizing granulomatous lymphadenitis were included. PR was defined as the worsening of preexisting lesions on the basis of clinical or radiological findings or the development of new lesions in patients who had received anti-TB medications for at least 10 days and initially improved. The predictive factors for PR were evaluated by using logistic regression analysis.

**Results:** A total of 404 patients were enrolled. The positive rate of acid-fast bacilli smear, culture and TB polymerase chain reaction were 12%, 11.8% and 55.4%, respectively. The number of patients with histologic evidence were 280 (69.3%). Among 39 *Mycobacterium* TB isolates with eligible anti-TB susceptibility data, the susceptibility rate of isoniazid, rifampin, ethambutol, pyrazinamide, streptomycin, and ofloxacin were 92.5%, 100%, 100%, 100%, 95.1%, and 100%, respectively. The PR occurred in 101 (25%) of 404 patients. Median interval (range) between the initiation of anti-TB medications and onset of PR was 74 (11–480) days. Median interval (range) between the onset of PR and improvement of PR was 69 (7–750) days. Multivariate analysis showed that younger age, larger node size and presence of swelling were predictive factors for PR. Although the duration of anti-TB medications were significantly longer in patients with PR (15.7 vs. 10.9 months,  $p < 0.001$ ), anti-TB treatment were completed successfully in all patients with cervical LN TB except 1 patients who died of acute leukemia.

**Conclusion:** Occurrence of PR during anti-TB therapy could be expected in more than one of fourth of LN TB with predictive factors. First line anti-TB drugs are useful to treat LN TB, based on the data of susceptibility and treatment outcome. Prospective study are warranted to investigate the optimal treatment options and duration in LN TB with PR.

#### **P1953 Tuberculous meningitis in HIV-negative adults. Retrospective analysis of 91 cases**

P. Pagliano\*, V. Attanasio, M. Rossi, R. Di Sarno, A. Pomicino, F. Fraganza, M. Conte, F. Faella (Naples, IT)

Tuberculous meningitis (TBM) is the most devastating manifestation of tuberculosis (TB).

**Objective:** Of the study was to describe the characteristics of TBM in HIV negative adults focusing on cases with severe presentation.

**Methods:** This was a retrospective study of TBM cases observed during the last 20 years in HIV negative adults referred to our department. TBM was defined by clinical signs of meningitis and characteristic cerebro-spinal fluid (CSF) findings coupled with microbiological, radiographic or epidemiologic evidences. Patients presentation was defined according to Medical Research Council (MRC) staging. Cure was defined by the absence of sequelae assessed 12 months after the end of therapy. Quantitative data are expressed as median and interquartile range (IQR). Statistical analysis was performed by two-tailed Chi-square test, Fisher's exact test, and Mann-Whitney *U*-test, as appropriate.

**Results:** Ninety-one cases were included (median [IQR] age 38 [24–52] years, 57% males). Risk factors were reported in 53 (58%) cases: immigration from areas with high incidence of TB in 21 (23%) cases, history of TB in 20 (22%), immunodepression in 14 (15%), close contact with active TB case in six (7%). Chest X-rays were suggestive of TB in 42 (46%) cases. Median (IQR) duration of general and neurological symptoms prior to admission was, respectively, 15 (7–30) days and 2 (1–5) days. Severe disease presentation (MRC stage III) was reported in 34 (37%) cases. Low CSF cells [median (IQR) cells/ $\mu$ L 135 (90–185) vs 190 (130–400),  $p = 0.04$ ] and longer duration of neurological symptoms prior to MTB diagnosis (median hours [IQR] 76 [50–196] vs 44 [24–70],  $p = 0.03$ ) were associated with severe disease. No difference in TBM presentation was reported in the other variables evaluated. Forty (44%) cases reported an unfavourable outcome. Outcome was unfavourable mainly in the cases presenting in MRC stage 3 [26/34 vs 14/57,  $p < 0.0001$ , RR 3.11, 95% CI 1.9–5.1]. Mortality was 4% for cases with less severe presentation and 38% for cases presenting in MRC stage 3.

**Discussion:** In our study, about 40% of TBM cases did not report risk factors for TB suggesting a high level of circulation of *Mycobacterium tuberculosis* outside target adult population. This evidence makes difficult successful prevention strategies. Long duration of neurological symptoms prior to diagnosis and lower CSF cells are associated to severe TBM presentation and poor outcome.

#### **P1954 Late presentation in patients with spinal tuberculosis**

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**Objectives:** New Zealand has a low burden of tuberculosis (TB) and spinal TB is an uncommon diagnosis that may not always be initially considered at presentation. The aim of this review was to describe the presenting features and outcomes of patients diagnosed with spinal TB in Auckland.

**Methods:** We retrospectively reviewed the clinical records, laboratory data and radiological imaging of all patients who were diagnosed with TB involving the spine from 1996 to 2011 in the Auckland region.

**Results:** During the 15 year study period, 45 patients were identified. The median age at presentation was 38 (range 21–84) years and 22 (49%) were male. The majority (47%) of the cohort were from India with a total of 29 (64%) patients originating from Asia and 13 (29%) patients originating from the Pacific region. Infection in the thoracic spine occurred in 28 (62%) and 10 (22%) patients had multiple spinal levels involved. Ten had psoas collections and five (11%) had pulmonary TB. Symptoms were present for 3 months or less in 27 (60%) patients, between 3 months and one year in 13 (29%) patients and for more than 1 year in five (11%) patients. Neurological deficits were found at presentation in 19 (42%) patients.

A biopsy was performed in 42 (93%) patients. The biopsy was radiologically guided in 35 (83%) and surgical in seven (17%) patients. *Mycobacterium tuberculosis* was cultured from 36 (80%). The median duration of treatment was 12 (range 6–24) months. Surgery was performed in 15 (33%) and radiological drainage in 12 (27%) patients. Thirty-one (69%) patients have completed treatment, eight left New Zealand, four died while on TB treatment and two are currently on treatment. Of the patients that completed treatment, 10 (32%) have persistent disability.

**Conclusion:** Although TB of the spine is an uncommon diagnosis, patients are usually young and are frequently diagnosed late and have a high incidence of persisting neurological disability.

**P1955 Bacterial adherence to different meshes for abdominal surgery**

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**Objectives:** To study the influence of morphology and material of surgical meshes to prevent or reduce bacterial adhesion of organisms involved in acute and chronic mesh infections.

**Methods:** Three different types of mesh were compared in order to study the adherence: (i) monofilament macroporous polypropylene mesh, (ii) monofilament microporous polypropylene mesh, (iii) multifilament highly hydrophobic mesh. Microbial adhesion tests were performed using collection strains of *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Mycobacterium abscessus* and *Mycobacterium fortuitum*. Tests were performed in triplicates using previously described protocols.

For the statistical study was using non-parametric tests. Mann-Whitney or Wilcoxon were used for two samples and the Kruskal-Wallis test was used for more than two samples. EPI-Info software version 3.5.1 (CDC, Atlanta, GA, USA) was used to perform the statistical studies.

**Results:** Both *Staphylococcus* sp. strains and *M. fortuitum* showed lower adherence to monofilament polypropylene meshes. *Mycobacterium abscessus* showed lower adherence to multifilament and hydrophobic mesh. Results indicate that both collection *Mycobacterium* spp. strains showed lower adherence than collection *Staphylococcus* spp. strains for all materials except for mesh 2. *Staphylococcus epidermidis* showed higher adherence for all materials than *S. aureus*. *Mycobacterium fortuitum* showed higher adherence to mesh 3 than *M. abscessus*, while this species showed higher adherence to mesh 1 than *M. fortuitum*.

Bacteria	Material	Mean ±Std Dev	p value*	p value**
<i>S. aureus</i>	Mesh 1	10350,0±6636,1	0,1851	0,0021***
	Mesh 2	3205,0±1551,2	0,0124***	
	Mesh 3	6887,0±4074,4		
<i>S. epidermidis</i>	Mesh 1	12618,5±5967,5	0,7573	0,0020***
	Mesh 2	3852,4±2534,7	0,0054***	
	Mesh 3	15600,0±12330,8		
<i>M. abscessus</i>	Mesh 1	1482,6±709,0	0,0023***	0,0092***
	Mesh 2	5066,0±3349,2	0,0007***	
	Mesh 3	613,9±349,5		
<i>M. fortuitum</i>	Mesh 1	878,0±189,6	0,0003***	0,0071***
	Mesh 2	5483,1±5856,7	0,2004	
	Mesh 3	6862,9±3572,3		

\* Mesh 1 or mesh 2 compared with mesh 3  
 \*\* Comparison between monofilament polypropylene meshes  
 \*\*\* Differences statistically significant

**Conclusions:** Depending on the type of bacteria, multifilament highly hydrophobic mesh significantly increase bacterial adherence in contrast to monofilament polypropylene meshes. Therefore, the surgeon should consider these results when choosing a mesh graft for limiting infection in abdominal wall repair.

**P1956 Comparative study of 6-month isoniazid and rifampin (6HR) vs. 6-month isoniazid, rifampin and pyrazinamide 2-month (6HR2Z) for pleural tuberculosis**

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**Objective:** To compare the long-term efficacy of 6HR vs. 6HR2Z therapy for pleural tuberculosis (PITB).

**Methods:** One hundred and seventeen HIV negative patients with PITB were prospectively followed in our tuberculosis-unit from 1991 to

2010 and entered a 6HR (H 5 mg/kg and R 10 mg/kg) or 6HR2Z (Z 30 mg/kg) non randomized treatment protocol. Patients with empyema and those with associated pulmonary infiltrates were ruled out. Incidence of primary isoniazid resistance is <4%. PITB diagnosis was based on culture positive for *M. tuberculosis* in 88 or presence of caseating granuloma in pleural biopsy in 29. Demographic as clinical variables were collected in each case, including: underlying disease, symptoms, pleural fluid analysis, PPD status, method of diagnosis, chest x-ray, as well as assessment of treatment adherence, tolerance and illness evolution. A descriptive and comparative study of the variables between the therapy groups was performed.

**Results:** Out of 117 patients followed, (76 males, 41 females, mean age 31.9 ± 18 years [13–86 year]), 50 received 6HR and 67 6HR2Z treatment. Patients characteristics were similar between the two groups for all the variables analyzed, except for lower frequency of fever (25/50 [50%] vs. 47/66 [71.2%]) and higher frequency of respiratory physiotherapy (16/49 [32.6%] vs. 10/66 [15.1%]) in 6HR therapy group, p < 0.05. All of the 48 *M. tuberculosis* strains with susceptibility tests performed were susceptible to first-line agents. 6HR therapy group developed lower frequency of adverse effects 11/50 (22%), (rash-itching 3 [6%], digestive intolerance 5 [10%]) than 6HR2Z group 20/67 (29.9%) (rash-itching 9 [13.4%], digestive intolerance 8 [11.9%], arthralgias 1 [1.5%], bouts of podagra 1 [1.5%]), without differences in the frequency of severe hepatotoxicity (3/50 [6%] vs. 4/67 [6%]). Five patients died during the treatment with 6HR2Z due to different causes from tuberculosis and all the other patients completed treatment. An overall success was achieved in all of them for a follow-up of 12.2 ± 5.4 years (0.5–19.4 year), without differences between the groups; 83% of the patients was followed for more than 5 years, and 58.9% for more than 10 years. Five (10%) patients in 6HR and 11 (17.7%) in 6HR2Z developed residual pachypleuritis, none of them required decortication.

**Conclusions:** Long-term efficacy of 6HR is similar to 6HR2Z for pleural tuberculosis, and it develops less adverse effects.

**P1957 Resistant mutants of *Mycobacterium tuberculosis* selected in vitro do not reflect the in vivo mechanisms of ethionamide and isoniazid resistance**

F. Brossier\*, E. Cambau, E. Tessier, V. Jarlier, W. Sougakoff (Paris, FR)

**Objectives:** To be active against *Mycobacterium tuberculosis*, isoniazid (INH) and ethionamide (ETH) need to be activated by the catalase/peroxidase KatG (for INH) and by the mono-oxygenase EthA (for ETH, regulated by EthR). ETH and INH both target the enzyme InhA involved in the cell wall biosynthesis. Clinical isolates resistant (R) to INH and ETH display a wide range of mutations altering (i) KatG (mutation S315T found in 70% of INH-R isolates), (ii) EthA (various mutations found in 50% of ETH-R isolates) and, (iii) InhA and the inhA promoter (found in 25% of INH-R and 65% of ETH-R isolates, and conferring co-resistance to INH and ETH). Here we have selected INH-R and ETH-R in vitro mutants in order to investigate, in an isogenic context, the effects of the mutations observed in clinical isolates.

**Methods:** Mutants were selected on Middlebrook 7H11 agar containing INH and ETH. The genes ethA, ethR, katG, inhA and the inhA promoter were sequenced for 115 *M. tuberculosis* in vitro mutants.

**Results:** The mutants selected on INH displayed alterations in katG only (46/69), with the following mutations: missense (12/69), nonsense (14/69), insertion (2/69), partial (13/69) and complete deletion of katG (5/69). The mutants selected on ETH showed alteration in ethA (11/46) and the ethA-ethR intergenic region (1/46), with the following mutations in ethA: missense (5/46), nonsense (4/46) and deletion (2/46).

**Conclusions:** The distribution of the mutations obtained in vitro does not reflect the mechanisms of INH- and ETH-R usually observed in clinical isolates. Surprisingly, the most common mutation found in the INH-R clinical isolates (KatG S315T) was not found in our INH-R

mutants which, by contrast, preferentially showed deletions in KatG (46%). Similarly, the -15c-> mutation in the inhA promoter, which is commonly found in clinical isolates showing co-resistance to INH and ETH, was not observed in our ETH-R mutants which displayed mutations only in EthA. Finally, for 33% and 74% of the INH-R and ETH-R mutants obtained in vitro, respectively, no mutation was found in ethA, ethR, katG, inhA and the inhA promoter.

**P1958** The emergence of clarithromycin resistance in *Mycobacterium avium* complex

D. Machado\*, L. Rodrigues, I. Couto, L. Amaral, M. Viveiros (Lisbon, PT)

**Objectives:** Emergence of resistance to macrolides in *Mycobacterium avium* complex (MAC) is problematic. Treatment options are a dilemma since MAC is usually resistant to conventional antituberculosis drugs. Clarithromycin (CLA) is crucial for MAC therapy; but resistance emerges promptly. Previously, we demonstrate that efflux pumps (EP) play a significant role in MAC resistance to macrolides. In this work, we evaluated the ability of efflux inhibitors (EIs) to reduce CLA acquired resistance in MAC strains.

**Methods:** Three reference strains CLAs and four clinical strains CLAr were characterized by CLA antibiotic susceptibility testing and minimum inhibitory concentration (MIC) determination of CLA (presence/absence of EIs verapamil, thioridazine, and chlorpromazine), rifabutin, moxifloxacin, and clofazimine, using the MGIT960/TB eXIST. Partial sequence of 23 rDNA gene was used to search for mutations. MAC CLAs were subjected to constant concentrations of CLA (4 µg/mL), in order to induce a phenotypic response through EPs. Detection of efflux activity was performed with a semi-automated fluorometric method using ethidium bromide as EP substrate.

**Results:** Exposure of CLAs strains to constant concentrations of CLA induced the activity of pumps that made the bacteria more resistant to this antibiotic. MICs of CLA for the adapted strains changed from 1 to 32 µg/mL, an intermediate level of resistance. The EIs used led to a significant reduction of resistance to CLA and efflux activity was demonstrated in all the adapted strains. In spite of the presence of a mutation in some CLAr strains, efflux activity was also detected. The MIC values for the other three antibiotics remained practically unchanged.

**Conclusion:** This work demonstrates that CLA is substrate for efflux in MAC, and phenotypic resistance can emerge in a susceptible isolate, preceding the genotypic resistance, via active drug-efflux. A period of intermediate resistance, sustained by the activity of EPs, after which the cells have an enhanced probability to mutate and acquire high level resistance, has been noted. The final resistance level of the CLAr strains is a balance between the resistance due to the mutation and due to the efflux-activity of this antibiotic. The latest can be reversed by EIs. In conclusion, we demonstrate that emergence of resistance to CLA in MAC is acquired by efflux during therapy and can be reduced by EIs as adjuncts of conventional therapeutic regimen.

**P1959** Delineating TB samples from Nepal using insertion site 6110 (IS6110) mapping PCR

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**Objectives:** Tuberculosis (TB), is the major cause of morbidity and mortality among the 29 million people in Nepal. The steady rise in the number of TB cases in the last few years has increased the challenges facing scientists and health professionals alike. With no data on the evolution of or distribution of strains available from the *Mycobacterium tuberculosis* complex (MTBC), the focus of this study is to categorise the TB samples in Nepal for the first time using IS6110 fluorescent amplified fragment length polymorphism (FAFLP) PCR into different genetic lineages.

**Materials and methods:** The bacterial DNA from 176 consecutive clinical isolates from Anandaban Hospital in Nepal extracted using the

CTAB method was subjected to fast ligation mediated FAFLP PCR using four differentially labelled selective primers and the samples were separated on the ABI genetic analyser 3730. The fragments generated were analysed using the PeakScanner software and identified using their fluorescent tag. The four-dye FAFLP data collected from the different profiles were then recorded and compared with the reference collection of TB samples.

**Results:** Out of 176 samples analysed, 64 samples belong to the Central Asian (CAS) lineage or principal genetic group 1 (PGG1), 33 samples belong to the Beijing lineage (PGG1) and the rest of the samples belong to other genetic groups- PGG2 and PGG3.

**Conclusions:** The majority (55%) of the samples fall under the CAS and the Beijing groups. The geographical location of Nepal likely explains the distribution of genetic lineages currently circulating, with a mixture of predominantly Beijing lineage coming from the North of the Himalayas and the CAS lineage from the south. As the prevalence of TB infection is high in the Nepalese population, this novel information will not only aid contact tracing but also shows a picture on the predominant PGGs found in Nepal which can be helpful in future epidemiological surveillance or outbreak investigation.

**P1960** EthR inhibitor BDM41906 boosts the in vivo antituberculous activity of ethionamide in a murine model

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**Objectives:** The use of ethionamide (ETH), one of the most efficacious second-line drugs for the treatment of multidrug-resistant tuberculosis is limited by its toxicity. ETH is a prodrug that needs to be activated by the monooxygenase EthA to exert its antimicrobial effect. The production of EthA is negatively controlled by the transcriptional regulator EthR, which then limits de bioactivation of ETH, and consequently the sensitivity of *M. tuberculosis* to ETH (Baulard *et al.*, J Biol Chem, 2000). A previous study has shown that parenteral drug like compounds able to inhibit the DNA-binding function of EthR boost the activity of ethionamide in mice (Willand *et al.*, Nat Med 2009). EthR inhibitors with improved efficacy, solubility, and pharmacokinetic parameters have been recently developed (Flipo *et al.*, J Med Chem 2011). In the present study, we evaluated the efficacy of the new EthR-inhibitor BDM41906 given orally in an in vivo murine model.

**Methods:** One hundred and fifteen Swiss mice were inoculated with the reference virulent strain *M. tuberculosis* H37Rv. Treatment was started one day after infection with either ETH alone (1-50 mg/kg) or ETH combined with BDM41906 (B 20 mg/kg). One negative control group was untreated and a positive control group was treated with isoniazid 25 mg/kg. After one month with or without treatment, mice

Groups	Mean CFU (log10 +/- SD)
Untreated	6.9 +/- 1.0
INH	2.9 +/- 0.4
ETH50	3.3 +/- 0.4
ETH25	3.7 +/- 0.3
ETH12.5	5.4 +/- 0.2
ETH10	5.8 +/- 0.3
ETH3	7.5 +/- 0.3
ETH1	7.4 +/- 0.1
ETH 25 + B	3.1 +/- 0.2
ETH12.5 + B	3.3 +/- 0.6
ETH10 + B	4.1 +/- 0.4
ETH3 + B	6.1 +/- 0.2
ETH1 + B	7.3 +/- 0.9

were sacrificed, lungs were collected, ground and plated to enumerate the bacterial load.

**Results:** ETH alone prevented mortality at 10 mg/kg whereas BDM41906 boosted-ETH prevented mortality at 3 mg/kg. Lung CFU counts reached 4.3 log<sub>10</sub> CFU at start of treatment. Lung CFU counts after one month are presented in the Table 1.

**Conclusion:** Our previous study demonstrated that the co-administration of ETH with BDM31343, when solubilized in DMSO and given intraperitoneally twice daily at 100 mg/kg, reduced the mycobacterial load as effectively as a three times higher doses of ETH monotherapy. Here, we show that an equivalent boosting effect of the ETH activity is obtained with compound BDM41906 given orally once per day, at 20 mg/kg, using beta-cyclodextrine as vehicle. This result opens new perspectives for the use of such combinations in humans.

**P1961** Direct detection of rifampin and isoniazid resistance of *Mycobacterium tuberculosis* complex in smear-positive clinical samples using low density DNA microarrays

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**Objectives:** Molecular methodologies for direct detection can reduce turnaround time to detect drug-resistant *M. tuberculosis* complex (MTBC) in clinical specimens. The objective of this study was to evaluate the usefulness of a low density DNA microarray (Array), which was designed in-house and developed in an external polymer support (Chipron, GmbH), for the detection of isoniazid (INH) and rifampin (RMP) resistance, directly on clinical samples with positive smears of MTBC.

**Methods:** Sixty-six clinical samples containing MTBC strains resistant to RMP and/or INH (64 to INH, 28 to RMP and 26 multidrug-resistant) and 88 specimens with MTBC isolates susceptible to INH and RMP, all smear-positive, were studied. The DNA from these samples was extracted using the QiAmp DNA minikit (QiaGen) and amplified by multiplex PCR using labelled primers for three regions: katG, inhA (INH) and rpoB (RMP). The Array design consisted on nine wild type probes (six rpoB, two inhA and one katG), and 17 probes (12 rpoB, three inhA and two katG) containing the main nucleotidic substitutions related to phenotypic resistance.

**Results:** The Array detected 47 mutations related to INH-resistance and 23 to RMP. Fifty resistant (75.8%) and 79 (89.8%) INH and RMP susceptible MTBC clinical samples showed a correct pattern of hybridization in the Array (compared to the sequencing results). Correlations with the phenotypic susceptibility and with sequencing are shown in the Table 1 (SE, sensitivity; SP, specificity; PPV, positive predictive value; NPV, negative predictive value).

A. Array results vs phenotype					
Drug		SE (%)	SP (%)	PPV (%)	NPV (%)
INH		73.4	88.9	100	88.8
RMP		82.1	96.7	88.5	98.3
B. Array results vs sequencing					
Drug	Target	SE (%)	SP (%)	PPV (%)	NPV (%)
INH	<i>katG</i>	83.3	85.7	96.2	100
	<i>inhA</i>	90.5	98.5	100	98.4
RMP	<i>rpoB</i>	76.7	94.3	100	91.9

**Conclusion:** This in-house-designed Array showed a good sensitivity and specificity for direct detection of mutations related to INH and RMP resistance, in smear-positive specimens. The lower correlation with the phenotype in the case of INH is due to the existence of other targets and mechanisms involved in the resistance which could not be included in the current design of the Array. This low-cost system is easy to perform and can reduce turnaround time of resistance detection from several weeks to 48 hours.

**P1962** The complete sequence of an Inc-P1 beta plasmid from *Mycobacterium abscessus* subsp. *bolletii*

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**Background:** We recently demonstrated that outbreaks of surgical site infection in different Brazilian states were caused by a single strain of *Mycobacterium abscessus* subsp. *bolletii*. In order to understand the biology of this outbreak strain we started a project to sequence its entire genome.

**Material and methods:** The *M. abscessus* subsp. *bolletii* outbreak strain F1725 was cultivated on sheep blood agar for 5 days at 30°C before DNA extraction. Genomic DNA was extracted as previously described by van Soolingen *et al.* In brief bacterial suspension was treated with lysozyme for 1 hour at 37°C before addition of proteinase K and incubation at 65°C for 10 minute. Prewarmed CTAB/NaCl solution was added before vortexing, addition of a solution containing chloroform/isoamyl alcohol and centrifugation at 12 000 g. DNA in the supernatant were precipitated with isopropanol, washed with 70% ethanol, dried and eluted in TE buffer. Genomic DNA was fragmented by nebulization with nitrogen before it was purified with Qiagen MiniElute PCR purification kit. Fragment ends were repaired and linked to an adaptor. Samples were added to AMPure beads, after small fragments removal. Emulsion PCR was conducted as described by Roche, following library enrichment and sequence primers annealing. Samples were loaded into picotiter plate before sequencing was performed using the GS Junior equipment. Plasmid sequence was assembled using proprietary software from Roche.

**Results and discussion:** The plasmid has 56 625 bp and is highly similar to pB10 plasmid isolated from waste-water treatment plan in Germany –GenBank AJ564903.1. It has a complete mating-pair-formation *trbA-trbP* operon, a class 1 integron, with sulfonamides, quaternary ammonium and kanamycin (*aacA4*) resistance genes. The *aacA4* gene is the only difference when comparing this sequence to that pertaining to the class 1 integron from plasmid pB10. It has a complete conjugative transfer operon genes *traC-traO*, genes for regulation of plasmid functions or stable inheritance – *korA*, *korB*, *korC*, *incC2*, *krfA*, *kleAEF* and *klcAB*. It has an insertion sequence element IS1071 but lacks the Tn1721-like tetracycline resistance transposon observed in pB10. pBRA100 has a truncated Tn5393c streptomycin-resistance transposon and a Tn501-like mercury-resistance transposon.

**Conclusion:** Brazilian outbreak strain *M. abscessus* subsp. *bolletii* F1725 has an Inc-P1beta plasmid that codes for aminoglycosides and mercury resistance.

**P1963** BCG S4-Jena strain: influence on the molecular mechanism of bladder cancer cell lines

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**Objectives:** The adjuvant intravesical therapy of non-muscle-invasive bladder cancer with mycobacteria (*M. bovis* var. BCG) is the gold standard for treatment of “high-grade” cancer (NMIBC) according to EAU guideline. The effects of BCG therapy on bladder cancer on molecular level are poorly understood.

**Methods:** Two permanent cell lines were cocultured either with viable or with heat-inactivated BCG (strains S4-Jena; OncoTICE, Apogepha). Proliferation was tested by cell proliferation reagent WST-1. Cell cycle arrest (propidium iodide DNA staining) and apoptosis (annexin V/propidium iodide staining) were analyzed by flow cytometry. Additionally, morphological hallmarks of apoptosis were shown by confocal laser scanning microscopy. BCG products were measured by HSQC-NMR and MS-spectrometry.

**Results:** The exposition of non-muscle-invasive bladder cancer to BCG has a direct impact on cancer cells and is not based on indirect immune stimulation exclusively. The first essential step is the adherence and followed by internalization of BCG into the bladder cancer cells. There was significant difference between living and heat-inactivated BCG. Furthermore it could be demonstrated that the internalization of



BCG by the cells is an active process. This process leads to inhibition of cell proliferation. As demonstrated by various methods this process is mainly based on apoptosis. The induction of apoptosis is correlated with IL-6 expression and triggers the internalization of BCG in other bladder cancer cells. In contrast to TICE BCG S4-Jena influences both cell lines. The inclusion of BCG into the cancer cell endosomes leads to their transformation into lysosomes with a decreased pH of 4.5-5. Because of the decreased pH-value BCG produces substances, which could be responsible for apoptosis. Regarding this the BCG strain S4-Jena also showed a greater effect than TICE.

**Conclusion:** The greater apoptotic effect of BCG S4-Jena on both examined cancer cell lines proves that S4-Jena is better suitable than TICE and probably other commercially available BCG strains. Furthermore the gene signature of BCG exposed cells reveals a base for the specific application of BCG in the adjuvant intravesical therapy of NMIBC to differentiate between responders and non-responders. Another aim of research should be the development of focused responder/non-responder array for the theranostic of NMBIC.

**P1964 Comparison of two PCR systems for the detection and management of tuberculosis infection**

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**Objectives:** The aim of the study was to undertake a comparative evaluation of the GeneXpert system and a novel assay, "Detect Ready", in diagnosing tuberculosis in the setting of a large London teaching hospital.

**Methods:** The Detect Ready assay uses one of the MIRU-VNTR typing targets to detect *Mycobacterium tuberculosis* in a variety of samples. A 44 bp region was amplified, using SYBR green for detection and melt curve analysis in a real time PCR with internal inhibition control. Molecular specificity was tested against seven non tuberculosis *Mycobacterium* spp. and 11 respiratory bacterial species, both pathogenic and commensal. The molecular sensitivity of the test was assessed by dilution endpoint analysis. one hundred and four samples were collected over a 6 month period; extraction following a heat killing stage 1 µL of sample was used directly in the PCR with no additional. Any inhibited samples were repeated using 1:10 and 1:100 dilutions. Clinical data regarding treatment of tuberculosis in these patients was also collected. The sensitivity, specificity, PPV and NPV were calculated with clinical treatment as the gold standard.

**Results:** The Detect Ready assay had a molecular sensitivity of 10 genome equivalents and cross-reacted with *M. kansasii*, *M. bovis* and *M. goodii* but no other respiratory pathogens or flora. Of the samples tested, 24 were positive by at least one routine diagnostic method and 76 were negative by all methods. One case of rifampicin resistance was detected by GeneXpert and confirmed by culture. Thirty patients were treated for tuberculosis. Nine with a negative smear result were positive by another method. Fourteen samples had discordant results among the other diagnostic tests. Five samples were culture positive for non tuberculosis mycobacteria, three were *M. kansasii*, one *M. chelonae* and one *M. triviale*, the only species that was detected by both PCR methods was *M. triviale*.

	Sensitivity	Specificity	PPV	NPV
Smear	53%	100%	100%	84%
Culture	60%	97%	90%	85%
Gene Xpert	60%	100%	100%	86%
Detect ready	50%	97%	88%	83%

**Conclusion:** Although the Detect Ready showed a slightly lower sensitivity it has the advantage of being able to be transferred onto different PCR platforms, both real time and gel based. The reagents can also be used for other real time assays, and are much cheaper than those of probe-based assays. The technical requirements are relatively undemanding and the simple extraction makes it very time efficient. The main disadvantage in comparison to the Gene Xpert is its inability to detect rifampicin resistance.

**P1965 Molecular analysis of human isolates belonging to *Mycobacterium avium* complex collected from 2003 to 2009 in the Czech Republic**

M. Slany\*, I. Slana, V. Ulman, E. Kalakayova, I. Pavlik (Brno, Ostrava, CZ)

**Objectives:** Members of *Mycobacterium avium* complex (MAC) are widespread in the environment, and have been isolated from many avian and mammalian species including man. The MAC includes two members, *M. avium* subsp. *avium* (MAA) and *M. a. hominissuis* (MAH) frequently associated with human diseases. The main sources of MAA infection are considered domesticated bird, while MAH is mainly acquired from soil. Avian mycobacteriosis is becoming disseminated within vulnerable populations (patients with HIV or chronic diseases). The aim of the present study was to determine distribution of the insertion sequence IS901 (MAA specific locus) in mycobacterial strains isolated from human patients in Moravian region identified as members of MAC by GenProbe.

**Methods:** A total number of 242 MAC isolates (124 patients) collected during years 2005–2009 were put to detailed analyses. Sputum samples were homogenized, decontaminated with sodium laurylsulfate, and cultured on Löwenstein-Jensen medium. DNA isolated from single bacterial colony was subjected to triplex qPCR based on insertion sequences IS901 and IS1245 according to the previously described method by Slana *et al.* (2008). Simultaneous sequence analysis (16S rRNA, ITS and hsp65) of IS901 and IS1245 negative isolates was carried out.

**Results:** The prevalence of IS901 (7%) was low, because only few patients included in the study reported close contact with birds (pigeons or hens). Other mycobacteria detected within analyzed group (124 patients) were: MAH (69%), *M. intracellulare* (18%) and newly described MAC members (6%; *M. arosiense*, *M. chimera*, *M. colombiense*, *M. marseillense*).

**Conclusion:** MAC members are second common cause of pulmonary mycobacterioses after *M. tuberculosis*. The most frequent MAC causing pulmonary infections was MAH. This fact was in accordance with published data. Low prevalence of MAA could be explained by only occasional contact of humans with infected birds, while exposition to contaminated soil or aerosols with MAH is more frequent. Triplex IS901/IS1245 qPCR is able to detect and differentiate between two etiological agents (MAH, MAA).

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**P1966 Characterisation of a novel gene, Rv2787, encoding ATPase in *Mycobacterium tuberculosis***

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**Objectives:** To clone, express and characterization of Rv2787 gene encoding ATPase activity in *M. tuberculosis*.

**Methods:** The Rv2787 gene from *M. tuberculosis* H37RV was amplified by PCR and cloned into a bacterial expression vector, pET15b, resulting in histidine-tagged Rv2787 recombinant gene. The plasmid was then transformed to *E. coli* BL21 (DE3) strain for protein expression. After, protein was purified with an affinity nickel column His-Trap-Chelating. The Rv2787 was further confirmed by Western blot and immunodetection with anti-histidine antibody. The purified recombinant proteins were renatured with dialysis against 50 mM Tris-HCl, pH 7.8 at 4°C for three times changes. The proteins were subjected to ATPase activity assay.

**Results:** The amplified products of Rv2787 gene with the corresponding length of 1764 bp was successfully clone into

expression vector pET15b. The optimal condition for the protein expression was at 30°C after induced by 1 mM isopropyl-D-thiogalactopyranoside (IPTG). The histidine-tagged Rv2787 was founded in SDS-PAGE gel electrophoresis with its corresponded molecular weight 64 kDa and maximum expression was detected at 4 hours after IPTG induction. Western bolt analysis of expressed protein was detected as a single band on nitrocellulose membrane with anti-histidine antibody. The enzyme activity was observed as  $K_m$  and  $V_{max}$  as being 43  $\mu\text{mol}$  and 16  $\mu\text{mol}/\text{minute}/\text{mg}$  of protein.

**Conclusion:** Our data showed that the Rv2787 can be expressed as recombinant proteins in *E. coli* BL21 (DE3) pLysS and it exhibited the ATPase activity. The studies here lay the groundwork for further investigation of the Rv2787 ATPase involved in chromosome partitioning processes or ion transporting system that are an important processes in *M. tuberculosis* for surviving and adaptation in host cells. Future functional analysis of Rv2787 will provide information that can be applied for development of anti-tuberculous agents affecting chromosome partitioning processes or ion transporting system.

## Infection control (miscellaneous)

### P1967 Readiness for infection control practice implementation: a survey among 15 European hospitals

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**Background:** The aim of PROHIBIT (Prevention of Hospital Infections by Intervention and Training) is to prevent healthcare-acquired infections (HAI) by understanding policies and best practices and by testing the effectiveness of two interventions to reduce catheter-related bloodstream infection (CRBSI) in intensive care units in 14 European hospitals. Work package four applies qualitative research to identify barriers and facilitators for implementing best infection prevention (IP) practices in six hospitals.

**Method:** Onsite investigators of the 14 intervention hospitals were invited to complete a 51-item online questionnaire with the following dimensions on a hospital and ICU level, respectively: annual clinical activity, staffing, status; organization and safety culture; quality of care and IP surveillance and improvement programs. Five-point Likert-scales were applied for opinion questions; all other questions could be replied by yes/no or a number. Scales were asymmetrically transformed to binary variables for analysis (agree and strongly agree = 1; remainder = 0). Each questionnaire was followed by a 30–45' semi-structured telephone interview.

**Results:** The response rate was 100%. The hospitals featured a median of 885 (range, 325–2167) beds and 2437 (572–7593) employees, of which 1095 (283–4200) were nurses, and 422 (90–1200) physicians. ICUs had a median of 23 (10–44) beds cumulating 5888 (2048–12 311) annual patient-days, 792 (336–3250) admissions, 2708 (640–8620) ventilator days and a mean nurse-to-patient ratio of 1:2.5. Hand hygiene

(HH) monitoring was performed by handrub/soap consumption in 8/14 ICUs, by observation in 11. HH is actively promoted in 13. CRBSI surveillance was performed in 12, while prevention programs were implemented in 8. Five onsite investigators would rank the overall support by the hospital administrative leaders as very good to excellent, the remainder as good (see Table 1 for more results).

**Conclusions:** The population is biased towards large tertiary care hospitals. Implementation of IP practices is difficult in many hospitals, most likely because of staffing and budget restrictions rather than leadership support. Most hospitals have established surveillance and promotion programs. In-depth inquiry in a purposefully chosen sample will deepen our understanding of the readiness of European hospitals for successful IC-programs.

### P1968 PROHIBIT – assessment of European practices in nosocomial infection control: reimbursement schemes and public interest

M. Martin\*, W. Zingg, C. Wilson, S. Hansen, P. Gastmeier, D. Pittet, M. Dettenkofer and the PROHIBIT consortium

**Objectives:** PROHIBIT “Prevention of Hospital Infections by Intervention and Training” aims to analyse influencing factors on implementing of infection control measures by European hospitals.

**Methods:** The European Centre for Disease Prevention and Control (ECDC) HAI surveillance National Contact Points (NCP) and HAI experts in 34 countries (27 EU member states – whereby UK counts as four countries, Croatia, Iceland, Norway and Switzerland) were invited to complete a questionnaire about financing of infection control (IC) activities in hospitals and the perception of HAI in the public. In a previous questionnaire the NCPs were asked to provide information about national guidelines for HAI prevention, national HAI surveillance systems, and policies of public reporting.

**Results:** Thirty-one of 34 NCPs (91%) completed the questionnaire. All but four countries issue IC measures by law. Seven European countries provide funding for infection prevention and control (IPC) programmes. Three countries reported specific incentive schemes for additional IC efforts. In three of 31 countries attributable costs of nosocomial infections are reimbursed. No European country established a pay-for-performance scheme, so far. HAI were taken up repeatedly by national media over the past 10 years, with only two NCPs reporting no public interest in this topic. Headlines in nation-wide media mostly covered multiresistant bacteria, especially MRSA, *C. difficile* and outbreaks of noroviruses. Half of the NCPs reported substantial influence of patient organisations on political decision making concerning HAI. HAI-surveillance-systems for public hospitals are established in 82% (27/33). Eight countries have established public reporting of HAI data from individual hospitals.

**Conclusions:** The majority of countries in Europe issue infection prevention and control programmes by law, but only seven countries provide funding for such activities in hospitals. On the other hand, attributable costs for HAI are not reimbursed; but no deductions for HAI or any pay-for-performance schemes are in place in European countries either. Hospitals in Europe may not be encouraged to implement IPC programmes as such activities are not financed and the benefits of prevention are difficult to calculate. However, factors such as negative media coverage or publication of HAI rates may still drive hospitals towards better IPC performance.

### P1969 Litigation and health-care associated infections in the English national health system (NHS)

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**Objectives:** Legal claims for clinical negligence, including those related to acquisition and management of Health-care Associated Infections (HCAIs) have been increasing in England.

**Methods:** We assessed costs of HCAI litigation by reviewing claims registered under the Clinical Negligence Scheme for Trusts (CNST) that

Statements	Number of respondents answering "Agree" or "Strongly agree"
It is very easy in my hospital to implement evidence-based recommendations to prevent healthcare-associated infection (HAI)	3 of 14
I receive a great deal of support from the leaders of my hospital when I want to implement an evidence-based recommendation to prevent HAI.	7 of 14
Leadership is driving us to be a safety-centered institution.	9 of 14
Staff members in my hospital are receptive to change in clinical processes.	4 of 14
Staff members in my organization have a sense of personal responsibility for improving patient care and outcomes.	10 of 14

mentioned methicillin-resistant *Staphylococcus aureus* (MRSA) or *Clostridium difficile* (CDI).

**Results:** Between 2001 and 2010 there were 252 claims mentioning CDI, of which 172 have been closed. Of these 172 closed claims, 130 (76%) were awarded costs and 42 (24%) were unsuccessful. Eighty claims were still open at time of analysis. Ninety-one cases (52%) resulted (directly or indirectly) in patient death. Mean damages paid were £20 357 (95%CI 14 505–26 210), mean defence costs were £4967 (95%CI 3605–6329) and mean claimant costs were £12 986 (95%CI 9831–16 141). Mean total legal costs were £29 649 (95% CI 22 249–37 048).

Between 1996 and 2010 there were 734 claims mentioning MRSA, of which 606 have been closed. Of the 606 closed claims, 388 (64%) had costs awarded and 218 (36%) were unsuccessful. One hundred and twenty-nine claims were still open at the time of analysis. 93 cases (15%) resulted (directly or indirectly) in patient death. Mean damages paid were £74 881 (95%CI 56 961–92 802), mean defence costs were £12,825 (95%CI 11 072–14 578), mean claimant costs were £37 664 (95%CI 31 343–43 984) and mean total costs were £82 469 (95%CI 65 981–98 956). The total number of successful claims per MRSA bacteraemia has decreased from a peak of 0.0082 in 2004/5 to 0.0005 in 2009/10.

There is evidence that recent significant declines in MRSA bacteraemia and CDI rates in England have been associated with reduction in HCAI negligence claims.

**Conclusion:** HCAs are associated with significant costs to society, individual patients and their families and healthcare organisations. Litigation is a significant but often overlooked cost of HCAI. Between 1996 and 2010, claims mentioning MRSA or CDI resulted in total litigation payments of £35.2 million. This does not include costs of abandoned claims. The recent significant reduction in HCAs due to MRSA and CDI in England has been associated with a potential reduction in HCAI litigation claims and costs. Further work is needed to confirm that this is a true casual association.

#### **P1970** Delivering an integrated programme to support improvement in independent sector nursing and residential homes – a key element in healthcare associated infection improvement across the whole health economy in Northern Ireland

L. Geoghegan\*, L. Patterson, G. Smyth, C. McGeary (Belfast, UK)

**Objective:** To describe the main components of an integrated programme supporting Healthcare Associated Infection (HCAI) improvement delivered to Independent Sector (IS) providers and to report main parameters agreed for continuous programme evaluation.

**Methods:** In April 2010 the HCAI Team established a programme delivering enhanced surveillance of *Clostridium difficile* infection (CDI) in community and primary care settings through the Health Protection Duty Room service. All suspected enteric outbreaks in community settings are also reported to the duty room. Our integrated programme delivering HCAI improvement support to IS nursing and residential homes includes – dedicated facility visits for all CDI cases and all enteric outbreaks; integrated CDI and outbreak surveillance information supporting all facility visits; competency based training and assessment of HCAI nursing services provided; twice weekly risk review meetings; weekly sharing of recommendations for further action with the regulator (Regulation and Quality Improvement Authority); twice monthly programme development meetings; integrated Root Cause Analysis (RCA); and establishment of a formal ‘‘link system’’ across IS providers. This paper will outline the main findings arising from detailed evaluation of the first three months of programme delivery (October through December 2011).

**Results:** Information presented will include activity related information (e.g. facility visits completed, HCAI nursing hours dedicated), themes identified for further action (e.g. assurance of best practice, information sharing), quality of support provided (e.g. competency assessments completed), capacity challenges addressed, learning arising from RCA, and team communications.

**Conclusion:** The HCAI Team within PHA has established and is delivering a unique programme of HCAI improvement support to IS nursing and residential homes across NI. There is early evidence to suggest that this programme has contributed to the prevention of a CDI outbreak and has shortened the duration of gastroenteritis outbreaks.

#### **P1971** A cluster of *Propionibacterium acnes* infection in post neurosurgical patients

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**Background:** *Propionibacterium acnes* generally considered a skin commensal has been recognised as a cause of post-operative infection in a number of surgical contexts, and is reported as one of the more common organisms responsible for neurosurgical, and more specifically post-craniotomy infections. We report the investigation and measures taken during a cluster of *P. acnes* intracranial infections following craniotomy in a large tertiary care hospital.

**Method:** Between July and October 2010, a cluster of nine cases of *Propionibacterium acnes* infection of the bone flap following craniotomy for tumour were identified. A series of measures and investigation were undertaken look into this cluster. All the isolates were genotyped by pulsed-field gel electrophoresis with endonuclease SpeI restriction enzyme.

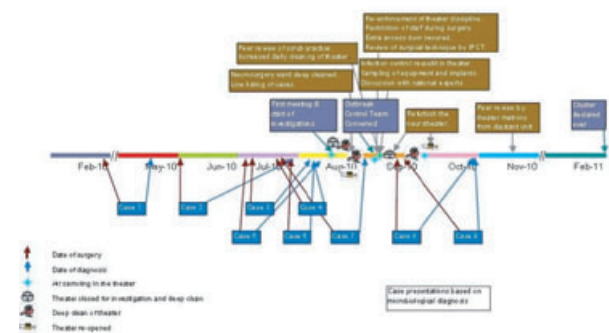
**Results:** *Propionibacterium acnes* infections accounted for 53% (nine cases) of craniotomies performed during January–October 2010. All these patients required repeat surgery to evacuate pus and infected tissue. The mean age of cases was 57 years. All cases were administered pre-operative prophylactic antibiotics and these isolates were fully sensitive to the antibiotics.

Control measures taken included audit of infection control practices and theatre practice, restriction of staff movements, adherence of theatre discipline. Investigations like air sampling, sterility checks and staff questionnaire for any skin conditions did not identify any concerns. The Pulse Field Gel Electrophoresis (PFGE) analysis showed that *P. acnes* isolates were unrelated. No further cases occurred during that year. Our intensive efforts failed to identify any single root cause source of the outbreak.

**Discussion:** *Propionibacterium acnes* is an emerging pathogen in post neurosurgical infections. There have been no clusters of *P. acnes* infection following craniotomy described in the literature.

This cluster is unusual because of the large number of cases occurring in a short period of time. Investigation of the theatre environment and audit of the postoperative care of patients did not identify any specific concerns.

Figure 1: Chronology of case presentations and key management interventions



**Conclusion:** The most likely origin of *P. acnes* is from endogenous scalp flora. Standardisation of pre-operative skin preparation and post surgical wound care need further investigations. No single factor was identified to which the cluster could be attributed. Maintenance of infection control standards were thought to be important in containing the cluster.

**P1972** Should lower respiratory tract secretions from intensive care patients be systematically screened for influenza virus during the influenza season?

M. Giannella\*, B. Rodriguez-Sanchez, P. Lopez Roa, P. Catalan, P. Muñoz, D. Garcia de Viedma, E. Bouza on behalf of the Gregorio Marañón Task Force for Pneumonia GANG

**Objective:** To assess the burden of influenza in adult intensive care units (ICUs) and the rate of overlooked and nosocomial cases during the influenza season.

**Methods:** Prospective study of adult patients admitted to three ICUs of our hospital from December 2010 to February 2011. All tracheal aspirates (TA) sent for suspicion of lower respiratory tract (LRT) infection were systematically tested for influenza. We defined influenza as unsuspected if testing was not requested and patient was not on empirical antiviral therapy after sample collection. Influenza was classified as nosocomial if symptoms started after the first 72 hours of hospital admission.

**Results:** We received TA from 100 patients with suspected LRT infection. Bacteria, viruses, and *Aspergillus* spp. were identified in 37, 30, and three patients, respectively. No significant microorganisms were found in the remaining 30 patients. Influenza was detected in 28 of the 30 patients with viral infection. Influenza was classified as unsuspected in 15 (53.6%) and as nosocomial in 11 (39.3%) patients. Compared to patients with suspected influenza, those with unsuspected influenza were more commonly admitted to the surgical ICU (40% vs. 0%,  $p = 0.001$ ), were classified as having nosocomial influenza (33.3% vs. 7.7%,  $p = 0.002$ ), and received antiviral treatment later after symptom onset (median 9 vs. 2.5 days,  $p = 0.001$ ). Overall, in-hospital mortality of patients with influenza was 60.7%. We could not demonstrate higher mortality among patients with unsuspected or nosocomial influenza.

**Conclusions:** During the influenza season, almost one-third of critical patients with suspected LRT infection had influenza; in 53.6% of them the influenza was unsuspected. Microbiology departments should consider including systematic influenza testing in LRT secretions from adult ICUs during influenza seasons.

**P1973** Management of a scabies outbreak in a university hospital

Y.K. Veenstra-Kyuchukova\*, G. Gezelle Meerburg, S.H. Kardaun, J.P. Arends (Groningen, NL)

**Objectives:** Scabies outbreaks in healthcare settings are difficult to control and associated with substantial work load. We describe the outbreak of scabies in the University Medical Center Groningen (UMCG), a hospital with 1339 beds, and the infection control measures that were taken to control further transmission.

**Methods:** The outbreak of scabies occurred at two intensive care units (ICUs) and one general ward of the UMCG.

Diagnosis of scabies was based on clinical presentation and identification of mites/eggs by skin scrapings by a dermatologist. Patients and healthcare workers (HCWs) regarded at risk and presenting with pruritus and rash, were considered to potentially have scabies.

**Results:** 21 July 2011 two HCWs were diagnosed with scabies by a dermatologist. This initiated establishment of a multidisciplinary Crisis Intervention Team (mCIT) and declaration of a scabies outbreak.

The index patient, a lung transplantation patient admitted for several months to two different ICUs and a ward in our hospital, was identified and diagnosed with crusted scabies, immediately treated, transferred to a single room, and nursed in strict isolation. All HCWs from the three affected wards were informed and offered consultation by a medical officer and, in case of complaints, a dermatologist. HCWs at risk or having complaints were offered treatment with ivermectin. They were ordered to use disposable aprons with long sleeves and gloves during contact with patients for 6 weeks. Seven HCWs were diagnosed infested and 40 considered potentially infested, based on clinical features. These were treated simultaneously with their family and prohibited from work for 24 hours. Moreover in total 183 HCWs preventively received ivermectin or, if pregnant, benzyl benzoate.

Patients that were exposed to the index patient on the same ward or were nursed by the same HCWs were traced as of 8 April 2011, the date of transplantation. Four hundred and sixty Patients and their general practitioners were notified. Five patients were treated preventively. 31 August 2011 the outbreak was considered under control.

**Conclusion:** Management of a scabies outbreak in a hospital requires immediate formation of a mCIT. The index patient has to be identified and treated. Intensive infection control measures, mass screening, treatment when in doubt, and proper internal and external communication are key factors to control an outbreak.

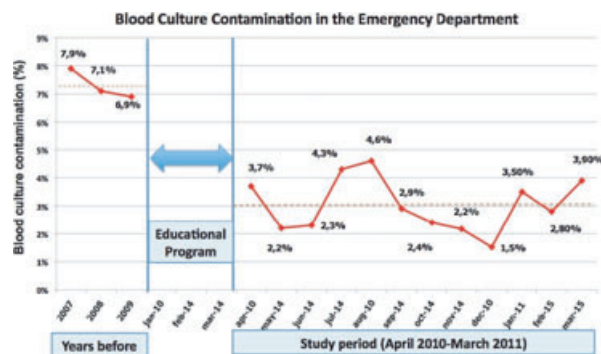
**P1974** Decreased frequency of blood culture contamination in an emergency department through the diffusion of a protocol among nursing staff

A. Garcia-Reyne\*, A. Igarzabal, A. Lalueza, J.M. Herrero-Martinez, J. Villa, M. Fernandez-Ruiz, B. de Dios, F. Lopez-Medrano, R. San-Juan, J.M. Aguado, F. Sanz (Madrid, ES)

**Objectives:** Blood culture contamination increases laboratory work and costs, prolongs lengths of patient stay and leads to inappropriate or unnecessary antibiotic use. All of these problems increase patient morbidity and hospital costs. The blood culture contamination rate in the emergency room of our hospital was much higher than the 3% recommended. The aim of this study was to decrease this contamination rate by conducting an education program for nursing staff.

**Methods:** First, we developed a blood culture protocol drawn following the current recommendations. The most important steps we included were (i) Hand hygiene with alcohol-based hand rub; (ii) The use of alcoholic 2% chlorhexidine as antiseptic, (iii) The performance of a sterile technique, (iv) Proper labelling of the samples, (v) Adequate registry of the extraction in the computer system and (vi) Proper handling and transport form of the blood culture bottles. Subsequently, between January and March 2010, there was a series of educational talks with all the nursing staff from the Emergency Department of our hospital, for the implementation of the protocol. Between, April 2010 and April 2011, we prospectively collected the blood culture contamination rates.

**Results:** In the following months, after the educational program, there was a significant decrease in blood culture contamination. The mean contamination rate of the 3 years before the intervention was 7.3% and the year after the educational program was 3% ( $p < 0.0001$ ). Increase in the months of July and August was attributed to the incorporation of temporary personnel who had not been instructed in the protocol. The number of blood cultures collected the years before the educational program and the study period were similar.



**Conclusions:** The implementation of a protocol for the extraction of blood cultures and appropriate diffusion is a very effective method to decrease the rate of blood cultures contamination. This information should be regular and given to the new nursing staff.

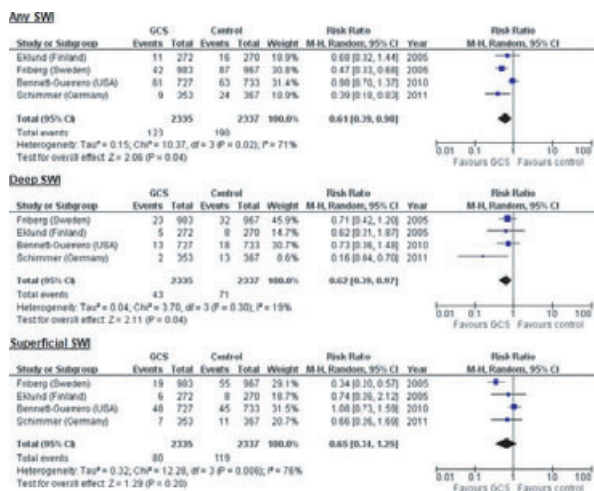
### P1975 Gentamicin collagen sponges for the prevention of sternal wound infection: a meta-analysis of randomised controlled trials

M. Mavros, P. Mitsikostas, V. Alexiou, G. Peppas, M. Falagas\* (Athens, GR)

**Objectives:** To determine if the application of gentamicin collagen sponges (GCS) reduces sternal wound infections (SWI) in cardiac surgery patients.

**Methods:** Meta-analysis of randomized controlled trials (RCTs).

**Results:** Four RCTs were eligible for inclusion. Pooling data from four RCTs (4672 per-protocol patients), GCS reduced deep SWI (relative risk [RR] = 0.62, 95% confidence intervals [CI]: 0.39–0.97,  $p = 0.04$ ) and any SWI (RR = 0.61 [0.39, 0.98],  $p = 0.04$ ). In contrast, no benefit was demonstrated regarding superficial SWI (four RCTs, 4672 patients, RR = 0.65 [0.34, 1.25],  $p = 0.20$ ) and all-cause mortality (three RCTs, 3994 patients, RR = 0.90 [0.57, 1.42],  $p = 0.66$ ). Based on data from two RCTs (3410 patients), GCS also appeared to reduce surgically treated SWI (RR = 0.59 [0.41, 0.86],  $p = 0.005$ ). The most commonly isolated pathogens were coagulase-negative *Staphylococcus* spp. (38.8%) and *S. aureus* (29.8%).



**Conclusion:** GCS appear to reduce SWI rate in cardiac surgery patients. The statistical heterogeneity among the existing trials underlines the need for additional large, high-quality RCTs.

### P1976 Endogenous and nosocomially transmitted exogenous *Clostridium difficile* infections in a low-prevalence setting

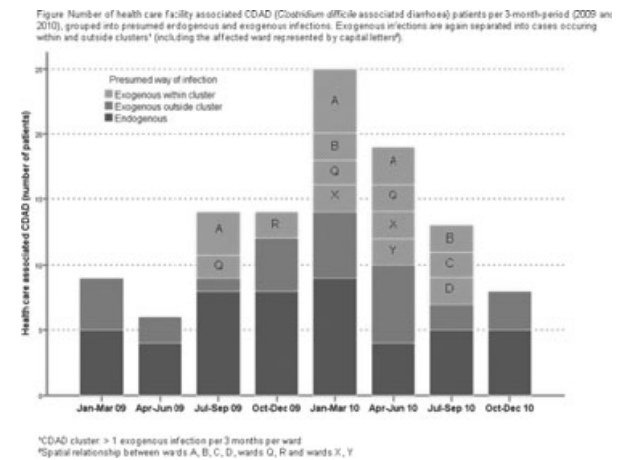
P. Kohler\*, A. Bregenzler-Witteck, P. Rafeiner, M. Schlegel (St. Gallen, CH)

**Objective:** In our institution, a tertiary-care hospital (700 beds) in Eastern Switzerland, only CDAD (*Clostridium difficile* associated diarrhoea) patients with stool incontinence or non-adherence to hygiene measures are being contact isolated. In order to evaluate our strategy, which is in contrast to current guidelines recommending contact isolation for every CDAD patient, we implemented a CDAD surveillance program discriminating exogenous (nosocomially transmitted) from endogenous infection.

**Methods:** We included every CDAD patient hospitalized in 2009 and 2010. Data on patients' characteristics, known risk factors for CDAD and all patient transfers (ward and room changes with date) between admission and discharge were collected retrospectively. Patients with health care facility associated (HCFA) CDAD were grouped into presumably exogenous and endogenous infections. Patients were considered infectious for a 14-day-period after symptom onset. Exogenous CDAD was postulated for patients who shared the room or the ward with an infectious patient before symptom onset, either at

the same time or within 30 days after discharge of the infectious patient. A CDAD cluster was defined as two or more exogenous infections on the same ward within 3 months.

**Results:** We registered 141 CDAD cases in the 2-year-period. 108 (76.6%) were HCFA, corresponding to an overall rate of 2.3/10 000 patient days. Per 3-month period, the number of presumably endogenous HCFA CDAD remained relatively stable over time, whereas the number of potential exogenous infections varied substantially, corresponding to the number of CDAD clusters (Fig. 1). Exogenous infections occurred most often through indirect patient contact (same ward, but not same room). Patients with exogenous infection were significantly older (median age 70.4 vs. 62.8 years,  $p = 0.04$ ), were more likely to have a Charlson Score >1 (92% vs. 67%,  $p = 0.001$ ) and tended to be less exposed to antibiotics prior to infection (duration >10 days, 42% vs. 60%,  $p = 0.05$ ).



**Conclusion:** Our overall CDAD rate of 2.3/10 000 patient days is clearly below the European average of 4.1. However, multiple ward clusters of exogenous infections suggest that our current infection control strategy fails to prevent indirect transmissions. Elderly patients with a higher comorbidity score are particularly prone to exogenous *C. difficile* acquisition. Consequently, we consider an intensification of our current infection control measures.

### Hand hygiene: what else?

#### P1977 Use of a novel tool for monitoring and improving hand hygiene in a surgical intensive care unit

E. Segal\*, D. Ben-David, A. Vatouri, P. Shleffer, D. Tau, Z. Rosenberg-Gilad, E. Hayam (Tel Aviv, IL)

**Objectives:** Hand Hygiene (HH) practices have been shown to impact outcome in critically ill patients and in surgical patients due to the effect of hand hygiene on nosocomial infection and on surgical site infections. The goal of improving HH is a constant challenge for clinicians, managers and infection control professionals yet despite instructions, training, monitoring and promotion of optimal HH practices, compliance continues to be lower than expected in many hospitals. We assessed the impact of a novel technology – a hand worn wrist watch like tool which provides immediate feedback to the clinician regarding HH (Hyginex Ltd, Tel Aviv, Israel) – to impact clinician's behavior and improving HH compliance.

**Methods:** Hyginex Ltd, developed a unique system to improve compliance with HH policies. The system consists of wrist worn bracelets similar to a wristwatch, and various sensors distributed in the clinical work area e.g. The bed spaces of the ICU and the general work areas. There are sensors in all the alcohol dispensers and the area is also networked with antennas which enable detection of the clinician wearing the wrist bracelet. Thus the system detects the presence of a

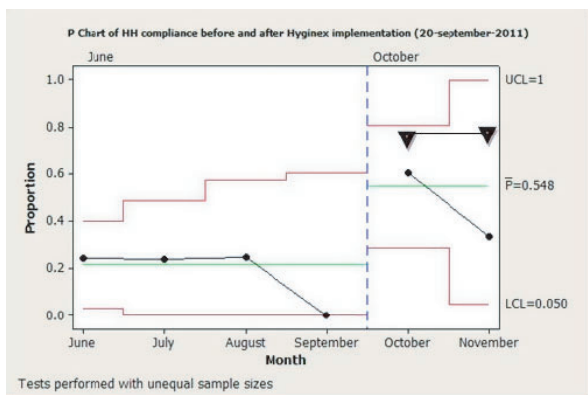
clinician at the bedside as well as the utilization of alcohol or soap, and the time spent on hand hygiene.

We compared the data from the Hyginex system to surveillance performed by infection control nurse and other nurses trained in surveillance of HH practices. We followed the compliance with a P-chart of the HH compliance as a proportion of the total number of observed incidences over a 6 month period compared to 2 months with the Hyginex system.

The surveillance was performed using the iScrub application on a smartphone or Ipad touch.

Control charts of the data were generated using Minitab statistical software.

**Results:** The compliance of the staff to HH was low on initial assessment of in/out monitoring. The average compliance before patient contact was 25%. After introduction of the Hyginex system the compliance went up to 44%. Measurements by the Hyginex system demonstrated an overall compliance of 75% (Fig. 1). This probably overestimates true compliance, but still has a recognizable effect on the standard surveys.



**Conclusions:** The implementation of Hyginex system had a favorable effect on HH as measured by surveillance monitoring. Further study of this technique will enable evaluation of the impact this may have on other compliance indicators and on nosocomial infections.

#### **P1978** Improvement of hand hygiene adherence in physicians after a hospital-wide campaign

P. Kohler\*, C. Kahlert, S. Simonet, G. Rettenmund, B. Schöbi, P. Rafeiner, M. Schlegel (St. Gallen, CH)

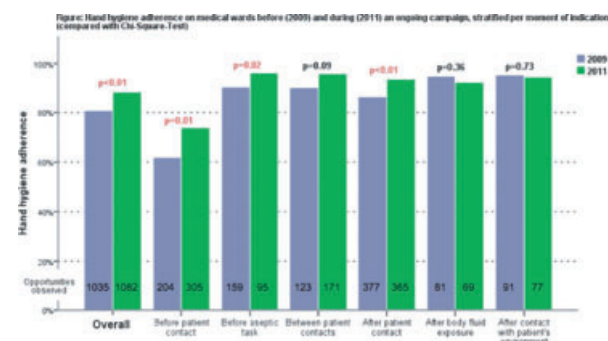
**Objective:** Hand hygiene is the most important single measure in preventing nosocomial infections. In 2009, we found at our 700 bed tertiary care hospital in Eastern Switzerland, a good overall adherence of 84%, except for the indication ‘before patient contact’. This prompted us to start a hand hygiene campaign. We present data of the medical wards before and at the end of a campaign.

**Methods:** The hospital wide, multimodal intervention included education and posters on the door of the patient rooms. Data on adherence were collected by direct observation at the end (spring/summer 2011) of the campaign. Electronic data collection by mobile phone allowed immediate presentation of adherence results to the observed health care workers.

Hand hygiene adherence was defined as percentage of the performed opportunities in relation to the total of opportunities. In addition to the five moments of the WHO, the opportunity ‘between patient contact’ (in case of multi-bed room and direct movement from one to another patient) was measured.

**Results:** Two thousand one hundred and seventeen opportunities in 736 physicians and 1381 nurses from 10 wards were observed. Overall adherence increased from 83.9% (858/1035) in 2009 to 90.1% (957/1082) in 2011 ( $p < 0.001$ ). Physicians improved significantly from 77.1% (168/218) to 90.7% (470/518),  $p < 0.001$ , whereas the

adherence of nurses remained unchanged (84.5% [690/717] to 86.3% [487/564],  $p = 0.3$ ). Physicians showed an increased adherence to the opportunities ‘before patient contact’ (from 53.7% [36/76] to 80.4% [156/194],  $p < 0.001$ ) and ‘after patient contact’ (from 85.5% [65/76] to 98.8% [170/172],  $p < 0.001$ ). Nurses improved their adherence to the opportunity ‘before aseptic task’ from 81.2% (125/154) to 92.6% (87/94),  $p = 0.01$ .



**Conclusion:** In hospitals with pre-existing high adherence to hand disinfection, additional improvement for specific professional groups and specific opportunities is feasible.

According to our experience, immediate feedback of results after observation is highly estimated and allows to identify misconceptions.

#### **P1979** Mixed messages: concurrent feedback of hand-hygiene compliance and handrub consumption seen from the hospital ward perspective

A. Stewardson\*, S. Touveneau, S. Longet-Di Pietro, N. Vernaz, W. Zingg, D. Pittet, H. Sax and the members of the Infection Control Program, University of Geneva Hospitals

**Background:** Evaluation and feedback of hand hygiene performance data is one of five components of the WHO Multimodal Hand Hygiene Improvement Strategy; with increased hand hygiene compliance through direct observation (DO) and increased usage of hand hygiene products (UP) cited as key success indicators. We compared performance feedback data produced by these two methods as seen from the ward perspective.

**Method:** At a 2200 bed tertiary-care facility, eight validated infection control nurses performed DO using the WHO ‘My 5 Moments’ method in 65 non-ICU acute care wards for 24 months from April 2009. Alcohol-based handrub usage and patient-days per ward were extracted from pharmacy and administrative databases to calculate UP (L/1000 patient-days) over the same period. Hand hygiene compliance (as percentage and quartile) and UP quartile were calculated for each ward for each 6-month period in order to simulate a realistic performance feedback scenario. Quartiles were employed to facilitate comparison between the two methods and to convert crude UP data into a more meaningful performance indicator. Descriptive statistics were used to demonstrate the concordance in quartile assigned by each method, and also the direction in change between consecutive periods (increase, unchanged or decrease). The null hypothesis that hand hygiene compliance is the same in wards in different quartiles of UP was tested for each 6-month period by the Kruskal–Wallis rank test.

**Results:** During the entire study period, 7719 HH opportunities were captured, and 22, 129L of alcohol-based handrub were used during 718 041 patient-days. The median HH compliance and UP for individual wards were 68% (IQR, 58–79) and 30L/1000-patient-days (IQR, 22–39), respectively. Wards were allocated to the same quartile by DO and UP on 25% (65/252) of occasions, a frequency that would be expected by random chance. Similarly, direction of change between consecutive periods was the same for the two methods on 31% (61/195) of occasions. During none of the four 6-month periods was there a difference in hand hygiene compliance between wards in different

product consumption quartiles ( $p = 0.17, 0.58, 0.53$  and  $0.27$ ), therefore a trend was not sought.

**Conclusion:** From the hospital ward perspective, there is poor correlation between hand hygiene performance feedback based on DO and UP. Concurrent use of both indices may result in confusion, and one should not be assumed to be an accurate surrogate of the other.

**P1980 Predictive model for high self-reported hand-hygiene compliance in intensive care units in Pakistan**

M. McLaws\*, N. Damani, B. Allegranzi, A. Raja, D. Pittet (Sydney, AU; Portadown, UK; Geneva, CH; Islamabad, PK)

**Objective:** To identify a change in perception predicting self-reported hand hygiene (HH) compliance  $\geq 60\%$  in healthcare workers (HCWs) pre- and post-implementation of the WHO hand hygiene improvement strategy (HHIS) in intensive care units (ICUs) in Pakistan.

**Methods:** HH perception questionnaires were distributed to HCWs in three ICUs pre- and post-intervention. Composite scales were developed from items included in the pre- and post-intervention surveys and were entered in a backwards multiple logistic regression analysis to predict high self-reported HH compliance ( $\geq 60\%$ ), while controlling for the effect of healthcare profession, service and ward. Alpha for perceptions was set at 6% level and odds ratios were standardised by the inter quartile ranges.

**Results:** Ninety-seven and 77 HCWs completed the questionnaire before and after implementation respectively. Prior to the HH intervention, HCWs who perceived that HH could impact on patient safety were nearly three times more likely (adjusted odds ratio [aOR] aOR 2.9,  $p = 0.01$ ) to report high compliance levels. HCWs who did not perceive that their peers expected them to comply with HH were 90% less likely (aOR 0.1,  $p = 0.023$ ) to report high compliance in the pre-intervention survey. After the intervention HCWs who perceived the HHIS components as effective were five times more likely (aOR 5.0,  $p = 0.015$ ) to report high compliance; HCWs who perceived ABHR as a cue to HH were five times more likely (aOR 5.0,  $p = 0.011$ ) to report high compliance.

**Conclusion:** All components of the WHO HHIS were found to strongly influence high self-reported compliance ( $\geq 60\%$ ) among ICU HCWs in Pakistan. This strategy, and in particular ABHR availability and use as a cue to memory to comply, should be continued for ensuring sustainable HH improvement.

**P1981 Transmission risk and indications for hand hygiene in outpatient settings: what global opinion leaders think**

H. Sax\*, S. Bagheri Nejad, M.N. Chraïti, B. Allegranzi (Geneva, CH)

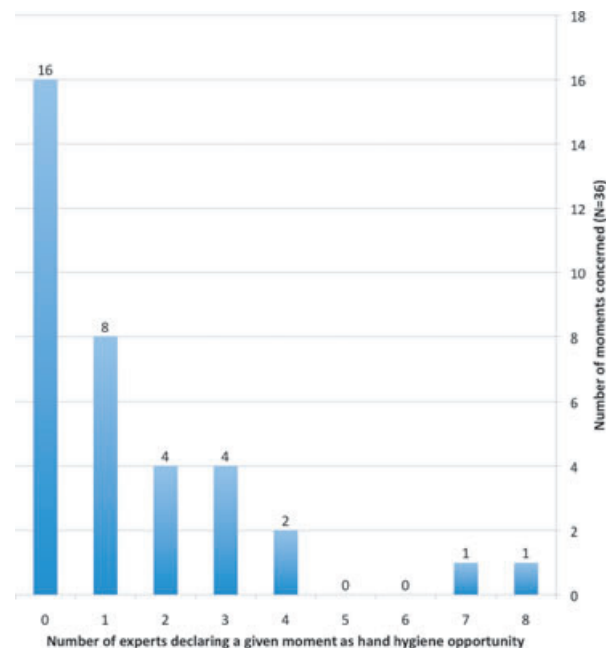
**Objective:** While the WHO “My 5 moments for hand hygiene” concept has been successfully implemented in many hospitals worldwide, its implementation in outpatient settings is challenging. In order to produce guidance on this issue.

We sought experts’ perception about infectious risk of hand pathogen transmission and specific hand hygiene (HH) opportunities (HH-OPP) in this setting.

**Methods:** Selected international HH experts from different countries and linked to the work of the WHO Clean Care is Safer Care program were asked to complete a self-administered survey. Experts had to quantify transmission risk according to a scale (1–10) and identify HH-OPP in three typical clinical outpatient scenarios that were presented in a step-by-step format: (i) mass-vaccination, (ii) patient blood sampling, and (iii) physical examination.

**Results:** Of 14 invited experts, eight responded from USA, Canada, Europe, Africa, and Asia. Mean ratings for negative infectious health impact as a consequence of hand transmission in scenarios 1–3 were 1.8, 2.5, and 2.5, respectively. Throughout the three scenarios, all eight experts agreed only on one moment being a HH-OPP among 36 possible options and on 16 being no HH-OP (see Fig. 1). Inter-rater agreement (Kappa) for scenarios 1–3 and overall, were 0.1, 0.3, 0.5, and

0.3, respectively. Median proposed HH-OPP per scenario were 2 (range, 1–3) for all three scenarios, with one expert systematically proposing only one HH-OPP after each care scene.



**Conclusions:** Agreement between HH opinion leaders on HH-OPP in outpatient settings is “poor” to “moderate.” While risk for transmission frequency and negative health impact are judged to be low by experts, proposed HH activity may consume a considerable proportion of the overall care time. More experts’ discussions and evidence are needed to understand the hand transmission risk in outpatient healthcare settings and to support implementation of HH improvement strategies.

**P1982 Do hand-hygiene product dispensers equipped with counting devices improve sustainability of hand-hygiene training sessions?**

D. Luft\*, M. Dettenkofer, R. Schulze-Röbbecke, A. Köpp, S. Lemmen (Freiburg, Düsseldorf, Aachen, DE)

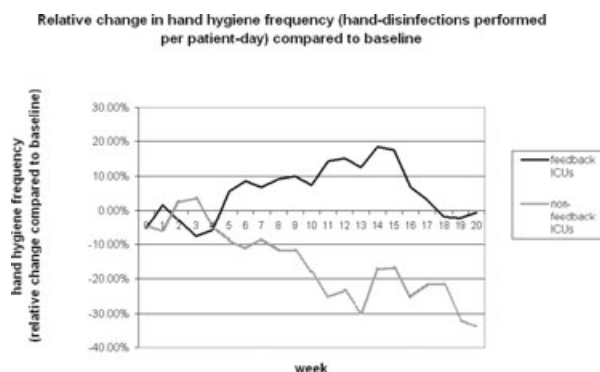
**Objectives:** Improving hand hygiene compliance is a constant challenge in infection control. Particularly obtaining sustainable effects tends to be difficult and technological tools creating data for feedback on hand hygiene performance might be helpful. We investigated the impact of hand hygiene product dispensers equipped with a digital counting device (HPD-Cs) on hand hygiene frequency and sustainability of training sessions.

**Methods:** Paired operative or multidisciplinary intensive care units (ICUs) in University Hospitals were equipped with HPD-Cs (referred to as non-feedback and feedback ICUs, respectively). Initially, the counters were concealed. Baseline data on numbers of hand hygiene actions performed and number of patient-days were collected for 4 weeks in both groups (week –4 to –1).

Medical staff of non-feedback ICUs received standardised training sessions on hand hygiene (starting week 0) followed by 20 weeks of data collection without further interventions.

On feedback ICUs counters were disclosed as an option for independent feedback at week 0. After 4 weeks of data collection staff was trained (starting week 4), including presentation of collected data and definition of an individual target frequency for hand hygiene. This was followed by 16 weeks of data collection with weekly feedback of targeted vs. actual performance.

**Results:** Two University Hospitals (four ICUs) were included into the analysis. Mean baseline rates were 49 hand disinfections performed per patient-day (HD/PPD) in non-feedback and 35 HD/PPD in feedback ICUs. Non-feedback ICUs showed a rather stable hand hygiene frequency after training sessions (week 0–3), followed by a marked decrease in HD/PPD resulting in a reduction of about 30% compared to baseline. Feedback ICUs showed a rather stable frequency of hand hygiene with disclosed counters (week 0–3). After start of training and weekly feedback in week 4, hand hygiene frequency steadily increased to a maximum rise of 18.5% on week 14 as compared to baseline. This was followed by a decline stabilising at baseline level (week 18–20).



**Conclusion:** Performing training sessions or disclosing counting devices only showed no sustainable promotional effect on hand hygiene. Combining training and feedback of data showed a considerable increase in hand hygiene frequency and prevented a decline as extensive as seen in non-feedback ICUs. Our baseline might be overestimated as installation of HPD-Cs by itself could have influenced hand hygiene frequency.

#### P1983 Hand hygiene in the emergency department: getting to the point

S. Scheithauer, V. Kamerseder\*, P. Petersen, H. Häfner, F. Hölzl, C. Mach, L. Lopez-Gonzalez, S. Lemmen (Aachen, DE)

**Objectives:** Compliance with hand hygiene (HH) is the cornerstone of infection control. However, in the emergency department adherence to the recommendations may be challenging like in other high intensity patient care areas.

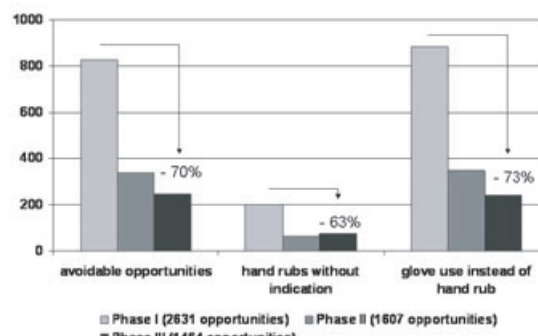
The number of opportunities and indication-specific compliance with HH in this setting are currently unknown. Additionally there is no information on the efficiency of HH implementation into the work-flow.

**Methods:** We conducted a prospective, three-phase, observational intervention study (controlled before after design) on HH interrupted by two intervention periods in the emergency department at a University Hospital. According to the WHO definitions opportunities, indications, activities, and additionally avoidable opportunities, hand rubs without indication and the glove usage instead of a hand rub were documented. Data were analysed by phase, profession, and patient-group, respectively. Interventions mainly consisted of teachings and implementation of standard operation procedures (SOPs) for common aseptic tasks from the HH perspective and for the complete work-flow of a standardized patient.

**Results:** A total of 5674 opportunities for HH were observed. After the first intervention we identified a significant decrease in the opportunities for an individual medical patient care from 21 (all numbers are medians; N = 77) to 12 (N = 75; p < 0.001) and for an individual surgical patient care from 11 (N = 50) to 6 (N = 51; p = 0.002), mainly due to a decrease in the avoidable opportunities. After the second intervention we observed a significant increase in the number of hand rubs performed from 4 (N = 75) to 5 (N = 76) for the medical patients (p < 0.001), and from 2 (N = 51) to 3 (N = 49) for the surgical patients (p = 0.048), respectively. Both together resulted in a 150%

improvement of overall HH compliance from 19% to 47% (p < 0.001). Despite a marked reduction of avoidable opportunities, unnecessary hand rubs and glove usage, there remains still potential for optimization without increasing the work-load (Fig. 1).

Fig. 1: Decrease of common mistakes during the study



**Conclusion:** This study provides the first detailed data on the number of opportunities and indications for HH and performed hand rubs in an emergency department. An about 150% increase in overall HH compliance could be achieved by a comparably moderate increase in hand rubs performed in combination with optimized HH standard operating procedures leading to a decrease in avoidable opportunities. However, there is still potential for optimization.

#### P1984 Changes in hand-hygiene compliance: professional status as a risk factor?

K. Graf\*, E. Ott, N. Tramp, M. Wolny, G. Porep, I.F. Chaberny (Hannover, DE)

**Objectives:** This study aims to evaluate the hand hygiene compliance in a German university hospital overall and in addition to the professional status after implementation of the national hand hygiene campaign "Aktion Saubere Hände" in 2008. To our knowledge, our observation is the largest nationwide survey on intensive care units (ICU) in a German university hospital.

**Methods:** During our investigation period (January 2008–December 2010), education and training of hand hygiene were implemented. Consumption rates of hand rub and gloves were collected and evaluated. Changes in healthcare workers behaviour were observed and compared to different professional groups and disciplines, compliance rates in hand hygiene of Healthcare personnel of twelve ICUs were evaluated before and after implementation of the campaign. Implementation included extensive education and training of all health care workers (at least three teaching units per ward). Five indications of hand hygiene were classified according to WHO definitions, compliance rates were evaluated for these indications. The health care workers were divided in three groups: physicians, nurses and other health care workers.

**Results:** Consumption rates of hand rub increased from 27 to 32 hand disinfections per patient day. No significant correlation between hand rub consumption and hand hygiene compliance was calculated. No significant change in use of gloves was detected.

Overall 4040 opportunities of hand disinfection were monitored. A significant improvement of hand hygiene compliance was assessed (from 54% to 61%, p > 0.05), the risk of no disinfection was reduced significantly (p > 0.05, 95%CI 0.74–0.85). The risk of no disinfection decreased significantly in the nurses group, but not in the physicians group (other health care workers were not evaluated due to the small number). There was no significant difference in hand hygiene compliance before the intervention between doctors and nurses, after the intervention, there was a significant difference in hand hygiene compliance (p > 0.05, 95%CI 1.42–1.85). Hand hygiene compliance is



higher in the nurses group, the risk of no hand disinfection in doctors is decreased, but there is no significance.

**Conclusion:** A significant change of hand hygiene behaviour was recognized for doctors and nurses. The hand hygiene compliance of nurses increased significantly higher, hence other training strategies for doctors will be essential.

## Typing of typical and atypical isolates from emerging diseases

### P1985 Dynamic distribution of *Neisseria meningitidis* serogroups in Italy, 2007–2011: increase of meningococcal Y isolates

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**Objectives:** The relative proportion of *Neisseria meningitidis* serogroups varies greatly over time. In Italy, although incidence has remained quite stable at around 0.3 cases/100 000 inhabitants in recent years, meningococcal serogroups showed a dynamic distribution: in particular, there was an increase of serogroups B and Y, paralleled by a decrease of serogroup C isolates. The aim of the present study was to investigate the phenotypic and genotypic characteristics of serogroup Y meningococci which are on the rise in Italy.

**Methods:** As reported in the data from the National Meningococcal Surveillance System in Italy, from 2007 to 2011 the serogroup, the serotype/subtype and the antibiotic susceptibility for penicillin, ciprofloxacin, ceftriaxone and rifampin were determined on 444 *N. meningitidis* isolates. Each isolate was genetically characterized by MultiLocus Sequence Typing (MLST) and by sequencing of *fetA* and *porA* (VR1 and VR2) genes. Each sequence was aligned and assigned an allele number using the *Neisseria* Typing website (<http://pubmlst.org/neisseria/>).

**Results:** During the study period the proportion of serogroup C isolates decreased from 32.1% in 2007 to 17% in 2011, whereas serogroup B increased from 60.7% in 2007 to 66% in 2011. The proportion of serogroup Y isolates also increased over time, from 3.6% in 2007 to 12.8% in 2011. Of the 28 patients infected by serogroup Y meningococci, 16 were women and 12 men, with a average age of 31 years, older than patients infected by other serogroups (average age of 19 years for serogroup B and 23 years for serogroup C). The most frequent clinical picture observed was meningitis (65% of patients). All the serogroup Y meningococci were susceptible to the drugs tested; however 14% of strains showed a decreased susceptibility to penicillin. Six different phenotypes were identified; in particular, 75% of isolates were Y:14:NST. Preliminary analysis of MLST data showed an homogeneity among the isolates analysed so far, due to the presence of three clonal complexes: ST-23 (83%), ST-167 (11%) and ST-41/44 (6%). The *PorA* VR1, VR2 and *FetA* variants more frequently detected were 5-2, 10-2 and F2-13.

**Conclusion:** In Italy, during the last few years, the proportion of serogroup Y has increased, as observed in other European Countries. The phenotypic and genotypic characteristics of the Italian serogroup Y meningococci define similar traits among the circulating isolates.

### P1986 Evaluation of molecular fine typing methods for outbreak-associated invasive *Neisseria meningitidis*

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**Objectives:** *Neisseria meningitidis* is a bacterial commensal of the human upper airway as well as a potential pathogen causing meningitis and septicaemia, with a high case-fatality rate (10%). A vast majority of all meningococcal infections are endemic or sporadic cases among infants, however, disease clusters occur and are most common among teenagers and young adults in educational or military institutions. In order to decide if prophylaxis in the form of vaccination should be considered, it is essential that strain typing is performed in a rapid and

accurate manner to distinguish a genuine outbreak from epidemiologically related isolates without genetic relatedness. Current genotyping methods such as multilocus sequence typing (MLST), sequencing of fine type antigens and the more outdated “gold standard” for outbreak investigations, pulsed-field gel electrophoresis (PFGE), are all considered laborious and costly. The aim of this study was to evaluate two new molecular fine typing methods; highly variable multiple-locus variable-number tandem repeat analysis (HV-MLVA) and the DiversiLab system based on repetitive-sequence-based-PCR, and compare them with the current recommended methodologies.

**Methods:** Invasive *Neisseria meningitidis* serogroup C isolates in Sweden from the years 2000–2006 (n = 33) were included in the study. Nineteen isolates were connected to six different epidemiological clusters and the remaining 14 isolates were sporadic isolates. PFGE had previously been performed on 26 of the 33 isolates. All strains were characterised by MLST and sequencing of the *fetA*, *fHbp*, *penA*, *porA* and *porB* genes, and typed with the DiversiLab system and HV-MLVA (Schouls *et al.* 2006).

**Results:** The DiversiLab system distinguished three different clusters. These results correlated with the results from the PFGE. Both methods found one pattern type which was prevalent over all six years investigated. HV-MLVA distinguished six different clusters which all had strong spatiotemporal connections. The MLST and sequencing of fine typing antigens did not yield as many clusters as HV-MLVA, and the results correlated more to those of the DiversiLab system and PFGE.

**Conclusions:** This study suggests that HV-MLVA is best suited for first hand rapid outbreak investigation because it is more discriminating, faster and more cost beneficial than all other methods investigated. A good interlaboratory reproducibility is provided with the allele designation similar to that of MLST.

### P1987 Phylogenetic analysis of *E. coli* causing bacteraemia in the UK and Ireland in 2001 and 2010

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**Objectives:** To compare the phylogeny of *E. coli* causing bacteraemias in the UK and Ireland in 2001 vs. 2010.

**Methods:** *E. coli* isolates (n = 409; 168 from 2001 and 241 from 2010) were from the BSAC Bacteraemia Surveillance Programme and had been submitted from 17 centres across the UK and from one in Ireland. They were assigned to major phylogroups (A, B1, B2 or D) by PCR. An initial subset of isolates from each year was analysed by multi-locus sequence typing (MLST) using the Achtman scheme (<http://mlst.ucc.ie/mlst/dbs/Ecoli>).

**Results:** The phylogroup distributions of isolates causing bacteraemias in 2001 and 2010 were similar, with group B2 the most common in both years (78% in 2001 vs. 71% in 2010), followed by phylogroup D (11% vs. 15%), B1 (7% vs. 8%) and A (4% vs. 6%). MLST was undertaken on 71 isolates (42%) from 2001 and 70 (29%) from 2010, with 50 sequence types (STs) identified: 22 STs were detected only in 2001, 16 only in 2010, and 12 in both years. ST73 phylogroup B2, was the most common ST in both years (24% of isolates in 2001 and 26% in 2010). The ST73 isolates were susceptible to most antibiotics. Numbers of isolates belonging to ST131 phylogroup B2 – an international clone that commonly now hosts CTX-M ESBLs – increased from 3/71 in 2001, all from separate centres, to 13/70 in 2010 (from 7/18 centres) (Fisher's exact test p = 0.008). None of the ST131 isolates in 2001 was ciprofloxacin-resistant or ESBL-positive, whereas eight were ciprofloxacin-resistant in 2010 and four (from multiple centres) had ESBLs.

**Conclusion:** As expected, phylogroups B2 and D were predominant in both years; these phylogroups are associated with virulent extra-intestinal *E. coli* strains. ST73, which is associated with urinary tract infections, was the commonest ST in both years. The urinary tract was the stated source of most bacteraemias caused by this ST. There also

was a significant increase in cases of bacteraemia caused by the international ST131 clone, with more members of this clone found resistant in 2010 than 2001.

**P1988** Local molecular epidemiology of *Escherichia coli* bacteraemia

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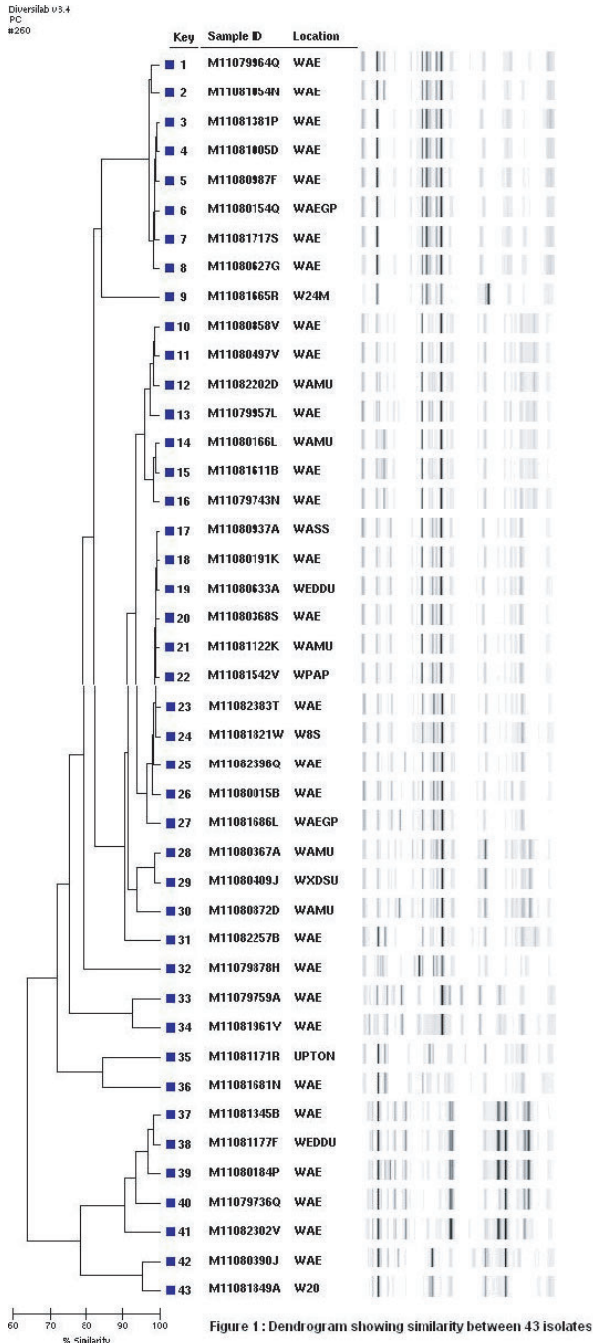
**Introduction:** *Escherichia coli* is one of the leading causes of bloodstream infections in UK. Mandatory reporting of *E. coli* bacteraemia (ECB) has been introduced in June 2011. The availability of local molecular epidemiological data of ECB is essential to design regional-specific prevention interventions.

**Method:** Forty three ECB isolates reported in the mandatory scheme during a 3 month period were strain-typed in-house using the

DiversiLab® (DL) repetitive sequence-based polymerase chain reaction (rep-PCR) system (BioMerieux). Comparison of isolates was performed with the web-based DL software, using the Pearson correlation similarity analysis. Rep-PCR patterns relationships were designated as recommended by the manufacturer. Clinical data was obtained from patients records and analysed.

**Results:** Rep-PCR analysis identified 22 unique patterns among all 43 isolates studied. With use of a similarity threshold of 95% to define clonal groups, 10 different clonal groups were identified (A-J). Overall, 40% (17) of isolates belonged to a markedly predominant clonal group D, consisting of five similar patterns differing by one to two peaks; 12% (five) to B, and the remaining 42% (21) were distributed in eight different clonal groups: A, F, G, I and J (two isolates each) and C, E and H (three isolates each). Of the 43 episodes of ECB, 67% (29) were community acquired (CA) and 33% non-CA: 11 were considered health care associated (HCA) (hospital stay within previous 30 days) and three hospital acquired (HA) (>48 hours of admission). Clonal group D isolates were 59% (10) CA and 41% non-CA: six HCA and one HA (p = 0.9). Urinary tract was the most common primary focus with 53% (23) of ECB episodes, 26% of which were urinary catheter related, 19% intra-abdominal source and 26% other sources. The majority of urinary tract primary source isolates (59%) belonged to clonal group D (95%CI 32.2–79.2, p = 0.06). Five out of all isolates were extended-spectrum beta-lactamase-producers, four of which were clustered into two indistinguishable patterns (with two isolates each) within clonal groups A and B.

**Conclusion:** These data demonstrate the complexity of the local epidemiological situation of ECB and this must be kept in mind when targets to reduce ECB are set in the future. Rapid strain-typing discrimination of isolates can be a useful tool to understand the local epidemiology of ECB and to enable the development of more accurate prevention strategies.



**P1989** Molecular and epidemiological analysis of an outbreak of extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* using repetitive extragenic palindromic-polymerase chain reaction

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**Objective:** Extended spectrum beta-lactamase (ESBL) producing bacteria are an increasing problem in clinical medicine. With the lack of development of novel antibiotics active against these organisms, infection control precautions are vital to prevent transmission. This study reports the microbiologic, clinical, and epidemiologic features of the infections caused by ESBL-producing *Klebsiella pneumoniae* (ESBL-Kp) in the neonatal unit of our hospital from June 2009 to December 2009. Fingerprinting analyses by automated repetitive extragenic palindromic-polymerase chain reaction (rep-PCR) were made for determining clonal spread.

**Methods:** ESBL-Kp affecting 27 patients were investigated using automated rep-PCR (DiversiLab System). Species identification and antimicrobial susceptibility testing were performed by WalkAway System. When necessary, ESBL production was confirmed using Etest. Medical records of colonised or infected patients were retrospectively reviewed.

**Results:** A total of 27 patients were found to be colonized/infected with ESBL-Kp. After an isolation, preventive contact precautions and surveillance cultures of rectal swabs from the patients were done every week until they were negative.

Risk factors for colonization/infection were parenteral nutrition (15.7%), catheters (41.2%), previous antimicrobial therapy (39%), surgery (3.9%) and mechanical ventilation (39%). Six out of the colonized patients were infected by ESBL-Kp: two pneumonias, two urinary tract infections, one bacteraemia and one wound infection.

Preliminary analysis using DiversiLab testing showed that 17 out of the 27 isolates were indistinguishable and belonged to a major cluster. Another cluster comprised two strains, whereas the rest of the strains were genetically unrelated.

**Conclusion:** A dominant cluster of ESBL-Kp that comprised over 63% of the strains have been identified. This study confirms the relevant role of ESBL-Kp as a pathogen in the neonatal unit. The risk of a rapid dissemination and the persistence of these multidrug-resistant strains through the time determine the need to implement measures for prevention of the spread of these microorganisms. The use of DiversiLab System for clonal strain typing may be a useful tool for fast and accurate management of nosocomial outbreaks caused by ESBL-Kp.

**P1990** **Characterisation of extended-spectrum beta-lactamase producing *Escherichia coli* isolated from patients with urinary tract infections in Korea: comparison between community isolates and nosocomial isolates**

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**Objectives:** This study was designed to characterise extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* isolates causing urinary tract infections (UTIs) and to compare the molecular characteristics of community isolates to those of nosocomial isolates at a single centre in Korea.

**Methods:** Urinary ESBL-producing *E. coli* isolates were prospectively collected at Daejeon St Mary's Hospital in Korea from January 2008 to September 2009. The ESBLs were characterised by PCR sequencing using specific primers. The genetic relatedness was determined by pulsed field gel electrophoresis (PFGE) and multilocus sequence typing (MLST).

**Results:** ESBL-producing *E. coli* accounted for 12% (111/925) of all UTIs caused by *E. coli*, including 76 community-onset infections and 35 nosocomial infections. A total of 78 non-duplicate urinary isolates (57 community and 21 nosocomial isolates) were included for this study. Of the 78 isolates, 30 (38.5%) were positive for CTX-M-15, 29 (37.2%) for CTX-M-14, and 17 (21.8%) positive for both CTX-M-14 and CTX-M-15. 25 isolates (29.5%) and 52 (66.7%) coproduced OXA-1 and TEM-1, respectively. PFGE revealed 37 different PFGE types with type 23 being the most predominant cluster (n = 14, 18.9%). By MLST, ST131 was the most common type (n = 18, 24.3%), followed by ST405 (n = 10, 13.5%) and ST38 (n = 8, 10.8%). *Escherichia coli* isolates belonging to ST131 were mainly grouped in PFGE type 23. ST131 isolates were more likely to produce CTX-M-15 (11/18, 61.1%) or coproduced both CTX-M-15 and CTX-M14 (5/11, 27.8%) than CTX-M-14 alone (2/18, 11.1%). Resistance to amikacin (9%) and piperacillin-tazobactam (19.2%) was low whereas resistance to ciprofloxacin (73.1%) was high. Resistance to ciprofloxacin was significantly higher in nosocomial isolates (90.4%) than in community isolates (66.7%). However, there was no significant difference in the distributions of CTX-M types, PFGE types, and STs between community and nosocomial isolates.

**Conclusion:** CTX-M-type ESBLs, primarily CTX-M-14 and CTX-M-15, have become the predominant types ESBL produced by *E. coli* isolates causing UTIs regardless of whether they are recovered from the community or hospital. ST131 has emerged as a dominant ESBL-producing *E. coli* clone not only in the community but also in hospitals, suggesting the widespread of this epidemic clone in Korea.

**P1991** **A study of the variation in genetic diversity of *Haemophilus influenzae* serotype B strains in the UK between 1987 and 2010 using multilocus variable-number tandem repeat analysis**

S. Mangani\*, D. Litt, S. Neal, M. Slack (London, UK)

**Objectives:** In 1992 the UK added *Haemophilus influenzae* serotype b (Hib) vaccine to the infant immunisation schedule, resulting in a dramatic decline in the incidence of invasive Hib disease. From 1999 there was a resurgence of invasive *Haemophilus influenzae* serotype B (Hib) disease in fully vaccinated children, older children and adults,

which has been controlled by the introduction in 2006 of a routine booster dose of Hib vaccine.

The objective of this study was to characterise over 700 Hib strains collected between 1987 and 2010 from UK cases of invasive disease, using multilocus variable-number tandem repeat analysis (MLVA) in order to study changes in the distribution of clonal groups in the bacterial population and in overall genetic diversity over a time period spanning before and after the introduction of the vaccine and between different patient groups.

**Methods:** A collection of 700 invasive isolates of *H. influenzae* type b submitted by hospital laboratories between 1987 and 2010 were analyzed. The isolates were divided into the following groups: Pre-vaccine and Post-vaccine children and Pre-vaccine and Post-vaccine adults and the isolates were characterised by MLVA typing. The Diversity for different groups was calculated and the distributions of MLVA types in different periods were compared.

**Results:** Our results revealed an increase in genetic diversity amongst strains isolated from children in the post-vaccine period compared to the pre-vaccine period. The reverse, however, was observed for strains isolated from adults. Nineteen MLVA types were only found in the post-vaccine population (compared to the pre-vaccine and non-vaccinated populations). Strains isolated from cerebrospinal fluid (CSF) demonstrated higher genetic diversity than those isolated from blood, and nine MLVA types were unique to strains isolated from CSF. Strains also demonstrated increasing genetic diversity in the following order of clinical presentation: septic arthritis, cellulitis, bacteraemia, pneumonia, epiglottitis and meningitis.

**Conclusion:** The introduction of Hib vaccine affected adult and patient groups differently and 19 MLVA types were found to be unique to the post-vaccine population. A higher genetic diversity was found for strains isolated from CSF than those isolated from blood and genetic diversity of strains varied with clinical presentation. The various MLVA clonal groups may possess diverse virulence determinants and pathogenic potential.

**P1992** **Genotypic characterisation of non-encapsulated *Haemophilus influenzae* strains isolated from invasive disease in Italy**

R. Cardines, M. Giufrè, M. Accogli, M. Cerquetti\* (Rome, IT)

**Objectives:** The introduction of *Haemophilus influenzae* serotype b (Hib) conjugate vaccines has changed the epidemiology of invasive *H. influenzae* disease. In Italy, most invasive infections are nowadays caused by non-encapsulated (ncHi) strains. The objective of this study was to investigate phylogenetic relationships among invasive ncHi strains. Moreover, variability in the promoter region of the blaTEM gene was examined in beta-lactamase producing ncHi strains, with the aim to find or not association between promoter types and either resistant phenotypes or specific successful clones.

**Methods:** A total of 91 ncHi strains isolated from invasive diseases in years 2007–2011 were analysed. Serotype was determined by PCR capsular genotyping. Minimum inhibitory concentration (MIC) of ampicillin was determined by Etest and interpreted using the EUCAST breakpoint. Beta-lactamase activity was detected by the nitrocephin test. The presence of the blaTEM or blaROB genes was investigated by PCR. The promoter region in the blaTEM gene was investigated by DNA sequencing. Genetic relationships among all 91 ncHi strains were assessed by PFGE. Beta-lactamase producing isolates were further analysed by multilocus sequence typing (MLST).

**Results:** Overall, ampicillin resistance was 18.7% (17/91). Thirteen strains were beta-lactamase positive (MIC range: 24–256 mg/L) and four were classified as BLNAR (MIC = 1.5 mg/L). All beta-lactamase producers harboured the blaTEM gene, except one with blaROB. In most strains (10/12), promoter regions of the blaTEM exhibited a 135 bp deletion (Pdel). The Pa/Pb overlapping promoter (originally associated with blaTEM in *H. influenzae*) was detected in two strains. No association was found between promoter types and level of ampicillin resistance. Overall, PFGE revealed a high degree of genetic diversity among the 91 ncHi strains, but 15 minor clusters were

identified, each including from 2 to 4 strains. By MLST, ST103 and ST165 clones predominated among beta-lactamase producer strains. The Pdel promoter was common to different MLST clones, including ST103 and ST165.

**Conclusions:** Although nHi strains showed a considerable genetic heterogeneity, the blaTEM gene was found to be preferentially carried by specific successful MLST clones. The Pdel promoter was widespread in *H. influenzae* blaTEM genes.

#### P1993 Genetic lineages and toxigenicity in *Staphylococcus aureus* from healthy humans and unrelated dogs: a comparative analysis

E. Gómez-Sanz, C. Lozano, C. Tenorio, C. Torres\*, M. Zarazaga (Logroño, ES)

**Objectives:** To investigate and compare the genetic lineages and virulence properties of *S. aureus* from the nares of healthy humans and healthy dogs to try to unveil potential associations between *S. aureus* of both origins.

**Methods:** Twenty-five *S. aureus* isolates from humans and 25 from pound-dogs (La Rioja, Spain), obtained in previous studies, were included. All 50 *S. aureus* isolates were characterized by agr- and spa-typing. One isolate per spa-type was characterized by MLST. PCR-based determination of 30 virulence genes (three leukocidins, five haemolysins, three exfoliatins, and 19 pyrogenic toxin superantigen genes) was performed in all isolates.

**Results:** Agr-types detected were (% human/dog): I (36/52), II (16/20), III (28/24), and IV (20/4). Seventeen distinct spa-types (plus one non-typeable, NT) and 13 STs (four new STs) [t002-ST5, t091-ST7, t012-ST30, t037-ST30, t440-ST30, t3711-ST34, t3916-ST34, t015-ST45, t073-ST45, t148-ST72, t209-ST109, t159-ST121, t2155-ST121, t571-ST398, NT-ST1733[new], t021-ST1645[new], t1054-ST2175[new], t1077-ST2177[new]] were identified among *S. aureus* from humans. This isolates belonged to nine clonal complexes (CCs) and three new singletons, being CC30, CC45 and CC121 predominant. Fifteen spa-types (two new) and 11 STs (two new) [t177-ST1, t2366-ST5, t084-ST15, t015-ST45, t040-ST45, t186-ST78, t1166-ST133, t002-ST146, t8764[new]-ST217, t034-ST398, t108-ST398, t5883-ST398, t189-ST1655[new], t8765[new]-new ST] were detected in *S. aureus* from dogs. Six CCs and one singleton were observed with CC15 and CC398 being predominant. Frequency (%) of virulence genes detected in *S. aureus* of human and animal origin were as follows (human/dog): lukE/D (40/72), hla (92/100), hlb (20/28), hld (96/100), hlg (48/36), hlgv (44/60), eta (8/0), etb (4/0), tst (36/0), [seg-sei-sem-sen-seo] (32/12), [seg-sei-sem-sen-seu] (8/16), [seg-sei-sem-sen-seu] (28/0), [seg-sei-sem-sen] (20/0), [sei-sem-sen-seu] (0/4), [sec-sel] (12/16), [sed-sej-ser] (0/4), sec (0/16), sep (8/12), seh (12/8), sea (8/4), sen (0/8), ser (0/4), sei (0/4), seu (0/4), see (4/0), and seb (4/0).

**Conclusions:** Based on our tested population, *S. aureus* genetic lineages CC15 or CC398 seem to be more associated to dogs whereas CC45, CC121, or CC30 to humans. Several toxin genes (lukE/D, hlgv, sec) were more common in *S. aureus* of dogs whereas the toxic-shock syndrome toxin gene, tst, and toxin gene clusters ([seg-sei-sem-sen-seo], [seg-sei-sem-sen-seu], [seg-sei-sem-sen]) were predominant in those of humans.

#### P1994 High-resolution melting curve analysis of the spa gene for rapid and cost-effective MRSA typing

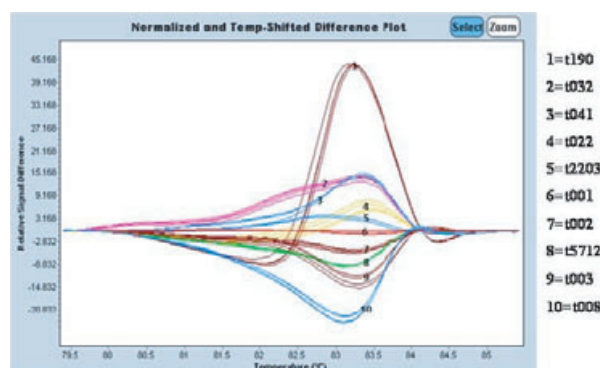
B. Mayerhofer, A. Pietzka\*, A. Stöger, E. Grund, F. Allerberger, E. Klinkacek, W. Ruppitsch (Vienna, Klagenfurt, AT)

**Objective:** *Staphylococcus aureus* is one of the most significant health care associated pathogens. Thus, *S. aureus* typing is an important tool for *S. aureus* surveillance. Sequence analysis of the hypervariable region X of the protein A gene (spa) is the current gold-standard for *S. aureus* genotyping. However, PCR based methods are still advantageous with respect to speed, costs and time. The suitability of

HRM-PCR analysis should be tested and evaluated for fast and accurate identification of the most frequent spa types from a hospital consortium in southern Austria.

**Methods:** In this study 212 arbitrarily chosen MRSA isolates collected from 2005 to 2010 comprising 39 spa types were analysed by high resolution melting curve analysis of the spa. PCR and HRM were performed in a single run using standard primers and the LightCycler 480 High-Resolution Melting Master mixture on a LightCycler LC480 instrument (Roche diagnostics Penzberg, Germany).

**Results:** High resolution melting curve analysis generated similar or even identical melting curves for spa types t001, t002 and t003, and spa types t008, t068 and t3220, and spa types t190, t919, t030, t037, and t011. Optimal reaction conditions were obtained by combined spiking of reaction mixtures with genomic DNA from spa types t003 and t030. Finally, this optimisation generated ten reproducible melting curve profiles for the ten most frequent spa types (t001, t002, t003, t008, t022, t032, t041, t190, t2203, t5712) (Fig. 1). Compared to sequence analysis HRM is approximately five times faster and 16 times cheaper.



**Conclusion:** The recent development of high resolution melting curve PCR represents a useful tool for accurate mutation detection. However, due to the enormous number of currently 9554 spa types, it is impossible to use HRM curve profiling for spa typing. Nevertheless, HRM curve analysis is a highly attractive typing technique due to the manageable number of spa types in a hospital or hospital consortium, the single closed tube format, the possibility for high-throughput screening, costs, speed, and its simplicity.

#### P1995 Development of an optimal MLVA typing scheme to investigate endemic MRSA

K. Hardy\*, M. Lewis, S. Shabir, C. McMurray, P. Hawkey (Birmingham, UK)

**Objectives:** To evaluate the discriminatory ability of loci from three multi-locus variable number tandem repeat analysis (MLVA) schemes for epidemiological typing of methicillin resistant *S. aureus* (MRSA) and develop an optimal typing scheme for investigation of cross transmission.

**Method:** A panel of 130 *S. aureus* isolates from different clonal complexes and with diverse pulsed field gel electrophoresis profiles, and separated in time and space were selected for evaluation of loci from three published MLVA schemes (Hardy *et al.*, Pourcel *et al.* and Schouls *et al.*). All isolates were typed with the 23 different loci using standardised PCR conditions and analysed using capillary gel electrophoresis on the QIAxcel. The numbers of repeats at each locus were calculated. The level of discrimination between loci was tested by Simpson's index of diversity using Ridom EpiCompare. Based on discriminatory ability and ease of analysis a panel of 11 loci were selected for further analysis in an endemic setting. Over a 16 month period 285 MRSA isolates were obtained from screening swabs on one ward. Typing using the Hardy *et al.* scheme (SIRU) demonstrated 96 isolates belonged to four predominant SIRU profiles and these were further typed using the panel of 11 loci.

**Results:** The discriminatory ability of all three schemes was high, Pourcel *et al.*, 99.3%, Schouls *et al.*, 94.2% and Hardy *et al.*, 93.9%. There was a high level of variation between the discriminatory ability of each individual locus, ranging from 92.9% to 29.5%. In some instances the amplification product in the STAR loci within the Pourcel *et al.* scheme was smaller than the size of the flanking region. Sequencing of the products from Sa0906 demonstrated a 221 bp deletion. A panel of 11 loci were selected for inclusion in the scheme based on individual discriminatory ability, ease of analysis, with loci with small repeat size being excluded. A total of 48 distinct SIRU profiles were identified with the scheme of Hardy *et al.*, with four predominant profiles, accounting for 96 isolates. Applying the additional loci enabled further discrimination within each of the four SIRU profiles and was concordant with transmission episodes on the ward.

**Conclusion:** A panel of 11 loci provided the optimal MLVA typing scheme, providing good discriminatory power between MRSA isolates within an endemic setting that fitted with cross transmission events.

**P1996 Multi-locus variable-number tandem-repeat analysis (MLVA) of methicillin-resistant *S. aureus***

M.E.O.C. Heck\* (Bilthoven, NL)

**Objective:** To control hospital infection a national of methicillin-resistant *S. aureus* (MRSA) surveillance program is carried out in the Netherlands. The program was started in 1988 and is still running. To study the spread and transmission routes of MRSA typing is necessary. In 2008 Multilocus Variable-Number Tandem-Repeats Analysis (MLVA) was introduced as a means of typing in the Netherlands. MLVA is a PCR method based on the amplification and fragment analysis of eight repeat loci, it generates an eight integer allelic string and is fairly easy to perform. This study shows the possibilities and the strengths of MLVA and its significance in epidemiological typing MRSA.

**Methods:** For this study we typed 12.600 *S. aureus* isolates. A survey was made of the incidence and the distribution of MLVA types in the Netherlands. To obtain a more detailed insight, subsets of MRSA strains were examined e.g. urban, rural and cross-border. Relationships between MLVA types were investigated by using the spanning-tree algorithm in the BioNumerics® software. The geographical dispersion of MLVA types with the help of a geographical tool on the Dutch MRSA website: <http://mrsa.rivm.nl>

**Results:** Among the 12.600 MRSA stains (screenings – and clinical isolates) we found 1191 MLVA types. The majority of these types i.e. 7735 (61%) could be assigned to complexes of closely related strains. (A complex was defined as a group of stains having only one difference in the allelic profile and consisting of at least five strains.)

We were able to define 16 of the complexes found. The remainder 624 (39%) strains did not fit in a complex. We have seen that MLVA complex 398, animal related, is the most frequent complex in the Netherlands. We found a difference in the distribution of MLVA types as well as MLVA complexes between urban and rural areas. In urban areas we found less animal related types and more variety of MLVA types. Among clinical strains we found less differences between rural and urban strains. In cross-border areas we found dominant MLVA types not found in other parts of the Netherlands.

**Conclusions and comments:** MLVA was able to define complexes of closely related strains. Animal related strains are the most frequent found in the Netherlands.

There is a difference the distribution of MLVA types among clinical and screenings strains. MLVA by means of its simplicity is suitable for web based queries and submission of data.

**P1997 Direct bacterial profiling by means of MALDI-TOF mass-spectrometry as a method of rapid differentiation of *Staphylococcus aureus* strains**

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**Objectives:** *Staphylococcus aureus* is a clinically relevant microorganism with an extensive strain diversity, primarily due to the

variability of virulence and pathogenicity factors. The aim of our study was to show a possible way for rapid differentiation of *S. aureus* strains based on MALDI-TOF MS direct bacterial protein profiling. In this particular work we selected beta-lactamase and alpha-hemolysin production, encoded by the blaZ and hla genes, respectively, as markers for the strain differentiation.

**Methods:** The study included 53 isolates of *S. aureus*. Mass spectra were collected from fresh bacterial cells by a Microflex MALDI-TOF MS (Bruker Daltonics, Germany). MALDI Biotyper 2.0 (Bruker Daltonics, Germany) was used for species identification. The presence or absence of genes hla and blaZ was established by the amplification with specific primers. Mathematical models for the classification of mass spectra obtained by direct mass spectrometric profiling of cell lysates (eight mass spectra for each strain) were generated using the software ClinProTools 2.1 (Bruker Daltonics, Germany) on the basis of a Genetic Algorithm.

**Results:** Mathematical analysis of MALDI mass spectra allowed the construction of two independent classification models that can differentiate the strains on the presence/absence of blaZ or hla genes, respectively. The most significant peaks (peptide/protein masses), which can be considered as markers of the strain differences in *S. aureus*, were identified using a statistical contribution of each mass peak in the models. Each diagnostic model was validated with other group of strains, not included in model creation. The established sensitivity and specificity were 97.5% and 82.5% for the classification of strains on the basis of beta-lactamase production, and 90.0% and 88.7% for the presence of alpha-hemolysin.

**Conclusions:** MALDI ToF MS based approach supported by mathematical algorithms can be very powerful tools for *S. aureus* strain differentiation.

**P1998 Evaluation of the Diversilab semi-automated repetitive-sequence-based PCR for typing isolates of *Pseudomonas aeruginosa* responsible for ventilator-associated pneumonia in different intensive care units**

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**Objectives:** The Rep-PCR (repetitive-sequence-based PCR) methods are rapid bacterial typing procedures that generate strain-specific band patterns, but are notorious for their susceptibility to minor variations in experimental conditions, resulting in poor reproducibility. Recently, the Diversilab (DL) system (bioMérieux) has semi-automated a rep-PCR method with a high level of standardization. The purpose of this study was to evaluate the usefulness of the rep-PCR-based DL system for typing *Pseudomonas aeruginosa* isolates from patients with ventilator-associated pneumonia (VAP). The applicability of the DL rep-PCR was compared with the reference method, pulsed field gel electrophoresis (PFGE).

**Methods:** A prospective multicenter study on patients with *P. aeruginosa* VAP was conducted in three intensive care units during a 31-month period. All isolates of *P. aeruginosa* obtained from respiratory samples in the three units were conserved (n = 710, corresponding to 175 patients). All *P. aeruginosa* isolates (n = 214, corresponding to 73 patients) from patients with two consecutive respiratory samples distant of more than 72 hour were subjected to DL rep-PCR, and 40 of these isolates corresponding to 20 patients were also subjected to PFGE-SpeI. DL rep-PCR and PFGE-SpeI restricted genomic DNA profiles were compared.

**Results:** 198/214 isolates (69 patients) showed DL profiles with >95% of similarity index with another isolate. Isolates of a same patient which showed identical profile in PFGE showed a single type in DL (>95% of similarity index for rep-PCR profile) (19 patients). Isolates for one patient which are different in PFGE were also different in DL. Three clusters of 13 isolates (six patients) showed rep-PCR profiles >95% and related PFGE profiles (≤3 bands of difference). Nine *P. aeruginosa* isolates (five patients) of three different units which were assigned to a single type in DL showed five different PFGE-SpeI profiles (>3 bands

of difference). Two PFGE-nontypeable *P. aeruginosa* isolates of the same patient could be typed in DL.

**Conclusions:** PFGE-SpeI had a better discriminatory power than DL rep-PCR for typing our *P. aeruginosa* isolates, but PFGE-nontypeable *P. aeruginosa* isolates could be typed in DL. The rep-PCR DL system showed fairly good but not excellent performance, making it a reliable typing tool for investigation of outbreaks caused by *P. aeruginosa*, even though it was less discriminating than PFGE analysis.

**P1999** Clonal epidemiology and resistance evolution in *Pseudomonas aeruginosa* strains colonising the respiratory tract of cystic fibrosis patients from the Balearic Islands

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**Objective:** To investigate the long-term clonal epidemiology and resistance evolution of *P. aeruginosa* within and across chronically-colonized cystic fibrosis (CF) patients from the Balearic Islands, in order to identify persistent and epidemic clones, linked or not antibiotic resistance phenotypes.

**Methods:** The collection studied included 10 sequential isolates from each of 10 CF patients. Each sequential isolate was separated by at least a 6-month interval, covering up to an 8-year period from 2003 to 2010. Colony morphotypes were recorded, and antibiotic susceptibility profiles (ceftazidime, cefepime, imipenem, meropenem, ciprofloxacin, tobramycin, and colistin) were determined by E-test, using EUCAST breakpoints. All isolates were typed by Pulsed-Field-Gel-Electrophoresis (PFGE) using SpeI as restriction enzyme. One representative isolate from each clone and patient was further analysed by Multilocus Sequence Typing (MLST) using available protocols and databases (<http://pubmlst.org/paeruginosa/>).

**Results:** Following PFGE results, 17 strains were analysed by MLST, leading to the identification of 14 sequence types (ST), eight of which (57%) were new. One of them (ST-274), was detected in three patients, and one more patient harboured a closely related clone differing only by single point mutations in two alleles. Remarkably, ST-274 has been also detected (MLST database) in several CF patients from Australia, Austria, and France. Two other patients shared the same clone (ST-299), whereas all other clones were found in single patients. In five of the patients a single clone was detected through the whole study period, remarkably including all four patients with ST-274 or the related clone. The remaining five patients showed the coexistence of several clones (2–4) or clonal replacements, including the superinfection with the Liverpool Epidemic Strain (LES-1, ST-146) in one of the patients. An important intra and inter clonal and patient variability of resistance patterns was documented, but with a significant trend towards the accumulation of resistance mechanisms, as evidenced by an average resistance to 0.7 antibiotics in the initial isolates from each patient compared to 2.0 in the last isolates.

**Conclusion:** Despite not particularly associated to resistance, this work alerts on the linkage to persistent colonization and wide dissemination among CF patients of ST-274. It also alerts of the first detection of the multidrug resistant LES-1 strain in Spain.

**P2000** Molecular typing of *Treponema pallidum* subsp. *pallidum*: comparison of CDC typing system with sequencing-based typing TP0136/TP0548/23S rRNA

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**Objectives:** PCR detection of *Treponema pallidum* subsp. *pallidum* (TPA), the causative agent of syphilis, represents a direct diagnostic method with high sensitivity. Moreover, PCR detection allows molecular typing of treponemal strains. Several previous studies indicated that different genetic variants of *T. pallidum* are present in different geographical areas. To distinguish the strains present in the

Czech Republic, we compared two typing schemes, the CDC typing and the sequencing-based typing.

**Methods:** The typing system approved by Centers for Disease Control and Prevention (CDC typing) determines the number of 60 bp repetitions in the arp gene and the restriction profile of 3 tpr genes (tprEGJ). Sequencing-based typing system (TP0136/TP0548/23S rRNA) determines the sequences of TP0136 and TP0548 genes and the restriction profile of 23S rDNA locus, where mutations at position 2058 or 2059 cause macrolide resistance of treponemal strains.

**Results:** A set of 144 PCR positive samples from 108 patients was collected between the years 2004–2011 in the Czech Republic. The sequencing-based typing system (TP0136/TP0548/23S rRNA) identified nine different genotypes among the 57 completely and 19 partially (only two out of three sequences of TP0136, TP0548 and 23S rRNA genes were determined) typed patients. The prevailing genotype was sequentially identical at the TP0136 and TP0548 loci to the SS14 strain but unlike the SS14 strain, the 23S rDNA encoded sensitivity to macrolide antibiotics. 42.9% of tested strains were resistant to macrolide antibiotics. In samples taken from 53 patients, the CDC typing was performed. Eight different subtypes were identified. Simultaneous application of both typing schemes identified 14 different genotypes of syphilis causing strains in the Czech population.

**Conclusions:** Our results showed that the sequence variants of TP0136, TP0548 and 23S rRNA genes combine independently with CDC subtypes, indicating their potential for more detailed genetic characterization of syphilis causing strains. Combination of both typing systems can be useful in genotyping of treponemes isolated from small populations.

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**P2001** Characterisation of *Chlamydia trachomatis* ompA genotypes among sexually transmitted disease patients in a city in the north of Spain

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**Objetives:** Lymphogranuloma venereum (LGV) is a sexually transmitted disease caused by serovars L1, L2, L3 of *Chlamydia trachomatis* (CT). It was considered a sporadic disease in developed countries, occurring endemically only in parts of Africa, Latin America and Asia. At the end of 2003, there was an outbreak of LGV in men who have sex with men (MSM) in Europe, indicated the emergence of a new epidemic in this high-risk group. However in Spain, only sporadic cases have been reported. We try to study the circulating genotypes in a group of male patients coming from a sexual transmitted diseases clinic and to explore the presence of serovars associated to LGV in our city.

**Methods:** We undertook a 4 years review, from August 2007 to August 2011. We included 235 male patients attended in a Unit of sexually transmitted infection (STI). To detect bacterial DNA in clinical specimens, the COBAS TaqMan CT Test was used (Roche). To genotype bacterial strains, a 990 pb-fragment of ompA gene was amplified by a nested PCR. The amplicons were purified by using a Montage DNA Gel Extraction Kit (Millipore) and sequenced with BigDye Terminator Cycle Sequencing Kit (Applied Biosystems). The individual sequences were compared to those available in the GenBank databases with the BLASTN program run on the NCBI Server.

**Results:** We found 31 of 235 (13.19%) rectal swabs positive for CT. A total of 27 of 31 (87.09%) could be analyzed for genotyping. The most prevalent genotype was E (48.14%) followed by D (22.22%), G (14.81%) and J (11.11%). We found a L2b genotype. The median age was 30.3 years (range 20–41).

**Conclusions:** Genotype E is the most frequent in this group of patients. Genotypes distribution is similar to other reports. We found one CT L2b variant related with LGV. Differentiation of CT serovars in clinical isolation may be important for a true understanding of the epidemiology and pathogenesis of genital chlamydial infections.

**P2002** Genotyping markers used for multi locus VNTR analysis with ompA (MLVA-ompA) retain stability through tissue culture adaptation and multiple passages in diverse *Chlamydia trachomatis* genotypes including the Swedish new variant nvCT

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**Objectives:** The aim of this study was to evaluate the stability of the *Chlamydia trachomatis* MLVA-ompA markers. Firstly, we analysed the stability of these markers through adaptation of *C. trachomatis* to tissue culture and secondly, we examined the stability of the MLVA-ompA markers after multiple passages in cell culture. Marker sequences were monitored to establish the stability of the individual markers within a numerical framework of bacterial divisions and this in turn informed us of the usefulness of using such typing systems for short and long term molecular epidemiology.

**Methods:** Southampton GUM clinic isolates from endocervical swabs collected from women who had contracted *C. trachomatis* were passaged through tissue culture. The MLVA-ompA genotyping scheme was assessed (Pedersen *et al.*, 2008); Sequence data from time zero and passage eight isolates were aligned with reference sequences to determine the stability of the markers. The Swedish new variant (nvCT) was grown through 72 passages in cell culture and the marker stability was similarly analysed.

**Results:** Analysis of the MLVA-ompA markers before and after the isolates were adapted to cell culture showed no change in sequence. The Swedish new variant that had been passaged 72 times over the duration of a year also showed no variation in the sequence of the MLVA-ompA markers.

**Conclusion:** The MLVA-ompA markers are stable upon *C. trachomatis* adaptation to tissue culture (McCoy cells) following isolation of strains from primary endocervical swab samples. In addition, these markers remain stable throughout multiple rounds of cell-division in tissue culture, concomitant with the incubation period and appearance of symptoms normally associated with host infection. Therefore this genotyping schemes is suitable for short and longer term epidemiological studies of *C. trachomatis*.

**P2003** Genotyping of Portuguese *Coxiella burnetii* isolates

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**Objectives:** *Coxiella burnetii*, the causative agent of Q fever in humans, is a zoonotic gammaproteobacteria with increasing interest in Europe due to the number and proportion of reported outbreaks. In Portugal, Q fever is a notifiable disease mainly reported in the Central and Southern Regions of the country. The disease is characterized by a low incidence rate of 0.08 cases (between 2004 and 2008) but could be largely underestimated. Moreover, there is no information available on the genotypic diversity of the agent that circulates in the country, important for both surveillance purposes and epidemiological investigation.

**Methods:** In the present study, a 6-loci Multiple-Locus Variable-number tandem repeat Analysis (MLVA) and Multi-spacer Sequence Typing (MST) were performed to characterize the Portuguese genotypes. Eleven cultivated human *C. burnetii* isolates, obtained from eight acute and three chronic Q fever cases, and, three positive DNA samples obtained from the blood of an additional acute Q fever patient and tissue samples from two stillborn goats were included in the study.

**Results:** Seven MLVA genotypes were observed that involved three different MST profiles (type 4, 8 and 13). Two different MLVA genotypes were present in the chronic Q fever isolates and goats ( $\geq 3$  markers differences), matching with MST type 8 and 13 that were also identified in human cardiac valves, sheep and goats from both France and Spain. Five other different but apparently closely related MLVA

genotypes (all belonging to MST type 4) were observed in acute Q fever patients. MST 4 has been identified before in animals and human blood samples in both France and Spain.

**Conclusion:** Our study identified a genetic diversity in Portuguese variants that is expected in a non-outbreak situation. Moreover, our results show the involvement of different genotypes in acute and chronic Q fever cases which has been described before in other Mediterranean countries. This is the first report of genotypic diversity among *C. burnetii* strains from Portugal. Integration of such data in international databases is important to understand the epidemiology of Q fever in Europe and beyond.

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**P2004** Genotyping of *Coxiella burnetii* from domestic ruminants in northern Spain

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**Objectives:** The information on the genotypic diversity of *Coxiella burnetii* isolates from infected domestic ruminants in Spain is limited. The aim of this study was to identify *C. burnetii* genotypes from livestock collected in Northern Spain and compare them to other European genotypes.

**Methods:** Fourteen placenta and vaginal mucus samples from sheep, five placenta samples from goats and 16 vaginal mucus and milk samples from cattle were included in the study. A commercial real-time PCR targeting the IS1111 insertion was used to confirm the presence of *C. burnetii* DNA. A 6-loci Multiple Locus Variable number tandem repeat analysis (MLVA) and Multispacer Sequence Typing (MST) were performed to identify the genotypes. The obtained genotypes were compared to *C. burnetii* genotypes from several other countries.

**Results:** Three MLVA genotypes were found in five goat farms, seven MLVA genotypes were identified in 10 cattle herds and two MLVA genotypes were identified in four sheep flocks. Clustering of the MLVA genotypes using the minimum spanning tree method showed a high degree of genetic similarity between most MLVA genotypes. Overall, 10 different MLVA genotypes were obtained corresponding to four different MST genotypes (MST-8, -13, -18 and -20). MST genotype 13, which has been identified before in human clinical samples from France, was found in all three ruminant species. MST genotypes 8, 18 and 20 have been identified before in animal and human clinical samples from France, Germany and Spain. Some of the MLVA genotypes had not been described before.

**Conclusion:** Genotyping reveals a substantial genetic diversity among domestic ruminants from Northern Spain. MLVA typing is less laborious and more discriminatory than MST.

**Acknowledgements:** This study was supported by INIA RTA 2009-00017-00.

**P2005** Clonal homogeneity of *Enterococcus faecium* isolates and comparative evaluation of DiversiLab typing and MLVA

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**Objectives:** *Enterococcus faecium* is an important microorganism causing health-care associated infections in Europe and is responsible for hospital outbreaks. In the last years, DiversiLab (DL) system (bioMérieux) has been introduced for clonal characterization of bacterial isolates. The aim of the present study was to investigate the usefulness of DL for subtyping *E. faecium* isolates for practical infection control purposes.

**Methods:** A total of 95 non-repetitive *E. faecium* isolates recovered from patients in the Dutch-German Euregio between 2007 and 2011 were analyzed in this study. The results obtained by DL were compared to those obtained by Multiple-Locus Variable Number Tandem Repeat Analysis (MLVA), well-established typing method for *E. faecium*. The

discriminative power of the typing methods was calculated by the Simpson's index of diversity and the concordance between methods was evaluated by adjusted Rand's and Wallace's coefficients. Moreover, distribution of the *vanA*, *vanB* and *esp* genes as well as the insertion element, IS16, by PCR screenings was determined.

**Results:** Eighty-one isolates presented *vanB* genotype, one isolate was *vanA* positive while 13 isolates were *vanA/B*/negative. All isolates carried IS16 indicating hospital-associated *E. faecium* isolates and in 90 isolates the *esp* gene was present. A typeability of 100% was obtained for DL, whereas a typeability of 95.8% was achieved for MLVA since a six-digit profile was obtained for 91 of the 95 isolates. The discriminatory power of DL was 0.875 (35 patterns) and its resolution differed substantially from that of MLVA which was 0.651 (eight types). Comparison of concordance data obtained by the two approaches and validated by *esp* gene typing allowed us to propose a 93% cutoff value of the similarity between any two DL patterns, which can be used in *E. faecium* epidemiological studies, including analyses of outbreaks and strain transmission events. Vancomycin-resistant and -susceptible *E. faecium* showed homoclonality in both typing systems, suggesting that both seem to transmit nosocomially.

**Conclusion:** DiversiLab is a rapid and highly discriminating tool to determine the clonality of *E. faecium* isolates and to trace their spread over periods of many months. Focus of prevention needs to be on *E. faecium* independently of vancomycin-susceptibility.

**P2006 Genetic and proteomic diversity of *Propionibacterium acnes* strains isolated from human skin in Japan and the comparison with European reports**

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**Objectives:** *Propionibacterium acnes*, an abundant commensal of human skin, is also known as an opportunistic pathogen of acne vulgaris (common acne), prosthetic hip joint infection, sarcoidosis, and prostate cancer and other infectious diseases. However, it is still not obvious if specific clones are associated with particular infections. Investigations using sequence data of several housekeeping genes (multilocus sequence typing, MLST) has been in progress. However, to date, such analysis has been deduced mostly for European strains, and it is unclear whether the clonal distribution seen in Europe is a global phenomenon.

**Methods:** We analysed 50 strains of *P. acnes* isolated from 10 healthy humans and four patients with atopic dermatitis (AD) in Japan, and carried out MLST analysis using seven housekeeping genes (*aroE*, *atpD*, *gmk*, *guaA*, *lepA*, *recA*, and *sodA*). Simultaneously, proteins were extracted from cells and analysed using a SELDI-TOF Mass Spectrometer with ProteinChip Arrays (H50, Q10, and CM10, Bio-Rad) to investigate their proteomic diversity.

**Results:** The genetic typing scheme successfully typed all strains into five types, IA, IB1, IB2, II and III. Types IA, IB1, IB2 and II are common in human skin in both Europe and Japan, while type III strains appear to be unique. In addition, 13 out of 14 strains in type IA were derived from the AD patients. In contrast, all five strains in type IB2 were from the non-AD group. Proteomic data suggested that the mass spectral profiles within cells differ widely between the five type groups, but are very similar between the strains of same type.

**Conclusion:** The present study revealed that the distribution of *P. acnes* types on the skin of those found in Europe are similar to those present in Japan, and the global existence of type III strains in human skin should be further investigated. In addition, type IA may be related to the pathogenesis of AD.

**P2007 Subtyping of *Legionella pneumophila* for epidemiological investigations by matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry**

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**Objectives:** *Legionella pneumophila*, the causative agent of legionellosis has its reservoir in natural and man made water networks. Through aerosol formation it can lead to sporadic and endemic infections mainly of immunocompromised patients. In an outbreak situation fast identification of the causing legionella strain is essential to identify and inactivate the source of infection as promptly as possible. Established methods for this purpose such as sequence-based typing (SBT), pulsed-field gel electrophoresis (PFGE) etc. or serotyping are either not discriminative or very time consuming and costly. Therefore we evaluated matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF) as an alternative tool for rapid identification of *L. pneumophila* on subspecies level.

**Methods:** *Legionella* samples from patients and environmental sources were used to compare strain differentiation ability of molecular methods (SBT) and MALDI-TOF. MALDI-TOF spectra were analyzed within the range of 5000 m/z and 11000 m/z. The study was repeated in two independent laboratories and by different operators.

**Results:** MALDI-TOF analysis yielded nine different groups in which the samples could be subdivided. These groups were matched to the results of sequence based typing according to the EWGLI scheme, as a standard typing method. In 88% the results of SBT and MALDI-TOF were identical. Three strains showed minor genetic differences that were not represented in the analyzed MALDI-TOF peak profile.

**Conclusion:** We concluded that MALDI-TOF has the potential to identify *L. pneumophila* subspecies level. This makes the technique an interesting candidate for reliable, fast and cost effective epidemiological investigations of *L. pneumophila*.

**P2008 A revised protocol for ribotyping of *Clostridium difficile***

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**Objective:** Ribotyping of *Clostridium difficile* is the primary way of differentiating isolates in many European countries. The standard method involves amplification of 16-23S rDNA spacer regions by PCR and separation of the resultant ribotype-specific products by electrophoresis in high resolution agarose. However this method is technically demanding, with the gel electrophoresis particularly difficult to standardise. The objective of this study was to develop and validate a simplified protocol.

**Methods:** Primers were redesigned and a fluorescent tag (TYE665) added to the forward primer. The PCR cycle optimised and PCR products detected using a fragment analyser (Beckman CEQ 8000). Control strains representing 24 of the most common *C. difficile* ribotypes and 187 isolates from a surveillance study were donated by the UK Anaerobe Reference Laboratory, Cardiff. The control isolates were used to construct a library of profiles and the study isolates were tested blind before referring results to Cardiff to break the code. For comparative purposes, some control isolates were processed using the standard method with gel electrophoresis to determine product sizes.

**Results:** The revised primers allowed products comparable to those produced by the standard protocol to be obtained under more stringent PCR conditions (higher annealing temperature and lower magnesium chloride concentration). Fragment analysis was simpler, required less hands-on time and was more reproducible in our hands, although the consumable costs were higher. Of the 187 study samples, 156 were ribotypes within the control panel, and 152 of these (97.4%) were correctly assigned. One of the remaining four was mis-assigned due to a clerical error; the others had reproducible PCR products which differed from the corresponding control, by only a single band in all but one cases.

**Conclusions:** Ribotype assignment using the revised protocol is highly concordant with the standard method. PCR products are assigned



molecular weights and so standardisation of the method should allow use of a shared standard database of ribotypes whereas centres currently have to produce their own database because of inter-centre variations in electrophoretic conditions. The revised protocol was more practical for the non-reference microbiology units who wish to use *C. difficile* ribotyping for local infection control purposes, although access to a fragment analyser is essential.

**P2009** The role of phylogenetic groups of *Escherichia coli* in childhood recurrent urinary tract infections

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**Objectives:** We aimed to compare the role of phylogenetic groups (PG) of *E. coli* in antibacterial (AB) susceptibility and containment of class I integrons in relation to first infectious attack and subsequent recurrences of urinary tract infection (UTI) caused by relapsing or re-infecting strains.

**Methods:** Altogether 89 urinary *E. coli* isolates from 41 children (35 girls and six boys) with a first acute pyelonephritis were derived from index (n = 41) and recurrent (n = 48) UTI episodes. PGs and occurrence of class I integrons were detected by PCR. E-tests were used for antimicrobial susceptibility testing. PFGE was applied to compare the consecutive 74 isolates of 26 patients with recurrences.

**Results:** More than half (51/89; 57%) of isolates belonged to PG B2. Group A (16/89) and D (18/89) were found with similar frequency, and B1 was the least common (4/89). AB resistance was detected to trimethoprim-sulfamethoxazole (28%), ampicillin (44%), cefuroxime (27%), cefotaxime (3%) and gentamicin (2%). There was no association between AB susceptibility and PG-s. PFGE revealed the unique clonal strains in 20/26 (77%) of patients, presenting as relapses. PG B2 caused more often relapses after the first attack than groups A, B1 and D (0/16 vs. 13/16, p < 0.001; 1/16 vs. 13/16, p < 0.001 and 2/16 vs. 13/16, p < 0.001; respectively). Clonal strains compared to individual ones had lower sensitivity to cefotaxime (MIC median 0.079 vs. 0.032, respectively; p = 0.004) and gentamicin (MIC median 0.75 vs. 0.5, respectively; p = 0.011). Strains of PG B2 consisted more often integrons than group A (45/51 vs. 8/16, respectively; p = 0.003). Intl pos compared to neg strains had higher MIC values to cefuroxime (MIC median 4.0 vs. 2.0, respectively; p = 0.001), cefotaxime (MIC median 0.079 vs. 0.032, respectively; p = 0.014), gentamicin (MIC median 0.75 vs. 0.44, respectively; p = 0.013).

**Conclusion:** Relapses of UTI are more frequent than reinfections. In children the PG B2 strains of *E. coli* containing integrons are prevalent at the first attack of recurrent UTI. Though the integron positive strains of *E. coli* express higher MIC values the PG diversity is not associated with AB susceptibility or development of resistance in recurrent strains.

## Emerging infectious diseases

**P2010** Survey on tick-borne encephalitis in Canton Valais, Switzerland

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**Objectives:** In 2009, a national survey demonstrated for the first time the presence of tick-borne encephalitis virus (TBEV) in field collected tick at two out of five investigated sites in the southern part of Switzerland in the canton Valais.

The aim of the present study was first to confirm the persistence of TBEV at the two positive sites (Raron and Salgesch) and second to detect the virus in other potential sites.

**Methods:** Over 2 years (2010–2011), 13 369 questing ticks (*Ixodes ricinus*) were collected by flagging low vegetation at 34 different sites located in the canton Valais. Ticks were counted and placed into tubes and stored at –80°C until further analysis.

Ticks were pooled (10–20 individuals for adults and 20–50 individuals for nymphs) because the prevalence of TBEV is usually very low. Then

they were crushed and lysed. DNA and RNA were extracted and TBEV was detected by real time RT-PCR.

**Results:** In 2010, a total of 6507 ticks was collected with a short majority of nymphs (50.8%, 3306/6507). A total of 1749 ticks was collected in Raron and 489 in Salgesch. The presence of TBEV in ticks at Salgesch and Raron was confirmed in 2010, with a prevalence of 0.23 positive ticks per 100 tested (95% CI: 0.04–1.01) and 0.88 (95% CI: 0.48–1.44), respectively.

In 2011, 6862 ticks were collected, two-thirds of which were nymphs (65.9% [(4518/6862) and 34.1% [2337/6862] were adults). The virus was detected again in ticks collected at Salgesch and Raron with a prevalence of 0.16% (95% CI: 0.04–0.41) and 0.69% (95% CI: 0.11–2.11), respectively. TBEV infected ticks were detected at two additional sites sampled in 2011 with prevalences of 0.19% (CI: 0.04–0.57) and 1.12% (CI: 0.19–3.46).

Of the 23 pools of ticks that tested positive for TBEV over the 2 years, 17 contained only adults (73.9%), four only nymphs (17.4%) and two (8.7%) were a mix of adults and nymphs.

**Conclusion:** These results represent a preliminary study but the repeated detection of the TBEV over three years at Raron and Salgesch confirms a persistence of the two foci. The detection of TBEV in ticks collected at two additional sites suggests the emergence of new risk areas in the canton Valais. Because of the high variability of TBEV in time and space, a surveillance of the infected areas and other way of investigations using rodents, domestic animals (e.g. goats) as well as blood donors as sentinels will be organized. Genotyping of 23 detected TBEV is under way.

**P2011** Characteristics of West Nile virus neuro-invasive disease vs. other viral meningitides

I.D. Oлару\*, A. Hristea, R. Moroti, C. Popescu, V. Arama, D.I. Munteanu (Bucharest, RO)

West Nile virus (WNV) is an arthropod-borne emerging pathogen. Large outbreaks from Romania in 1996, and Greece, 2010, have led to the recognition of WNV infection as a public health concern. Between 2005 and 2011, there were 74 recorded cases of WNV neuroinvasive disease (NID) in humans, in Romania, with over 70% of cases occurring in 2010.

**Objective:** Compare characteristics of patients with West WNV NID vs. other viral meningitides.

**Methods:** Retrospective, observational study of patients admitted with viral meningitis or meningo-encephalitis between 2005 and 2011 in a tertiary infectious diseases hospital. We excluded patients who received antimicrobial treatment (possible bacterial or fungal meningitis) and patients without a cerebrospinal fluid (CSF) analysis. Serology for WNV was performed in all patients hospitalized with meningitis during the period of vector activity (May–November) as part of an

Table. Patient characteristics

	West Nile virus NID Median, IQR/ N(%)* N=14	Other viral meningitides Median, IQR/ N(%)* N=244	Statistic (p, 95% CI)
Age	68.5 (52-83)	22.5 (17-31)	< 0.001
Male sex	10 (71%)	146 (59.8%)	0.388
Symptom onset	3.5 (2-6.5)	2 (1-4)	0.015
Fever	14 (100%)	228 (93.4%)	0.398
Headache	11 (79%)	240 (98.4%)	0.004
Neurological impairment	9 (75%)	22 (9%)	< 0.001
CSF cell number	55 (35-120)	150 (86-329)	0.009
CSF protein (mg/dl)	122.5 (91-157)	78 (59-108)	0.006
CSF glucose (mg/dl)	70.5 (48-79)	60 (53-67)	0.278
Unfavorable outcome	3 (21%)	1 (0.4%)	< 0.001

\*Continuous variables are described using the median and interquartile range (IQR), and dichotomous variables - number and percentage, NID- neuro-invasive disease, CSF - cerebrospinal fluid.

epidemiological surveillance program for WNV infection. Differences between groups were analyzed using the Mann–Whitney *U* test for continuous variables and the chi-square test for dichotomous variables. Multivariable analysis was performed using logistic regression.

**Results:** We identified 14 patients with WNV NID and 244 cases of other viral meningitides. Nine cases of WNV infection occurred in 2010. The characteristics of patients from the two groups are shown in Table 1. On multivariable analysis, only older age was associated with WNV NID (95%CI,  $p < 0.001$ ).

**Conclusions:** On univariate analysis older age, a longer symptom duration, the absence of headache, neurological impairment, lower CSF cell number, higher CSF protein, and an unfavorable outcome were associated with WNV infection. On multivariable analysis only older age remained statistically significant.

#### **P2012** Outbreak of West Nile virus infection in humans in southern Greece, August–October 2011

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**Objectives:** West-Nile virus (WNV) is a reemerging zoonotic pathogen of public health importance. During summer-autumn 2011, an outbreak of WNV infection occurred in southern Greece, following the first large 2010 outbreak in the north of the country. WNV infection in humans has not previously been documented in southern Greece according to Hellenic Centre for Disease Control and Prevention (HCDCP). We conducted a study to describe the geographic distribution of WNV cases reported from southern Greece and to determine the clinical manifestation of these cases.

**Methods:** Specimens (serum and CSF) from in- and outpatients suspected with WNV infection, originating from southern Greece, were tested for the presence of WNV-specific IgM and IgG antibodies using commercial ELISA kits (WNV IgM capture DxSelect and WNV IgG DxSelect, Focus Diagnostics Inc, Cypress, CA, USA). Laboratory diagnosed cases were notified to the HCDCP.

**Results:** From August to October 2011, 32 laboratory-diagnosed cases of WNV infection, all from patients hospitalized in Athens, were diagnosed by Department of Microbiology, Medical School, University of Athens, and reported to the HCDCP. Twenty one of those presented with WN neuro-invasive disease (12 meningitis, seven meningoencephalitis, one encephalitis and one acute flaccid paralysis) and 11 as non-neuroinvasive, which experienced mild non-specific disease symptoms. For the 32 laboratory confirmed cases, 28 serum samples and 20 CSF were available; for 16 patients both CSF and serum specimens were provided, while for four patients only CSF was available. WNV-specific IgM antibodies were detected in all 28 serum samples and in 19 CSF specimens, while WNV specific IgG antibodies were detected in 11 serum and five CSF specimens. Tauhe median age of cases was 65 years (range 10–86). Seventeen cases were reported from Eastern Attiki, approximately 43 km from the metropolitan area of Athens. Hypertension ( $n = 8$ ), diabetes mellitus ( $n = 6$ ) and coronary disease ( $n = 2$ ) were the most commonly reported underlying conditions. Eleven patients were admitted to an intensive care unit and seven had a fatal outcome.

**Conclusion:** In 2011 WNV dispersed southward to newly affected areas, including Eastern Attiki, which is in close proximity to the metropolitan area of Athens. WNV serological evaluation remains important to guide epidemiological surveillance as well as patients' management and disease control.

#### **P2013** Human bocavirus infection in hospitalised children: molecular characterisation and clinical associations of a newly emerging respiratory virus

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Human bocavirus (HBoV) has recently been detected in children with respiratory tract infections (RTI). Due to the frequent co-detection of

other viruses and its detection in healthy children, the clinical relevance of this virus is still unclear.

**Objective:** The aim of our study was to describe the clinical, epidemiological and molecular characteristics of this new virus in hospitalized children and to determine its pathogenic role in RTI.

**Methods:** A total of 270 samples of nasopharyngeal aspirates from children  $\leq 5$  years hospitalized for respiratory tract infection or suspected sepsis with some respiratory symptoms, and 51 samples from healthy children were studied. HBoV detection was performed using conventional polymerase chain reaction (PCR). Viral load quantification of HBoV by real-time PCR and phylogenetic analysis of HBoV sequences were performed. Other common respiratory viruses were detected using immunochromatographic, immunofluorescence or real-time reverse transcriptase-PCR (RT-PCR) assay.

**Results:** HBoV was more frequently detected in hospitalized children than in healthy children (24% vs. 12%,  $p = 0.046$ ). It was the second most frequent virus after respiratory syncytial virus (RSV). HBoV showed a seasonal distribution with December as the peak month, being more frequent in children older than 6 months ( $p < 0.001$ ). Bronchiolitis was the most frequent diagnosis (55%). HBoV was detected in 56% with other viruses. More severe disease was found in patients with RSV single infection than in HBoV single infection. Adjusted for age and gender, the detection of HBoV as a sole agent was associated with RTI (OR: 9.17, 95% CI: 2.1–39.8,  $p = 0.003$ ). A high viral load was not associated with the severity of the disease. Phylogenetic analysis confirmed the co-circulation of both HBoV ST1 and ST2 groups described so far.

**Conclusion:** HBoV was frequently found in children hospitalized with acute lower RTI. HBoV can be detected in healthy children but with a significantly lower frequency than in children with RTI. Despite the high rate of co-detection, our results suggest that HBoV may play a role as an etiologic agent of RTI in children. Additional studies are required to completely define the epidemiological profile of this newly recognized pathogen.

#### **P2014** The evaluation of ribavirin use in patients with Crimean-Congo haemorrhagic fever

N. Tulek, B. Ozturk\*, C. Bulut, G. Tuncer Ertem, FS. Erdinc, S. Altun, S. Kinikli, AP. Demiroz (Ankara, TR)

**Objectives:** Crimean Congo Hemorrhagic Fever (CCHF) is an important viral hemorrhagic disease with comparatively high mortality. While supportive care is the basic treatment, ribavirin usage as antiviral treatment is a debate. The aim of this study is to investigate the effect of ribavirin on mortality and morbidity in the patients with CCHF treated in our hospital.

**Methods:** A case–control study was conducted retrospectively between the years 2007 and 2011. In our hospital there are two independent infectious diseases departments. One of the departments had ribavirin in the treatment protocol of CCHF patients. Supportive care protocol was similar in both of the departments. The patients who had received at least 48 hours of ribavirin were included in the ribavirin group and they were monitored in the sense of probable adverse effects.

Table. Comparison of age, gender distributions and laboratory findings of both groups

	Ribavirin group Mean (SD)	Control group Mean (SD)	p value
Gender M/F (n)	47/44	75/77	0.728
Age (years)	48 (17)	51 (18)	0.261
Hgb ( $10^3/\mu\text{L}$ )	13.6 (1.8)	13.5 (1.9)	0.646
PLT ( $10^3/\mu\text{L}$ )	54644 (40924)	56204 (34946)	0.267
WBC ( $10^3/\mu\text{L}$ )	2459 (1494)	2837 (2134)	0.259
AST (U/L)	254 (246)	316 (640)	0.650
ALT (U/L)	110 (103)	137 (161)	0.254
LDH (U/L)	733 (566)	747 (729)	0.783
CK (U/L)	623 (700)	606 (1140)	0.056
PT (s)	13.1 (3.4)	12.0 (2.7)	<b>0.001</b>
aPTT (s)	43.2 (11.0)	40.3 (13.2)	<b>0.004</b>

The two groups were compared according to clinical course and laboratory findings. SPSS 15.0 package program was used for statistical analysis.

**Results:** The study was conducted with 243 patients (122 male, 121 female) after excluding seven deaths because of <48 hours hospitalization. The average age was 49.8 years (SD = 17.7). The ribavirin group was composed of 91 patients (37.4%). Both of the groups were similar in terms of age, gender distribution and laboratory results except PT and aPTT mean values (Table 1). For blood and blood products requirement, there was no statistically significant difference. One patient (1.1%) in ribavirin group and eight patients (5.3%) in the other group died ( $p = 0.096$ ). In ribavirin group, 60% of the patients had received ribavirin in the first 4 days after onset of symptoms. No adverse effect was observed in the patients of ribavirin group during treatment.

**Conclusion:** Although no statistical significance was detected in our study, ribavirin seems noteworthy in survival of CCHF patients. Besides, as we did not observe any adverse effects, ribavirin should not be excluded from the CCHF treatment protocol until convincing studies performed.

#### P2015 Relation of the viral load and laboratory and clinical findings in Crimean-Congo haemorrhagic fever

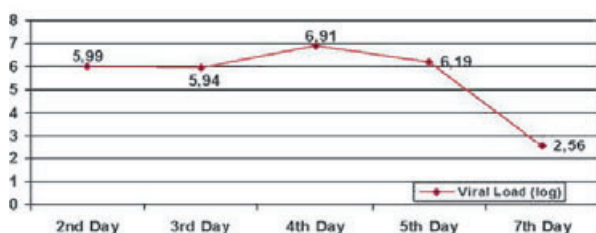
Z. Kocak Tufan\*, K. Ergunay, C. Bulut, H. Turan, A. Ozkul, S. Kinikli, A. Demiroz (Ankara, TR)

**Objectives:** Crimean Congo hemorrhagic fever (CCHF) is an emerging disease in Turkey since 2006. In this study, we aimed to evaluate the possible relations between CCHFV viral load and clinical and laboratory findings.

**Methods:** Confirmed CCHF patients, who were hospitalized in the second infectious diseases and clinical microbiology department of our hospital between May and August 2011, were included into the study. Clinical records and hospital database were used to investigate the clinical and laboratory findings. RNA extraction from patient sera was performed via a commercial spin-column system (High Pure Viral Nucleic Acid Kit; Roche Diagnostics, Germany) and viral load determination was performed employing a quantitative one-step reverse transcription real-time PCR assay in a Rotor-Gene 6000 instrument (Corbet Research, Australia). Patients were divided into two groups according to their viral load: (i) Patients with higher viral load ( $>10^7$ ) and (ii) patients with lower viral load ( $<10^7$ ), to compare the clinical and laboratory findings.

**Results:** A total of 37 CCHF patients were included into the study. Nineteen of them (51%) were male. Mean age was  $53.1 \pm 16.5$ . CCHFV-PCR was positive in 23 patients (62%). Mean duration between the onset of the symptoms and hospitalization were 7.4 and 4 days in PCR- negative and PCR-positive patients respectively ( $p < 0.05$ ). Viral load according to the day of the disease was shown in Fig. 1. The highest viral load value was detected on the 4th day of the illness. When PCR-positive and negative groups compared, mean length of hospitalization, mean prothrombin time (PT) and activated partial thromboplastin time (aPTT) levels were higher in the PCR-positive group ( $p < 0.05$ ). Mean duration of hospitalization, mean aPTT levels and mean INR levels were found to differ significantly between high and low viral load groups ( $p < 0.05$ ) (Table 1).

Figure 1. Viral load (log) vs days of the symptoms



	Patients		P
	Viral load $>10^7$	Viral load $<10^7$	
Age (years)	55,8±14,6	49,1±13,5	0,291
Hospital stay (days)	8,7±3,6	5,8±2,4	<b>0,039</b>
WBC ( $10^3/\mu\text{L}$ )	2,4±1,1	3,1±2,2	0,441
Hb (g/dL)	14,6±2,2	13,6±1,5	0,226
PLT ( $10^3/\mu\text{L}$ )	33,0±15,9	45,8±28,8	0,247
SGOT (U/L)	1354,7±2516,7	347,0±302,1	0,178
SGPT (U/L)	276,7±350,4	158,0±126,3	0,242
ALP (U/L)	91,9±28,4	85,8±35,1	0,669
GGT (U/L)	127,4±84,5	112,5±99,0	0,717
LDH (U/L)	1743,0±1921,9	932,7±527,7	0,182
CK (U/L)	619,8±704,3	761,6±1166,7	0,747
PT (sec)	13,9±3,052	11,7±2,5	0,082
aPTT (sec)	47,1±10,8	36,9±7,6	<b>0,015</b>
INR	1,2±0,2	0,9±0,2	<b>0,029</b>

**Conclusion:** Our study showed that higher viral load causes longer hospital stay and recovery time. Since only one patient died among our patients, viral load and mortality relationship could not be evaluated. Interestingly, viral loads were not correlated with any of the laboratory findings, suggesting possible immune response variations among patients. Further studies including animal models are needed to clarify this issue.

#### P2016 Certain cytokine levels in sandfly fever caused by sandfly fever Turkish virus

Z. Kocak Tufan\*, C. Bulut, S. Yagci, S. Kinikli, A. Demiroz (Ankara, TR)

**Objectives:** Sandfly fever is caused by Sandfly Fever Virus (SFV). Sandfly Fever Turkey Virus (SFTV) is a variant of SFV Sicilian serotype and recently identified in Turkey. Although the sandfly fever has been described as a self-limited disease with a benign clinical course, the SFTV causes more severe findings. The aim of this study is to investigate certain cytokine levels which are involved in the inflammatory response during the acute phase of the disease.

**Patients and methods:** Thirty-three patients with sandfly fever were included into the study. The control group comprised of 26 otherwise-healthy individuals. Interferon gamma (INF gamma), which has anti-viral effects; IL 6, which can act as both pro-inflammatory and anti-inflammatory; IL 10, which has anti-inflammatory effects; and the main pro-inflammatory cytokine tumor necrosis factor alpha (TNF alpha) levels were assessed by ELISA (Biosource, USA).

**Results:** The differences of the serum levels of IL-6, IL-10 and INF gamma between patients and control group was found statistically significant. Especially the serum concentrations of IL 10 and IL 6 were strikingly higher in the patient group (median [range], 5.8 pg/mL [11.45] vs. 2.45 pg/mL [8.14],  $p < 0.0001$  and 3.32 pg/mL [1049] vs. 0.11 pg/mL [1049],  $p = 0.001$  respectively, Table 1). Correlation analysis showed moderate relations between IL 10 and TNF alpha ( $r = 0.35$ ,  $p = 0.042$ ) and between IL 6 and TNF alpha ( $r = 0.52$ ,  $p = 0.002$ ).

The mean levels of the cytokines were also checked and found as follows: INF gamma:  $32.1 \pm 30.4$  pg/mL vs.  $17.1 \pm 10.3$  pg/mL; IL

Table 1. The cytokine levels of the sandfly fever patients and the controls.

Cytokine	Median (min-max) pg mL		P
	Patient group (n=33)	Control group (n=26)	
INF gamma	22.83 (0.07-130.97)	17.20 (0.08-43.29)	0.044
IL 10	5.81 (0.94-12.39)	2.45 (0.0-8.14)	<0.001
IL 6	3.32 (0.0-1049.22*)	0.11 (0.07-1049.22*)	0.001
TNF alpha	9.97 (1.94-201.58)	12.17 (5.71-41.58)	ns

\*The maximum detectable level of the test for IL 6 was 1049.22.

10:  $6.1 \pm 2.8$  pg/mL vs.  $2.7 \pm 2.1$  pg/mL; IL 6:  $257.9 \pm 449.4$  pg/mL vs.  $107.9 \pm 306.8$  pg/mL and TNF alpha:  $26.9 \pm 39.9$  pg/mL vs.  $12.6 \pm 8.1$  pg/mL in the patients and control group respectively.

**Conclusion:** IL 6, IL 10 and INF gamma levels were found to be higher in sandfly fever patients with respect to healthy subjects, whereas no difference observed between groups for TNF alpha. In this study the cytokine levels were measured during the clinically active phase of the disease, when the patient applied to the clinic. So we can assume that these three cytokine may have a role on the symptomatic phase of the disease with fever. However, cytokine levels for subsequent days and the convalescent phase are lacking. To understand the immune modulation of the SFTV and the disease better, detailed studies on cytokine response vs. clinical course, including animal models are needed.

#### **P2017** Anaplasma seropositivity in Crimean-Congo haemorrhagic fever patients

F.S. Erdinc, S. Kilic, C. Bulut\*, B. Celebi, N. Tulek (Ankara, TR)

**Objectives:** Turkey is an endemic country for Crimean-Congo haemorrhagic fever (CCHF) but there is no reported human anaplasmosis case ever. However, one human and one animal serological surveillance study have been reported with seropositivity of 8% and 15%, respectively. Ixodes ricinus, the tick vector for *Anaplasma phagocytophilum* is widespread in the country. Clinical findings of the both diseases seem similar with fever, headache, myalgia, anaemia, leukopenia, thrombocytopenia and hepatic transaminase elevation. In this study the aim was to investigate the probable seropositivity of anaplasmosis in patients hospitalized with probable or confirmed CCHF diagnosis.

**Methods:** The study is a retrospective serological survey of serum samples collected from 78 CCHF suspected cases and followed up in our clinic between 2008 and 2011. The samples were kept at  $-20^{\circ}\text{C}$ . The history of patients including tick exposure, clinical and laboratory findings were recorded. Serological survey of IgG or IgM antibodies reactive with *A. phagocytophilum* antigen is performed by immunofluorescence antibody test (IFAT) (Fuller Lab., USA).

**Results:** Among 78 serum samples, 13 had either IgM or IgG seropositivity for *A. phagocytophilum* with  $\geq 64$  titer. Range of ages of the positive patients was 36–72 years. The patients had been referred to our clinic from hinterland Black Sea Region and hospitalized in May, June and July. Two patients had isolated IgG positivity and 11 patients had isolated IgM positivity for *A. phagocytophilum*. IgG titers were detected as 1/64 and 1/128 but the test could not be repeated to predict the increase in titers. Among IgM positive patients, three had 1/64, two had 1/128 and 6 had 1/256 titers. Of the 78 patients, a total of 68 including anaplasma seropositive patients had CCHF confirmed by PCR and/or ELISA IgM. The time of tick exposure implied by 11 of anaplasma positive patients were between 2 and 20 days before the onset of symptoms.

**Conclusion:** Although it has a potency to be seen as the area is relevant, anaplasmosis is a less known disease in Turkey. CCHF and anaplasmosis are two different diseases transmitted by different tick species but they may have similar clinical expression. People with high risk of tick exposure may have possible risk of all tick bite associated diseases. Although that 17% seropositivity needs to be proven by increased IgG titers, our results indicate to be on alert for anaplasmosis in our country.

#### **P2018** Tularemia: evaluation of the patients and antimicrobials

B. Ozturk\*, M. Demirelli, Z. Kocak Tufan, S. Altun, C. Bulut, N. Tulek, F.S. Erdinc, G. Tuncer Ertem (Ankara, TR)

**Objectives:** *Francisella tularensis*, is a Gram-negative, aerobic, facultative intracellular bacteria. It causes the disease "tularemia" with mild to severe clinical course. Tularemia is a re-emerging disease in our country as well. The most common mode of transmission is

arthropod vectors and consumption of contaminated food or water. Streptomycin, gentamycin and tetracycline are among the therapeutic agents of tularemia. Here, we evaluated the clinical-epidemiological characteristics of our patients with tularemia.

**Methods:** Prospective review of clinical records of patients diagnosed with tularemia admitted to our hospital in Ankara, from January 2010 to December 2011 was done. Suspected clinical course plus epidemiology (coming from epidemic/endemic area) along with positive serology (antibodies to *F. tularensis*  $>1/160$ ) was accepted as case definition. Patients were divided into two groups according to the response to treatment; complete and partial response. Development of a new lymphadenopathy during the therapy period was defined as partial response. SPSS15.0 for Windows was used for statistical analysis.

**Results:** Total 77 patients (34 males, 43 females) with a mean age of  $42.7 \pm 19.0$  years were included into the study. Half of the patients were living in endemic areas. Consumption of contaminated food or water was the most commonly noted exposures. Thirty-four patients reported to see a rat around their living area. Clinical symptoms of the patients were shown in Table 1. Sixty-two patients were accepted as having complete response (80.5%) while 15 patients had partial response (Table 1). When these groups were compared, only ESR was found to be statistical high in partial response group ( $p = 0.048$ ). Moxifloxacin was found to be the most effective drug among others.

Table. Risk factors that affect the response for tularemia treatment

	Complete response (n=62)	Partial response (n=15)	p value
Age (mean)	43,2 = 18,9	40,8=19,7	0,648
Gender F/M	34/28	9/6	0,718
Sore throat	51	13	0,683
Fever (>38,5)	51	13	0,683
Conjunctivitis	48	1	0,163
Lymphadenopathy	57	15	0,255
WBC ( $10^3/\mu\text{L}$ )	9,2=2,6	9,2=3,0	0,827
CRP	3,38 (4,36)	3,54 (3,55)	0,538
ESR (mm/h)	45,7=28,0	64,7=31,7	0,048
Drugs			
Siprofloksasin	25 (78,2)	7 (21,8)	
Moksifloksasin	13 (42,0)	1 (7,1)	
Doksiziklin	20 (87,0)	3 (13,0)	-
Streptomycin	4 (66,7)	2 (33,3)	
Gentamisin	-	2 (100)	

**Conclusion:** Although first line therapy for tularemia is streptomycin, moxifloxacin can be a good treatment option.

#### **P2019** Ocular tularemia

S. Eren Gok\*, O. Evren Kemer, A. Kocagul Celikbas, N. Baykam, A. Atay Buyukdemirci, M. Eroglu, B. Dokuzoguz (Ankara, TR)

**Objectives:** Tularemia is a zoonotic disease caused by *Francisella tularensis*. Depending on the route of entry, tularemia occurs in several clinical forms. The oculoglandular form of tularemia is accounts for 3–5% of all diagnosed tularemia cases. In this report we discuss our findings and management of seven patients with ocular tularemia.

**Methods:** During an outbreak of tularemia in the district of Ankara in Turkey, 48 patients were followed up in our clinic between November 2010 and February 2011. The epidemiological and clinical findings, laboratory assays and drugs used for the treatment of patients were recorded prospectively. The diagnosis of tularemia was confirmed with microagglutination test as well as clinical findings.

**Results:** The oculoglandular form of tularemia was diagnosed in seven of the patients. In our patients the rate of oculoglandular tularemia was 14.58%. The most common symptoms were fever, ocular findings and lymphadenopathy. Ocular findings included periorbital edema, conjunctival injection and chemosis. The most distinctive ophthalmic feature was follicular conjunctivitis accompanied by conjunctival epithelial defects. The lymphadenopathies were localized especially in preauricular, postauricular and less frequently in submandibular and jugular region. Only one patient had tonsillopharyngitis.

The time between the onset of the symptoms and diagnosis was 3–21 days. Among the laboratory tests; white blood cell count were normal, ESR and CRP values were elevated, the micro agglutination test titers for tularemia varied between 1/20 and 1/320.

In treatment; topical ciprofloxacin or tobramycin eye drops, ophthalmic ointment and systemic streptomycin or ciprofloxacin were used. Ocular findings were regressed in 7–10 days of therapy. The patients followed up for three months and fully recovered.

**Conclusion:** The oculoglandular tularemia is one of the rarest clinical form which shouldn't be misdiagnosed. Although admission to hospital in the early phase of the disease and immediate therapy are important for complete resolution, lack of sufficient antibody production may be a difficulty in diagnosis of these patients. The management of the patients should be performed by ophthalmologists and infectious diseases doctor with topical and systemic antimicrobial agents.

**P2020 Evaluation of proteinuria and erythrocyturia as prognostic factors of renal function in leptospirosis patients and correlation between number of inducing serovars and severity of leptospirosis**

M. Madelane\*, I. Kozlovska, A. Broduza, B. Rozentale (Riga, LV)

**Objectives:** To analyze clinical parameters correlation with inducing leptospirosis serotypes amount; to analyze bacteruria, proteinuria, erythrocyturia as prognostic factors for urea and creatinine levels in patients with leptospirosis.

**Methods:** One hundred and thirty-seven medical records of patients with leptospirosis at the Infectology Center of Latvia during 1997–2010 were analyzed. Count of leucocytes, erythrocytes, thrombocytes, level of BIL, ALAT, urea, creatinine, bacteruria, proteinuria, erythrocyturia were analyzed in regard to number of inducing serotypes, as well as bacteruria, proteinuria and erythrocyturia in regard to the levels of urea and creatinine during disease. Confirmed leptospirosis was based on the positive leptospirosis Microagglutination Test titer of  $\geq 1:100$ . Exclusion criteria- patients with any proved chronic disease.

**Results:** One hundred and twelve cases were reviewed. The patients ranged in age from 18 to 80 (mean 49). Urban and rural area ratio was 53/59. Totally 13 different serovars of *Leptospira* were detected. In 46 cases (41%) one serovar induced disease, in 66 cases (59%) combination of two or more different serovars induced disease. Statistically significant link could be established between number of inducing serovars and erythrocyte level in the peripheral blood ( $p = 0.014$ ) at the 1st day in hospital. No other statistically significant links or correlations were found for clinical parameters and number of serovars. Bacteruria was present in 47 patients (42%). Proteinuria was present in 77 cases (69%).

Statistically significant correlation was found for patients with higher proteinuria levels and higher levels of urea (cor.coef. = 0.381) and creatinine (cor.coef. = 0.518) during the hospital setting. In patients with erythrocyturia link was found for higher urea ( $p = 0.003$ ) and creatinine ( $p = 0.003$ ) levels. No statistically significant link could be established suggesting bacteruria as a prognostic factor for urea and creatinine levels during periods of illness. Statistically significant link could be established between number of highest levels of urea ( $p = 0.013$ ) and creatinine ( $p = 0.001$ ) in patients living in rural area.

**Conclusions:** Number of inducing illness serotypes do not correlate with severity of leptospirosis. Proteinuria and erythrocyturia could be a prognostic factor for suggesting higher urea and creatinine levels during the hospital period. Bacteruria in leptospirosis patients does not have impact on urea and creatinine levels during the disease.

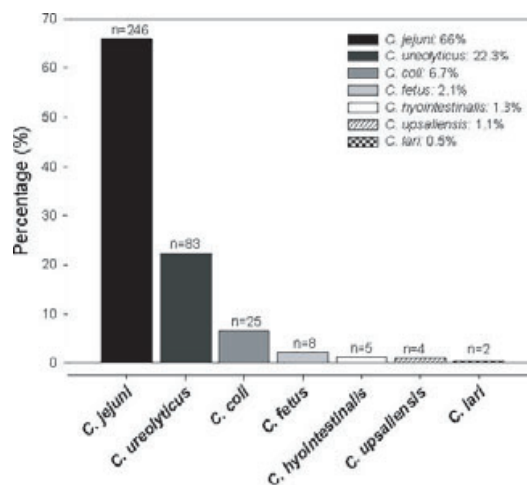
**P2021 *Campylobacter ureolyticus*: an emerging gastrointestinal pathogen**

S. Bullman\* (Cork, IE)

**Objectives:** The focus of the current study was to compare traditional culture based *Campylobacter* detection methods with EntericBio; an automated molecular based detection system. The principal objective was to identify previously undetected/unreported strains which may pose a significant public health concern.

**Methods:** Seven thousand one hundred ninety-four faecal samples, collected over a 1 year period from patients presenting with diarrhoea, were screened for *Campylobacter* spp. using the EntericBio® system. All *Campylobacter*-positive samples were cultured retrospectively and uniplex *Campylobacter* species-specific PCR screening for clinically significant enteropathogenic *Campylobacter* species was conducted on all strains initially detected using the EntericBio system. Furthermore, based on these results, a detailed bioinformatics analysis facilitated the development of a *C. ureolyticus* species-specific PCR assay targeting a 429 bp region of the heat shock protein (hsp60) gene of *C. ureolyticus*.

**Results:** A total of 349 samples were determined *Campylobacter*-genus positive by the EntericBio molecular method. Targeting the hsp60 gene: *C. ureolyticus*, was detected in 83 samples, representing 23.8% of all *Campylobacter*-positive samples. Of these, 30 were found to exist as mixed isolates with other *Campylobacter* species. In total, 53 cases of *C. ureolyticus* was isolated from faecal samples in the absence of the other common bacterial enteric pathogens. Routine culture on the other hand, failed to detect the organism from samples that were PCR positive for *C. ureolyticus* only. We are the first to report *C. ureolyticus* as being the second most common *Campylobacter* species (22% of all *Campylobacter* infections) detected in the faeces of patients presenting with GI illness present, surpassing *C. coli*. After analysis of the patterns of infection for the *Campylobacter* spp detected, we report a prominent seasonal distribution in addition to a bimodal age distribution. More than 50% of all *Campylobacter* infections occurred at extremes of age;  $\leq 5$  and  $\geq 70$  years of age.



**Figure 1**  
The speciation of *Campylobacter* by PCR: Percentage contribution of each species to the total number identified (n=373).

**Conclusions:** The EntericBio system displays significantly improved sensitivity over traditional culture based methods for the detection of diverse species of *Campylobacter*. Furthermore, the EntericBio system enabled the identification of *C. ureolyticus* in the faeces of patients presenting with diarrhoeal illness, suggesting its possible role as a novel gastrointestinal pathogen and an emerging *Campylobacter* species.

**P2022** Assessment of the prevalence and diversity of emergent campylobacteria in human stool samples using a combination of traditional and molecular methods

L. Collado\*, M. Gutierrez, M. Gonzalez, H. Fernandez (Valdivia, CL)

**Objectives:** Emergent campylobacteria, including *Arcobacter* and *Campylobacter* species other than *Campylobacter jejuni* and *Campylobacter coli*, are increasingly being associated with human and animal diseases. However, the traditional culture methods used in clinical laboratories are generally unable to detect these emerging pathogens. Therefore, the aim of this study was to assess the prevalence and diversity of *Campylobacter* and *Arcobacter* species in human stool samples, using a combination of traditional and molecular methods.

**Methods:** Ninety-one human faecal specimens from patients with diarrhoea (Group A) and 63 faecal samples obtained from healthy volunteers (Group B), collected in the city of Valdivia (Chile) from September 2010 to February 2012, were analyzed. *Campylobacter* and *Arcobacter* genus-specific PCRs were used to analyse DNA extracted directly from samples. In parallel, each sample was inoculated in selective and non-selective medium which were incubated under microaerobic conditions, at 37°C for up to 5 days. The obtained isolates were characterized at species level by multiplex-PCR and PCR-RFLP. The chi-square test was used to evaluate the detection capacity of both detection methodologies. A p-value of <0.05 was considered statistically different.

**Results:** Traditional method detected campylobacteria in 13.2% of the group A and 3.2% of the group B. In contrast, molecular methods detected campylobacteria more often than culture (p < 0.0001) with a prevalence of 31.9% and 4.4% respectively in both groups. The ability to detect various species also differed between traditional and molecular methods. Only the species *C. jejuni*, *C. coli* and *Arcobacter butzleri* were recovered using the traditional approach, whereas by using molecular methods, the emergent species *C. concisus* and *C. ureolyticus* were also detected.

**Conclusion:** The present study demonstrates that the additional use of molecular methods in the analysis of clinical samples gives a greater representation of the prevalence and diversity of *Campylobacter* and *Arcobacter* species. To our knowledge, this is the first report of the detection of *C. concisus* and *C. ureolyticus* from human clinical samples in Chile and Latin America.

This work has been supported by the postdoctoral grant Fondecyt Nordm;3110016.

**P2023** Ability of the zoonotic emerging food-borne pathogen *Arcobacter butzleri* to survive inside the free-living amoeba *Acanthamoeba castellanii*

H. Fernandez\*, M.P. Villanueva (Valdivia, CL)

**Objective:** *Arcobacter butzleri* is considered as a zoonotic emerging food borne human enteropathogen. This species recognizes a broad spectrum of mammals and birds as their natural reservoirs, being also isolated from shellfish and transmitted by food -mainly of avian origin- and by water.

This species has been related to water environments where they may interact with other microorganisms which are natural inhabitants of hydric ecosystems, such as free-living amoebas.

Free-living amoebas could play a role in the transmission of some bacteria. Recently, the survival of *C. jejuni* (a species closely related to *A. butzleri*), inside *Acanthamoeba castellanii*, has been demonstrated. The aim of this study was to demonstrate the ability of *A. butzleri* to survive inside *A. castellanii*.

**Methods:** Three strains of *A. butzleri*, one isolated from human stools (strain F-215), one from chicken meat (PUA-190) and the reference strain LMG 10828 were studied.

Axenic cultures of *A. castellanii* were inoculated with the strains under study and incubated at 26°C under aerobic conditions for 240 hours, in presence of gentamicin (100 µg/mL). The interaction bacteria/amoeba was monitored by phase contrast microscopy and the bacterial survival

rates inside the amoebas were assessed by colony forming unit (CFU) count before and after amoebal lysis.

**Results:** All the *A. butzleri* strains were able to survive for at least 240 hours inside the amoebas; with the intracellular bacterial numbers always being higher than the extracellular ones. Intracellular bacteria ranged from 12 500 to 70 000 CFU/mL and extracellular bacteria from 1000 to 2300 CFU/mL.

**Conclusion:** Our results allow us to infer that *A. butzleri* is able to establish endosymbiotic relationships with *A. castellanii* surviving for at least a 10 days period inside the amoebas. The fact that the number of intracellular bacteria was always greater than that of extracellular bacteria indicates that *A. castellanii* could protect *A. butzleri* from this adverse environmental condition. Hence, free living amoebas could be a potential environmental reservoir and a vehicle for this microorganism. Financial Support: Grant FONDECYT 1110202.

**P2024** Shiga toxin-producing *Escherichia coli* O104:H4 in Turkey

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**Objectives:** Following the outbreak of Shiga toxin-producing *Escherichia coli* (STEC) O104:H4 in Germany, May 2011, active laboratory-based surveillance of haemolytic uraemic syndrome and STEC infections were started in Turkey.

The aim of this study was to examine the frequency and distribution of human shiga toxin-producing *Escherichia coli* (STEC) O104:H4 in Turkey and, to examine the presence of virulence genes and antimicrobial resistance in O104:H4 isolates.

**Methods:** Human stool samples were investigated to detect STEC or their toxins by using both phenotypic and genotypic methods. Isolated strains were studied to presence of verotoxin by ELISA and, toxin and virulence genes by PCR.

**Results:** Between June and October 2011, a total 75 human stool samples from 13 provinces in Turkey were received to National Reference Laboratory for Enteric Pathogens. After the cultivation of stool samples in sorbitol McConkey agar, eight STEC strains (10.7%) were isolated, three of these were STEC O104:H4. The remaining five isolates were O157:H7 (n = 2) and non-O157 *E. coli* (n = 3). All O104:H4 strains were positive for *stx2* and *aggR* genes, but negative for *eae* and *hlyA* genes. Two *E. coli* O104:H4 isolates were resistant to ampicillin, streptomycin, sulfonamides, trimethoprim/sulfamethoxazole, tetracyclin and nalidixic acid, while one isolate was resistant to only ampicillin and nalidixic acid. None of the *E. coli* O104:H4 strains were presence of extended-spectrum beta-lactamase.

**Conclusions:** To our knowledge, this is the first isolations and characterisation of of *E. coli* O104:H4 strains in Turkey. It was noted that an increase in the number and variety of STEC and *E. coli* O104:H4 isolates. However, there is need to further investigation to see if this trend is sustainable or not.

**P2025** Extra-intestinal infections caused by *Vibrio* spp. in southern Spain

P. Aznar-Marin, I. Guerrero-Lozano, P. Marin-Casanova, F. Galan-Sanchez, M. Rodriguez-Iglesias\* (Cadiz, ES)

**Objectives:** The Genus *Vibrio* includes halophilic (salt-tolerant) Gram-negative bacteria found naturally in temperate marine and estuarine environments. These species are recognised as human pathogens, and the incidence of infection significantly increases during summer months. *V. alginolyticus* is the more frequent and is ubiquitous in seawater and tends to cause superficial wound and ear infections (otitis media and otitis externa). Other species are *V. parahaemolyticus*, *V. vulnificus* and *V. fluvialis*. We report the strains of *Vibrio* spp. isolated in the last 10 years in a coastal area in the Southern of Spain.

**Methods:** Samples of patients were processed by conventional culture-based methods that involve selective pre-enrichment of samples, plating

onto selective solid media followed by morphological, biochemical and serological characterization. Identification and antimicrobial susceptibility tests were determined by a commercial microdilution system (Wider, Spain), using saline serum as diluent and incubated at least 48 hours. In summer months a TCBS plate was added.

**Results:** We have isolated 40 strains of *Vibrio* spp. from 39 patients in the following samples: ear exudates (35), wound (3), respiratory (1) and blood (1). *V. alginolyticus* was the more frequent with 31 strains isolated from ear (28), wound (2) and respiratory (1). *V. parahaemolyticus* was isolated from ear (5) and blood. Two strains of *V. vulnificus* was found in ear and one of *V. fluvialis* in wound. The incidence is increasing and 2009 was the year with more strains isolated. *Vibrio* isolates are more frequent in the summer months and there are not isolations from January to April. The age of patients was ranged from 2 to 80 years but was more frequent between 20 and 30 years. In men the infection was more prevalent (67%).

**Conclusion:** *Vibrio* species grow naturally in estuarine and marine environments worldwide, and are able to survive and replicate in contaminated water with increased salinity. In our area can be responsible of rare infections related with swimming and aquatic leisure in summer months, then, ear infection is the more prevalent clinical presentation (87%). *V. alginolyticus* was detected in 78% of the cases followed by *V. parahaemolyticus*. We recommend the use of TCBS plate especially in ear samples during warm months.

**P2026 Genetic variation and dynamic changes of *Yersinia pestis* strains isolated in Tuva natural focus of plague**

M. Afanas'ev\*, D. Verzhutckii, S. Balakhonov (Irkutsk, RU)

**Objectives:** Tuva natural focus of plague is one of the active natural plague foci in Russian Federation and northernmost part of Central Asia natural focality region. Since its finding in 1964, the focus demonstrates epizootic activity almost every year. In the present study we examined genetic variability of the *Y. pestis* strains isolated in the Tuva focus for an extended period of observation from 1964 to 2007 as well as dynamic changes in the populations of the pathogen during the dissemination of new sites on the natural focality.

**Methods:** *Yersinia pestis* strains (n = 102) have been selected during epidemiological and epizootological monitoring of 32 epizootic active sectors in Tuva focus from 1964 to 2007. Strains isolation and identification was performed according to standard microbiological protocols. Standard procedure of VNTR typing, that based on amplification of 25 loci followed by capillary electrophoresis was carried out as described previously. Geographical coordinates of sectors, where strains were isolated, defined by GPS receiver and plotted on focus map in the DMS format. Strain *Yersinia* ssp. caucasica was used as outgroup in cluster analysis.

**Results:** All studied Tuvianian strains belonged to *Yersinia* ssp. pestis (biovar antique). Seventy-seven strains were isolated from ectoparasites (*Citellophilus tesquorum* prevailed among them (48/77; 62,3%)) and 25 – from small mammals (*Spermophilus undulatus* prevailed [21/25; 84,0%]).

Nineteen different MLVA profiles were identified (seven of them [7/103; 6,8%] were unique). Ninety-five strains were grouped into 12 clusters. We observed significant association between genotypes and place of strain isolation.

Strains with one genotype, which formed main clonal complex (CC), have been persisting since 1964, when focus was discovered. New genotypes were detected among strains isolated in the eighties and nineties. At the beginning of two thousandth new CC forming genotype was revealed. Spatio-temporal pattern of *Y. pestis*' spreading was constructed.

**Conclusion:** Specific genotypes distribution in the natural focus from 1964 to 2007 testifies to active *Y. pestis* transmission and reflects dynamic dissemination of plague through epizootic areas.

**P2027 Emergence of *Klebsiella pneumoniae* liver abscess in southwestern Sydney, Australia: a retrospective case series**

R. Chavada\*, J. Ng, M. Maley (Liverpool, AU)

**Objectives:** To describe the clinical characteristics of KPLA (*Klebsiella pneumoniae* liver abscess) over a 10 year period (2001–2010) from south-western Sydney and compare the local experience with the published literature.

**Methods:** A retrospective descriptive cross sectional study of patients with KPLA over a period of 10 years from south-western Sydney was undertaken by cross-referencing ICD-10 hospital admission codes for liver abscess with KP (*Klebsiella pneumoniae*) blood culture and liver aspirate isolates. Data on demographics, clinical features, test results, management as well as outcomes were extracted, recorded and compared with the reviewed literature.

**Results:** Twenty-one patients (15 males, six females) aged 56.2 ± 14.1 years (mean ± SD) presented with KPLA during the study period comprising 12% of all liver abscesses admitted to hospitals in south-western Sydney. The mortality rate was 4.7% (1/21 patients). Metastatic infection occurred in 24% (5/21) of patients but there were no cases of metastatic endophthalmitis or meningitis. 4/5 patients with metastatic infection occurred in the latter half of the study period. 33% (7/21) of KPLA patients had diabetes mellitus including 40% (2/5) with metastatic infection. A history of travel from Asia or contact with travellers from Asia was present for all patients with metastatic complications (p = 0.01) and liver abscess size >5.0 cm appeared to correlate with a risk for bacteraemic spread of infection (p = 0.03).

**Conclusions:** KPLA is an emerging infectious disease in south-western Sydney with a high rate of metastatic complications. Metastatic infection should be actively sought in patients returning from Asia as well as patients with diabetes mellitus or large liver abscesses.

**P2028 Sporadic *Pantoea agglomerans* bacteraemia: clinical significance and utility of 16s rRNA gene sequencing**

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**Background:** There are three case reports of adults with sporadic bacteraemia due to *Pantoea agglomerans* in the English literature.

**Methods:** From our microbiology database we identified 18 adults with *P. agglomerans* bacteraemia from 2000 to 2010. We performed 16s rRNA gene sequencing to ascertain whether this tool is able to correlate with microbiological and clinical features.

**Results:** Of 18 patients, 83% (n = 15) had active gastro-oesophageal disease treated with antacids. Two-thirds of patients had indwelling central lines and advanced cancers. None of the removed catheter tips yielded *P. agglomerans* and line persistence was not associated with adverse outcomes. Initial disease severity was low; median Pitt bacteraemia score was 1, hypotension was rare (n = 4), and no patients died from bacteraemia. All patients were successfully treated with antibiotics. Recurrence occurred in only one patient after an interval of 11 months, in whom the focus was septic arthritis. Ampicillin non-susceptible proportions increased from 36% pre- to 71% post-2005. 16s rRNA sequencing identified only half of the isolates as *P. agglomerans*. The closest matches all with at least 99% maximal identity for the remaining nine patients were *Enterobacter* spp. for six (33%), *Pantoea ananatis* for two (11%), and *Exiguobacterium profundum* for one (6%). Trends for fewer patients with liver cirrhosis (0% vs. 33%), thrombocytopenia (22% vs. 77%), and ampicillin resistant isolates (33% vs. 67%) were observed among the group molecularly identified as *P. agglomerans* compared to those with miscellaneous genotypes. However the patient with recurrent bacteraemia had three sequential isolates matched to three different accession numbers; the second was alternatively identified as 99% *Enterobacter* spp. but shared acquired resistance to ampicillin and

cefazolin with the last isolate (obtained 5 days later) identified as 94% *P. agglomerans*.

**Conclusions:** *P. agglomerans* is an opportunistic pathogen of low virulence even amongst immunocompromised hosts. Bacteraemia outside the context of outbreaks is associated with receipt of antacids. Since *Pantoea* spp. is commonly found on plants, it may be introduced by ingestion of vegetables or fruits. Intravenous catheter removal appears unnecessary. Deep-seated foci should be evaluated in rare cases of recurrence. 16s rRNA genotyping does not correlate well with inter- and intra-individual differences in microbiological and clinical characteristics.

**P2029** Emergence of carbapenem-resistant *Acinetobacter baumannii* producing OXA-23 gene in a major Saudi Arabian hospital

A. Alsultan\* (Alahsa, SA)

**Objectives:** *Acinetobacter baumannii* is an important and opportunistic pathogen associated with immuno-compromised patients in intensive care units (ICUs) worldwide. The increase of carbapenem resistance in *Acinetobacter baumannii* is a global concern since it limits the range of therapeutic alternatives. Carbapenem resistance in *Acinetobacter baumannii* is largely manifested by class D beta-lactamases, comprising OXA-23-like, OXA-40-like, OXA-51-like and OXA-58-like beta-lactamases. Some of these enzymes are able to hydrolyze carbapenems and responsible in multi-drug resistance. OXA-51-like beta-lactamases are present in all isolates of *A. baumannii* and carbapenem resistance has sometimes been associated with this gene. The emergence of MDR *A. baumannii* has been reported in several hospitals in Kingdom of Saudi Arabia, the aim of this work is to investigate the disseminations of carbapenem resistance in *A. baumannii* in a major Saudi Arabian hospital.

**Materials and methods:** A total of 29 non-repetitive, strains collected between January 2011 and April 2011 from different specimens from King Faisal Specialist Hospital and Research Centre (KFSHRC) in Riyadh. All isolates were identified presumptively by the Vitek compact II system. PCR was used to identify not only the intrinsic blaOXA-51-like gene but also the genes encoding the blaOXA-23. The MIC of antibiotics was determined by dilution test according to BSAC guidelines.

**Results:** Twenty-nine clinical isolates were identified as *A. baumannii* by having the intrinsic of blaOXA-51-like gene. All isolates except one were resistant to imipenem (MIC > 16), three of which were highly resistant (MIC > 32 mg/L). The sensitive strain had an MIC ≤ 1 mg/L. All isolates were also resistant to meropenem, 25 of which had MICs > 32 mg/L, two isolates had MICs = 16 mg/L. One strain was intermediate (MIC > 4 mg/L) and another sensitive (MIC MIC ≤ 0.5 mg/L). The OXA-23 beta lactamase was the cause of imipenem and meropenem-resistance in 14 strains, which were resistant to carbapenems because they carried this gene of beta-lactamase and no other; there was one strain harbouring the OXA-23 beta-lactamase which was sensitive to both carbapenems.

**Conclusion:** The high level of multi-resistance in carbapenems in *A. baumannii* responsible for infection in those patients due to the dissemination of the OXA-23 beta lactamases in this Saudi Arabian hospital.

**P2030** Clinical and microbiological profile of cystic fibrosis patients in adults unit, Madrid

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**Objective:** Cystic fibrosis (CF) is the most common fatal genetic disorder affecting Caucasian populations. CF is a multisystem disease involves the pancreas, liver, and gastrointestinal tract among other organs. There is a perception that the epidemiology of respiratory tract infection in persons with CF is changing in recent years. The aim of this

study was to describe the most prevalent microorganisms and the relation with clinical symptoms and CF genotype from adult CF patients.

**Methods:** Patients were examined in a CF Unit at Hospital La Princesa (Madrid) from January to October 2011, all sputum samples were cultured in different selective media (MRSA, blood agar, chocolate agar with bacitracin, sabouraud, BCSA and MacConkey) with serial dilutions with acetylcysteine. The identification was performed with WalkAway (Siemens). Identification of some fastidious microorganism was carried out in a reference laboratory (Majadahonda, Madrid) by sequencing of ribosomal 16S. Patients were classified in four groups: Group I (18–24 years old), Group II (25–34 years old), Group III (35–44 years old) and Group IV (more than 44 years old). Statistical analyses were performed using SPSS 15.0 and Microsoft Excel 2003.

**Results:** A total of 59 adult CF patients were studied: 50.8% were women. The average age was 28.38 (DS: 8.88) years old and pulmonary function, expressed in average FEV1 was 63.57% (DS: 22.4). 18.6% patients had diabetes and 93.2% had pancreatic insufficiency. 35.6% had F508del/F508del mutation and 41.4% had F508del/other mutation. The microorganisms most frequent isolated were: *Staphylococcus aureus* (59.3%), *Haemophilus Influenzae* (52.5%), *Pseudomonas aeruginosa* (35.6%), MRSA (13.6%), *Achromobacter xylosoxidans* (13.6%), *Burkholderia cepacia* complex (10.2%) and *Stenotrophomonas maltophilia* (8.5%). We appreciated a relation between women and colonization with *S. maltophilia* ( $p < 0.05$ ). Most of *P. aeruginosa* isolates belonged to Group II ( $p < 0.05$ ). Patients colonized by *S. aureus* had a best pulmonary function and patients colonized by MRSA had worse pulmonary function ( $p < 0.05$ ).

**Conclusion:** *S. aureus* and *H. influenzae* were more prevalent than *P. aeruginosa*, in spite of studying adult patients. This may be due to the application since many years of aggressive protocols against *Pseudomonas*. It begins to detect an increase in colonization by multiresistant pathogens such as MRSA or *A. xylosoxidans*, so there will be to develop new strategies against them.

## Experimental and clinical immunology

**P2031** Identification of adhesion proteins involved in crossing of blood-brain barrier by *Francisella tularensis* subsp. holarctica

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**Objectives:** Tularemia (rabbit fever) is a serious infectious zoonotic disease caused by *Francisella tularensis*. It is already known that *Francisella* readily adhere to various cells like macrophages, epithelial and endothelial cells to evoke self-internalization or crossing of various cell barriers. Underlying molecular principle of adhesion of *Francisella* to various cells as well as protein candidates, which play crucial role in the adhesion process need to be revealed.

**Methods:** To identify interacting proteins ligand capture assay was employed, wherein whole cell lysates of two *Francisella tularensis* subsp. holarctica strains (LVS and Tul4) were separated by SDS-PAGE, proteins were electro-transferred on nitrocellulose membrane. Non-specific sites were blocked with ultra-pure BSA fraction V and membrane was hybridized with whole cell lysate of brain microvascular endothelial cells (BMEC) isolated from rat. Non-interacting proteins were washed out, while interacting proteins were stripped with stripping buffer (patent pending, Slovak patenting agency). Stripped proteins were fractionated on SDS-PAGE and subjected for MALDI-TOF-MS peptide mass fingerprinting (PMF). MALDI-TOF based peptide mass fingerprinting of ~60 kDa protein gave maximum identity with ICAM-1 protein. To confirm the interaction between ICAM-1 and *Francisella* surface proteins, His-tagged ICAM-1 was overexpressed in *S. cerevisiae* expression system, purified and immobilized on cobalt-magnetic beads (magnetic beads based immobilized metal ion affinity chromatography, Bruker Daltonics). Bound His-tagged ICAM-1 was hybridized with *Francisella* LVS whole cell lysate, unbound proteins



were washed and His-tag ICAM-1-LVS ligand assembly was eluted with elution buffer and separated on SDS-PAGE.

**Results:** We found that Tul4 strain (*Francisella tularensis* subsp. holarctica) lacks proteins, which are able to interact with surface proteins of BMEC. On the other hand, prominent protein of ~60 kDa was found interacting with proteins LVS strain. ~60 kDa protein representing ICAM-1 and ~40 kDa protein, were observed.

**Conclusion:** ICAM-1 seems to be an important binding partner for *Francisella* in CNS invasion. This is the first report where adhesion of *Francisella* to brain microvascular endothelial cells has been revealed at proteomic level.

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**P2032 Protective immunity against *Coxiella burnetii* in BALB/c mice induced by mouse bone marrow-derived dendritic cells activated with *Coxiella* outer membrane proteins, Com1 and Mip but not GroEL**

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**Objectives:** *Coxiella burnetii* is the causative agent of Q fever, a worldwide zoonotic infectious disease. This study is to identify novel protective antigens of *C. burnetii* as candidates of subunit vaccines against Q fever.

**Methods:** BALB/c mouse bone marrow-derived dendritic cells (BMDCs) were stimulated in vitro with *Coxiella* recombinant outer membrane proteins, GroEL, Com1 and Mip, and then these antigen-pulsed BMDCs were transferred into naïve BALB/c mice and the recipient mice were subsequently challenged with *Coxiella* virulent organisms, respectively. The protections conferred by *Coxiella* protein-activated BMDCs were measured by quantitative PCR assays and the interactions between *Coxiella* protein-pulsed BMDCs and CD4+/CD8+ T cells isolated from mice receiving homologous protein-pulsed BMDCs were investigated by flow cytometric assays.

**Results:** The *Coxiella* loads of mice receiving with Com1- or Mip-pulsed BMDCs, but not GroEL-pulsed BMDCs, were significantly lower than that of negative controls. After in vitro interaction with homologous antigen-pulsed BMDCs, the percentages of CD69-positive cells and TNF-alpha-positive cells in CD4+ and CD8+ T cell from mice receiving Com1- and Mip-pulsed BMDCs as well as GroEL-pulsed BMDCs were markedly increased, and the percentages of IFN-gamma-positive cells in CD4+ and CD8+ T cells from mice receiving Com1- and Mip-pulsed BMDCs, but not GroEL-pulsed BMDCs, were also dramatically increased, respectively. However, the percentage of IL-4-positive cells in the CD4+ T cells from mice receiving GroEL-pulsed BMDCs was significantly higher than that of mice receiving Com1- or Mip-pulsed BMDCs.

**Conclusion:** Our results demonstrate that Com1- or Mip-activated BMDCs have the unique ability to activate T cells and to drive CD4+ T cells and CD8+ T cells toward Th1 and Tc1 differentiation, respectively, in the protective immune response to *C. burnetii* infection.

**P2033 T regulatory cells dampen inflammatory responses in murine *Mycoplasma pneumoniae*: promotion of IL-17 AND IFN-g production, but not IL-10 or TGF-b**

A. Odeh, J. Simecka\* (Fort Worth, US)

Mycoplasmas cause respiratory diseases in humans and animals and are characterized by persistent infection and chronic airway inflammation. Previous studies showed mycoplasma lung disease is immunopathologic, with Th cell populations critical in controlling the severity of disease, as well as development of resistance to infection. Th2 cell responses appear to promote immunopathology, while Th1 cells help confer resistance to infection. Although other cell populations can modulate mycoplasma disease, the role of Treg cells in mycoplasma respiratory diseases have yet been examined.

Given the persistence of mycoplasma infections and the development of chronic inflammatory lesions, it was hypothesized that Treg cells control the severity of the inflammatory lesions through production of IL-10 or TGF-beta, but in doing so, Treg cells could inadvertently promote persistence of infection, as found in other diseases. To address this hypothesis, we examined the role of Treg cells in murine *Mycoplasma pneumoniae* due to the natural pathogen, *Mycoplasma pulmonis*. Our studies demonstrate that Treg cells do play an important role in controlling damaging immune responses in mycoplasma respiratory infection. Depletion of Treg cells, by giving mice anti-CD25 antibody, prior to infection lead to markedly increased disease severity, increased immune cell infiltration into the lungs, and a higher overall level of activation of the immune system. In contrast to our hypothesis, depletion of Treg cells did not affect the numbers of mycoplasma recovered from the lungs, and therefore Treg cells do not contribute to persistence of infection. In studying possible mechanisms, we did not find that Treg cells produce IL-10 or TGF-beta, suggesting an alternative mechanism for their activity. Surprisingly, the Treg populations that respond during infection express IFN-gamma or IL-17. In additional studies, depletion of Treg cells in mice were shown to increase the Th2 (IL-13) responses. Data from in vitro and in vivo experiments suggest that Treg cells can also promote the secretion of IFN-gamma and/or IL-17 by other cell types, and these responses may dampen the immunopathologic Th2 immune response to mycoplasma lung infection. These findings may represent a novel mechanism through which Treg cells can dampen immune mediated inflammatory disease associated with mycoplasma infection, and could play a role in other infections.

**P2034 Lipocalin-2 is a potent chemo-attractant for neutrophils in humans and mice**

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**Objectives:** Neutrophil gelatinase-associated lipocalin (NGAL, 24p3 or lipocalin-2, Lcn2) is a 21 kDa protein of the lipocalin superfamily. NGAL has been shown to affect cellular iron homeostasis transport, cellular apoptosis, and the course of infections with gram negative bacteria via binding of bacterial siderophores. Since lipocalin is produced and released in large quantities by granulocytes, we questioned whether this peptide may affect the immunological function of these cells.

**Methods:** Human or murine neutrophil granulocytes were purified and attracted in vitro with varying concentrations of hIL-8, mIL-8 (KC), mLn2, hLn2 and/or an anti-mLn2-Ab. Neutrophil migration assays were performed using a modified 48-well Boyden microchemotaxis chamber.

To investigate the adhesion of neutrophils we coated 12-well plates with mouse serum and allowed cells to adhere for 40 minute. To study neutrophil function upon microbial challenge a single intradermale injection of *Salmonella typhimurium* (300 CFU) was given to wild-type and Lcn2<sup>-/-</sup> C57BL6 mice. After 24 hour, the skin at each injection site was excised and fixed in formalin for histological analysis.

**Results:** We found that the migration of human and murine granulocytes was significantly increased upon the addition of Lcn2, which was not linked to increased formation of IL-8/KC. Mechanistically, this could be traced back to Lcn2 mediated changes of ERK1/2 signaling in these cells. Accordingly, we found significantly reduced neutrophil migration into intradermale bacterial lesions of Lcn2<sup>-/-</sup> as compared to their wild type littermates. In addition to a reduced chemotactic activity, neutrophils from Lcn2<sup>-/-</sup> mice also presented with an impaired adherence to surfaces.

**Conclusion:** We herein describe a novel role of Lcn2 as an important paracrine chemo-attractant and an indispensable factor for neutrophil function in bacterial infections.

**P2035** An example of collateral damage: cytomegalovirus infection and immune senescence

A. Mekker, V. Tchang, L. Haeberli, A. Trkola, U. Karrer\* (Zurich, Winterthur, CH)

**Introduction:** Immune senescence defined as the age-associated dysregulation and dysfunction of the immune system is characterised by a loss of protective immunity and by decreased efficacy of vaccines. Recent clinical, epidemiological and immunological studies suggest that Cytomegalovirus (CMV) infection and CMV-driven memory T cell accumulations may be associated with accelerated immune senescence, possibly by restricting the remaining naïve T cell repertoire. However, direct evidence whether and how CMV-infection is implicated in immune senescence is still lacking.

**Objectives:** In this study, we have investigated whether latent mouse CMV (MCMV) infection alters antiviral immunity of young and aged mice.

**Results:** After infection with lymphocytic choriomeningitis virus (LCMV) or recombinant Vaccinia virus (Vac-LCMV-GP) specific antiviral T cell responses were significantly reduced in aged MCMV-infected but not in young mice. More importantly, control of LCMV-replication was more profoundly impaired in aged MCMV-infected mice compared to age-matched or young mice. In addition, MCMV-infection reduced immunisation efficacy in old but not young mice. In contrast to the prevailing hypothesis, we find similar total naïve T cell numbers in MCMV-infected compared to non-infected mice. Instead, MCMV-infection significantly expands the total CD8+ T cell pool by a massive accumulation of effector memory T cells.

**Conclusions:** Based on these results, we propose a new model of increased competition between CMV-specific effector memory T cells and any "de novo" immune response after infection or immunisation of aged individuals. In summary, our results demonstrate for the first time in a mouse model that CMV-infection impairs immunity in old age.

**P2036** Serum interleukin-8 levels may predict relapse in brucellosis

N. Aktug Demir, O. Ural\* (Adiyaman, Konya, TR)

**Objectives:** Elimination of brucella occurs in conjunction with macrophage activation which is, in turn, induced by Th1 cell-mediated immunity. Cytokines released during this stimulation play a critical role in pathogenesis of brucellosis. Former trials were not able to clarify the relation between clinical course/relapse and cytokines in acute brucellosis.

This study aimed to investigate whether cytokines are effective in predicting relapses among patients with acute brucellosis.

**Methods:** This trial was conducted on 42 patients who were being followed up with diagnosis of acute brucellosis. Relapse was diagnosed in seven patients.

Diagnosis of acute brucellosis was confirmed by an increase in serum agglutination titers (STA)  $\geq$  and/or  $>1/160$  or 4-times increase in STA titers evaluated 2 weeks apart and/or positivity in blood cultures, in addition to clinical symptoms. In patients whose clinical symptoms recurred in a year after treatment and exhibited infectious parameters in compliance with brucellosis, rivanol tube STA test was performed and diagnosis of relapse was based on brucella IgM. Serum samples were obtained on days 0 and at the time of relapses. Serum samples were evaluated for various parameters, namely TNF-alpha, IFN-gamma, IL-2, IL-4, IL-6, IL-8, IL-10 and sIL-2R. Data were evaluated by SPSS 13.0 for Windows (Real State Corporation, England) program. Statistical analysis was carried out using the same program. For data analysis, Mann-Whitney U-test with Bonferroni correction and Wilcoxon Signed-Rank Test were used and Friedman test was utilized for repetitive measurements.

**Results:** No difference was found between relapsing patients and totally recovered patients in terms of age, gender, leukocyte levels, CRP values. Comparison of TNF-alpha, IFN-gamma, IL-2, IL-4, IL-6, IL-8

and IL-10 values on day 0 (day of enrollment) revealed two-fold higher IL-8 values among relapsing patients as compared to totally recovered cases. IL-8 was suggested as significant in terms of predicting relapse.

IL-2	33.3±24.9	38.9±36.1	>0.05
IL-4	27.0±38.94	17.4±10.5	>0.05
IL-6	6.1±6.4	5.0±2.6	>0.05
<b>IL-8</b>	<b>32.8±17.3</b>	<b>60.1±21.3</b>	<b>0.004</b>
IL-10	9.9±6.5	8.3±5.3	>0.05
sIL2R	3.0±4.5	2.3±1.4	>0.05

RC.relapsing cases, TRP. totally recovered patients.

**Conclusion:** In conclusion, currently a few studies are available indicating correlation between cytokines and relapse in brucellosis. Predicting relapse by certain laboratory evaluations may be beneficial in preventing clinical relapses by re-arranging treatment and monitoring strategies of patients. We suggest that high IL-8 value may be influential in indicating relapse.

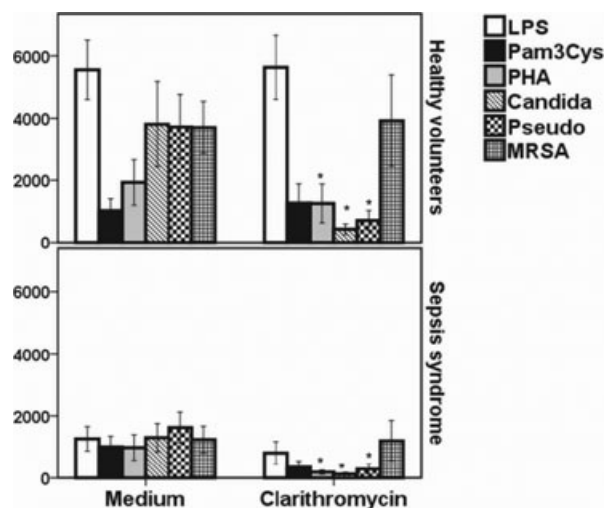
**P2037** Modulation of cytokine release of monocytes of septic patients by clarithromycin

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**Objectives:** A former randomized study of our group (CID 2008; 46: 1157) showed that intravenous administration of clarithromycin reduced relative risk for death by septic shock and multiple organ dysfunctions in patients with ventilator-associated pneumonia. The efficacy of clarithromycin in modulation of cytokine release by monocytes of sepsis patients was studied.

**Methods:** Peripheral blood mononuclear cells (PBMCs) were isolated by gradient centrifugation over Ficoll from eight healthy controls and 12 patients with sepsis and 24 patients with severe sepsis/shock (ACCP/SCCM 1992 classification) within 24 hours from diagnosis. PBMCs were stimulated at a density of 5 000 000 CFU/mL with various stimuli and heat-killed isolates of *Candida albicans*, of *Pseudomonas aeruginosa* and of methicillin-resistant *Staphylococcus aureus* (MRSA) in the absence/presence of 10 mg/L of clarithromycin. Concentrations of pro-inflammatory cytokines were measured in cell supernatants by an enzyme immunoassay.

**Results:** Clarithromycin modulated release of interleukin (IL)-1beta from PBMCs of controls and of patients by the pattern shown in Fig. 1



where asterisk denotes respective significant differences. Release of IL-6 was not affected. Release of TNF $\alpha$  was decreased only in LPS-stimulated PBMCs of controls and of patients

**Conclusions:** When added ex vivo, clarithromycin modulates release of IL-1 $\beta$  and to a lesser extent of TNF $\alpha$  by a pattern depending on the applied stimulus. These results may be promising for the application of clarithromycin in the management of sepsis.

#### **P2038** Potential role for CXCR7 in idiopathic CD4+ T-cell lymphopenia

G. Kumar\*, H. Schmid-Antomarchi, A. Schmid-Alliana, P.-M. Roger (Nice, FR)

**Objectives:** Idiopathic CD4+ T-cell lymphocytopenia (ICL) is defined as an absolute CD4+ T-cell count of <300 cells/mm<sup>3</sup> or <20% of total T-cells associated the occurrence of opportunistic infections. To date the pathophysiology of ICL remains unclear, but recent work reported defective surface expression of CXCR4 and abnormal accumulation of intracellular CXCR4 and its ligand CXCL12. CXCR4/CXCL12 binding contributes to proliferation of T-cells. CXCR7 also binds to CXCL12 but acts as an antagonist, thus inhibiting proliferation of T-cells. We report herein the case of a 60 year old female patient with ICL for whom immunological explorations showed imbalance between CXCR4 and CXCR7.

**Methods:** Blood was taken from the patient as well as from five healthy volunteers (HV). PBMC was isolated by ficoll separation. T cells were analyzed for their phenotypic marker, both ex vivo and after overnight stimulation with CD3 and CD28. HLA-DR and Ki67 was used as marker of activation and proliferation respectively. T-regulatory cells were determined by CD4 T cells which were double positive for CD25 and Foxp3. For gene expression analysis, first total RNA was purified from PBMC and then cDNA was made by reverse transcriptase after which gene expression was analysed by qPCR for both HV and ICL patient.

**Results:** The data of the ICL patient and five HV are presented in the following table. The first column represents the median value. qPCR results confirmed the expression of the genes CXCR4, CXCR7 and CXCL12. The delta cross-over threshold (dCT) value for CXCR4, CXCR7 and CXCL12 was 3.11, 10.37 and 16.16 respectively in comparison to the house keeping genes Actin and GAPDH in the patient. On comparison to HV the absolute value of ddCT for CXCR4, CXCR7 and CXCL12 was 0.29, 0.45 and 1.24 respectively.

	CD4		CD8	
	HV % (range)	ICL	HV % (range)	ICL
HLA-DR+	1.5 (1-3)	16.3	1.5 (1-4)	6.8
Ki67+	6.4 (5-9)	4.9	5 (4-8)	2.1
CD152+	0.2 (0.1-0.4)	1.5	0.1 (0-0.2)	0.5
CXCR7	1.4 (0.6-1.9)	5.7	0.4 (0.3-1.4)	0.9
CXCR4	1 (0.3-1.5)	2.3	0.4 (0.1-1)	1.1
T-reg	3.7 (2-7)	17.1	-	-

**Conclusion:** We found an increased activation without proliferation, notably of CD4 T-cells. This result appeared associated with a high expression of CXCR7, playing an inhibitory role for the CXCR4/CXCL12 proliferation pathway. A potential link between CXCR7 expression and the large amount of T-regulator cells that we found remains to be elucidated.

#### **P2039** Enterococci as probiotics or means of vaccine delivery. Can medicine give them a chance?

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Enterococci belong to the genus of bacteria causing significant number of nosocomial infections affiliated with multiple resistances to antibiotics. At the same time enterococci are the part of human normal microbiota, used for a long time in food industry for food manufacturing and are very successful as probiotics used for the treatment of

bacterial infections, intestinal dysbiosis and stimulation of innate immunity (Suvorov *et al.* 2011, Yermolenko *et al.* 2011, Tarasova *et al.* 2010). The aims of the study was to understand the differences between pathogenic and probiotic strains and evaluate the possibilities of their usage for vaccination.

Clinical *E. faecium* strains were obtained from the Institute Experimental medicine strain collection. Two probiotic *E. faecium* strains were isolated from probiotics "Linex" and "Laminolact" (*E. faecium* L3). These probiotic enterococci together with a set of clinical strains were subjected to genetic analysis for the presence of virulence genes and enterocins with specific DNA primers. Genomes of two probiotic strains and one clinical strain were studied employing optical mapping. Strain L3 was tested as a target for vaccine delivery. Portion of group B streptococcal (GBS) gene bac was inserted in the middle of enterococcal gene encoding for the surface protein orf 2356, cloned and expressed in *E. coli* and then introduced into enterococci by electroporation. Bac protein expression was tested by ELISA and IgA binding. Resultant enterococcal recombinants were tested for viability, colonization ability and immunogenicity employing vaginal immunization mice model and GBS infection model.

Comparison of the probiotic *E. faecium* with a set of clinical strains showed that they are free from the virulence markers and possess several enterocin genes. Optical mapping allowed constructing three complete genomic maps of enterococci. Sizes of probiotic genomes were found to be considerably smaller than the enterococcal average (2600 kb relatively to 2900 kb). GBS bac gene inserted in frame into enterococcal surface protein gene was expressed both in *E. coli* and in Enterococci. Recombinant enterococcal strain was introduced into mice. All the animals under study were successfully colonized but differed in colonization rate, Bac immunogenicity and GBS protection level. Perspectives of usage of enterococcal probiotics as vaccine targets are discussed. Work was supported by grant 10-04-00750a.

#### **P2040** Palmitoylethanolamide stimulates phagocytosis of *Escherichia coli* K1 and *Streptococcus pneumoniae* R6 by microglial cells

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**Objective:** Microglial cells play a crucial role in the inflammatory response and can phagocytize and kill invading pathogens. Palmitoylethanolamide (PEA), an endogenous lipid and a congener of anandamide (arachidonylethanolamide, AEA) has been shown to reduce allergic reactions, elicits analgesic effects and has anti-inflammatory and anti-nociceptive properties. We investigated whether PEA influence the phagocytosis of *Escherichia coli* K1 and *Streptococcus pneumoniae* R6, which can cause CNS infections in immunocompromised patients, by microglial cells in vitro.

**Methods:** Primary mouse microglial cells were stimulated with PEA for 30 minute. After stimulation, microglial cells were washed and infected with  $6 \times 10^6$  CFU/well with the encapsulated *E. coli* K1 for 90 minute or with the unencapsulated *S. pneumoniae* R6 for 30 minute. Extracellular bacteria were killed by incubation in culture medium (DMEM) containing 100  $\mu$ g/mL Gentamicin for 60 minutes. Microglial cells were lysed with distilled water, and the number of intracellular bacteria was determined by quantitative plating of serial 10-fold dilutions on Sheep blood agar plates. Data are presented as means  $\pm$  SD. ANOVA followed by Bonferroni's multiple comparisons test was used to compare groups.

**Results:** Unstimulated cells (DMSO group) ingested bacteria at a low rate (mean  $\pm$  SD) ( $100.0 \pm 17.29\%$ , *S. pneumoniae* R6) ( $100.0 \pm 59.8\%$ , *E. coli* K1). Pre-stimulation of microglial cells with different concentrations of PEA showed an increase of ingested bacteria in all PEA tested groups in a dose dependent manner. Pre-stimulation with 100 nM PEA significantly increased the phagocytosis rate of *S. pneumoniae* R6 approximately three fold ( $303.5 \pm 179.5\%$ ,  $p < 0.0007$ ). The highest phagocytosis rate for *E. coli* K1 was seen after pre-stimulation with 300 nM PEA ( $206.9 \pm 101.0\%$ ,  $p < 0.0068$ ).

**Discussion:** Pre-stimulation with PEA increases the phagocytosis of *Escherichia coli* K1 and *Streptococcus pneumoniae* R6 by murine microglia cells in vitro. Our results suggest, that the endocannabinoid PEA enhances the cellular innate immune response increasing phagocytosis of invading bacteria and acts as an endogenous protective factor in the brain.

**P2041** The impact of adherence of *Escherichia coli* strains from children with inflammatory bowel syndrome on actin cytoskeleton of intestinal epithelial cells

G. Gosciniak\*, A. Duda-Madej, B. Sobieszczanska (Wroclaw, PL)

**Objectives:** Bacterial adherence to intestinal epithelial cells, determined by specific adhesins, is a key factor in the development of an infection. Many adhesins detected in pathogenic *Escherichia coli* (*E. coli*) strains. In the study we determined (i) adherence patterns of *E. coli* isolates examined to HEP-2 cells; (ii) distribution of genes: aggA, aggR, afaA, afaC, afaD, daaD, eae (iii) impact of adherence of *E. coli* strains on actin cytoskeleton of intestinal epithelial cells.

**Methods:** The study was conducted on 55 *E. coli* strains isolated from children with Crohn's disease (CD) (n = 20), ulcerative colitis (UC) (n = 14), indeterminate colitis (IC) (n = 11) and from children with polyps (PO) (n = 11) as control group. The adherence patterns were determined in an in vitro assay with HEP-2 cell line in the presence of D-mannose. The genes encoding adhesins were detected in *E. coli* examined by PCR assay, whereas the ability of *E. coli* strains to induce specific histopathological lesions AE (attaching and effacing) was determined using FAS assay (falloidine-actin staining) performed on intestinal epithelial Int407 cell line.

**Results:** The results of adherence assay are presented in Table 1. As many as 24 (43.6%) *E. coli* strains examined demonstrated the presence of eae gene, but only 19 (34.54%) isolates were positive in FAS assay as other five (9.1%) of strains were hemolytic and cell-detaching. All eae-positive strains were associated with localized or mixed, diffuse-localized adherence patterns.

	Adherence patterns determined						
	No. of strains (%)						
	UD	DA	AA	DA/LA	LA	CD	NA
<b>CD</b>	4	7	2	3	3	1	0
n=20	(20)	(35)	(10)	(15)	(15)	(5)	
<b>UC</b>	4	0	3	2	3	1	1
n=14	(28)		(21)	(14)	(21)	(7)	(7)
<b>IC</b>	2	1	4	2	0	1	1
n=11	(18.2)	(9.1)	(36.3)	(18.2)		(9.1)	(9.1)
<b>PO</b>	1	2	0	1	0	2	1
n=10	(10)	(20)		(10)		(20)	(10)
<b>Total</b>	11	10	9	8	6	5	3
n=55	(20)	(18.2)	(16.4)	(14.5)	(11)	(9.1)	(5.4)

**Conclusion:** The eae gene seems to be widely distributed among *E. coli* from children with persistent infections although its presence is not always associated with expression that should be confirmed by FAS Test.

This study was supported by the Ministry of Science and Higher Education grant No 0187/B/P01/2009/37.

**P2042** Baseline vitamin D deficiency is not associated with poor clinical outcomes in osteoarticular infections

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**Objectives:** Although vitamin D is recognized as an important factor in bone health its role in osteoarticular infections is unclear. We hypothesized that vitamin D deficiency is associated with lower likelihood of treatment success in patients with osteoarticular infections.

**Methods:** We conducted a retrospective cohort study of patients (pts) seen by the Washington University bone & joint infectious diseases consult service that had a vitamin D (25-hydroxy-ergocalciferol) level drawn at baseline. Successful treatment (clinical improvement; improved follow-up markers & imaging; no readmission for treatment) was determined at early follow-up (3–6 months) and late follow-up (6+ months) after completing intravenous antibiotics. Vitamin D baseline levels were correlated with treatment success.

**Results:** We included 159 pts with bone and/or joint infections and baseline vitamin D levels seen during a 9-month period in 2009/2010. The mean age was 56 years (SD ± 16); patients were predominantly male (84; 53%) and white (117; 74%) and had a median BMI of 29 kg/m<sup>2</sup> (16–60). Most infections were caused by *Staphylococcus aureus* (64; 40%) or coagulase-negative staphylococci (23; 14%). There were 34 (21%) polymicrobial, 39 (25%) culture-negative, and 103 (65%) hardware-associated infections. As part of the inpatient management, vitamin D deficiency was identified and treated. The median vitamin D level was 20 ng/mL (5–112); 121 (76%) pts had levels <30 and were deficient. Risk factors for vitamin deficiency included seasonality (84 [82%] with deficiency in fall/winter vs. 37 [65%] in spring/summer [p = 0.02]). Overall, treatment success was 90% (113/125) at early and 88% (104/119) at late follow-up. Vitamin D baseline levels were similar in those with or without successful clinical outcomes, both at early (21 ng/mL [5–112] vs. 21 [9–42]; p = 0.8) and late follow-up (22 [5–112] vs. 17 [6–32]; p = 0.08). Follow-up levels were only obtained in 40 (25%) pts, with a median level of 31 ng/mL (8–91); 17/40 (43%) were still deficient at that point. There was no difference in early (p = 0.9) or late (p = 0.3) follow-up levels whether pts had successful clinical outcomes or not.

**Conclusion:** To our knowledge this is the first report on vitamin D deficiency and its impact on outcomes of osteoarticular infections. Vitamin D deficiency was frequent in this cohort. With vitamin D supplementation, there was no difference in treatment success whether patients had baseline vitamin D deficiency or not.

## Experimental and diagnostic aspects of Lyme borreliosis

**P2043** OspA:CD40 interaction plays role in crossing of *Borrelia* cross of brain microvascular endothelial cell

L. Pulzova\*, A. Kovac, R. Mucha, S. Hresko, P. Mlynarcik, E. Bencurova, M. Bhide (Košice, Bratislava, SK)

**Objectives:** Lyme disease is the most common tick-borne disease in northern hemisphere and has neuroinvasive character. Invasion of CNS is associated with translocation of *Borrelia* across the blood–brain barrier (BBB). This translocation is a multi-stage process with unclear molecular principle. Main prerequisite of successful borrelial traversal seems to be adhesion of *Borrelia* to brain microvascular endothelial cells (BMECs). The objective was to identify molecules that take part in the adhesion.

**Methods:** Ligand capture assay (LCA) based unfolding of interaction between ligands of borreliae and receptors of BMEC revealed that OspA of neuroinvasive strain SKT-7.1 (*B. garinii*) possess a potential to interact with BMEC. No BMEC interacting protein of non-neuroinvasive strain SKT-2 (*B. burgdorferi sensu stricto*) was observed. To identify BMEC receptor interacting with OspA His-tagged OspA proteins of both strains were constructed and hybridized with whole cell lysate of rat BMEC. For OspA of non-neuroinvasive strain no co-immunoprecipitant was observed which confirmed the results of LCA.

**Results:** Co-immunoprecipitant of OspA of neuroinvasive strain SKT-7.1 was detected on polyacrylamide gel and further identified as CD40. Differential binding ability of OspA of SKT-7.1 and SKT-2 to CD40 indicates that OspA:CD40 dyad is crucial in the adhesion of spirochetes to BMEC, which is a fundamental step in the borrelial translocation across BBB. To assess the cause of differential CD40 affinity, the amino-acid sequences of OspA of both strains were aligned. We found four amino-acid changes (Y165F, V166T, T172A, V179K) in the

endothelium binding site (AA 144–183), which might be responsible for differences in adhesion to BMEC and subsequent BBB translocation.

**Conclusion:** Taken together, study demonstrated that OspA provides *Borrelia* with an essential function in adhesion and neuroinvasion thus it can be concluded that adhesion is mediated via OspA:CD40 dyad.

**Acknowledgement** Work was supported by research grants: APVV-0036-10, VEGA -1/0621/09, 1/0608/09.

**P2044** Experimental validation of the predicted binding site of outer surface protein A of neuroinvasive *Borrelia garinii* to CD40 of brain microvascular endothelial cells

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**Objective:** *Borrelia* is capable of penetrating the blood–brain barrier (BBB), either through transcellular and/or the paracellular route. In our previous study we showed that interaction between CD40 of rat brain microvascular endothelial cells (BMECs) and outer surface protein (OspA) of *B. garinii* (strain SKT-7.1) is crucial for the transient tethering of *Borrelia* to the endothelium. To this background, objective was set to predict and identify the domain/s of OspA that binds CD40 molecule.

**Methods:** Nucleotide sequence of OspA of SKT-7.1 was in silico translated and amino-acid sequence was subjected to search for endothelium binding sites, antibody binding pockets and hypervariable antigenically important regions in OspA based on database search (Uniprot, SMART) and data mining. Three putative endothelial cell binding sites were identified: 18–44 AA (putative tick gut endothelium binding site, TGEBS), 85–103 AA (putative TGEBS) and 144–183 AA (HUVEC binding site). Three his-tagged forms (N-terminal tag) of the proteins encompassing these three putative endothelial cell binding sites of OspA were overexpressed in *E. coli* (SG10003 strain) using pQE-30 vector (Qiagen) and isolated using affinity tag chromatography. Binding affinity of truncated forms of OspA was assessed with western-blotting in which BMEC proteins were immobilized on nitrocellulose membrane. To confirm the results of western-blotting, His-tagged proteins were immobilized on Talon beads (Clontech) hybridized with cell lysate of primary cultures of rat BMEC. Protein complex was eluted and candidate proteins were identified on MALDI-TOF.

**Results:** Truncated OspA candidate encompassing amino acid residues 144–183 AA showed affinity to BMECs in western-blot analysis, while other two truncated forms of the OspA did not show binding ability to any of the BMEC proteins. As expected, in the Talon based MALDI assay, peak at 11 kDa corresponding to truncated OspA protein and its binding partner at ~30 kDa were found. 30 kDa protein was identified as CD40 with peptide mass fingerprinting.

**Conclusion:** Results indicate that interaction between CD40 and OspA is mediated through the OspA domain encompassing 144–183 AA. Thus this domain may be the crucial for transient adhesion of borreliae to BMECs and may be essential for subsequent BBB translocation of *Borrelia*.

**Acknowledgements:** Work was supported by research grants: APVV-0036-10, VEGA- 1/0621/09, 1/0608/09, 2/0121/11.

**P2045** Human vitronectin protects *Francisella* and *Borrelia* against complement mediated lysis

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**Objectives:** *Borrelia burgdorferi* (B.b) and *Francisella tularensis* (F.t.) are the tick-borne pathogens that cause chronic disseminated infections in human and animals. To survive in the host both pathogens need to evade the immune system. Vitronectin is a multifunctional glycoprotein, which also has regulatory function of the complement system through binding with C5b67 by C8 competitive inhibition. Here

we demonstrate possible exploitation of vitronectin by B.b. and F.t. to inhibit complement activation pathway and bacterial lysis.

**Methods:** To explore whether different species of *Borrelia* or *Francisella* bind vitronectin on their surface, whole cell proteins of 17 *Borrelia* and six *Francisella* strains were separated by SDS-PAGE, electro-transferred and used for far-western blotting to assess their differential vitronectin binding ability. Normal human serum was the source of vitronectin and human anti-vitronectin monoclonal antibody in mouse (Abcam, UK) was the primary antibody and HRPO anti mouse in goat was used like secondary antibody in far western blotting.

**Results:** A ~64 kDa protein of LVS and two proteins (~160 and ~58 kDa) of Tul4-hu, both F.t. subsp. holarctica strains, showed affinity to human vitronectin what indicates that these strains may bind vitronectin on their surface and may inhibit formation of MAC complex. Eight *Borrelia* strains possessed protein ligands capable of binding human vitronectin (proteins with their approximate molecular weight are indicated in the parenthesis): *B. afzelii* strain SKT4 (~130, 30 kDa), *B. garinii* strains Rio2 (~55 kDa), PBi (~45 kDa), G117 (~45 kDa), SKT3 (~37 kDa); *B. valaisiana* strain VS116 (~48 kDa); *B. andersonii* 21123 (~43 kDa) and *B. anserina* ES-1 (~51 kDa).

**Conclusion:** Binding of vitronectin may allow borreliae and francisellae to inhibit activation of the terminal complement pathway and allow their survival in the blood during the early stage of infection as well as during chronic infections even in the presence of specific antibodies.

**Acknowledgements:** Work was supported by research grants: APVV-0036-10, VEGA -1/0621/09, 1/0608/09, 2/0121/11.

**P2046** The role of DbpA and B adhesins of *Borrelia burgdorferi* sensu lato in the pathogenesis of Lyme borreliosis

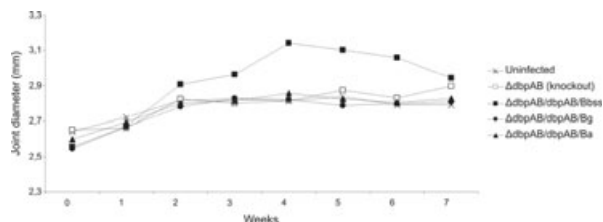
J. Hytönen\*, J. Räikkä, M. Söderström, M.K. Viljanen, J. Salo (Turku, FI)

**Objectives:** Lyme borreliosis (LB) is an infectious disease caused by the spirochete *Borrelia burgdorferi* sensu lato. It is the most important tick-transmitted disease in Europe. Despite decades of active research on the pathogenesis of LB a multitude of bacteriologically and clinically relevant questions remain unanswered, like: o Are borrelia spirochetes able to evade antibiotic treatment? If yes, what are the molecular mechanisms behind this? o Why is borrelia able to persist in an infected host regardless of a strong immune response? o What is the molecular mechanism of the different tissue tropism of borrelia genospecies? There are three major borrelia genospecies, *B. burgdorferi* sensu stricto (Bbss), *B. garinii* (Bg) and *B. afzelii* (Ba), which are known to cause disease in humans. Decorin binding proteins (DbpA and B; Dbps) of the three genospecies differ in the amino acid sequence, and our earlier results show that they, indeed, have different binding properties to decorin potentially leading to differences in dissemination, tissue tropism and treatment resistance of LB. The present study aims at clarifying the role DbpA and B adhesins of borrelia in the above mentioned questions.

**Methods:** We have constructed a range of genetically engineered borrelia strains. The DbpA and B deficient background strain is modified to express dbpAB operon of Bg, Ba and Bbss. C3H/He mice are infected with the engineered bacteria and followed up for up to 14 weeks before they are killed. A subgroup of mice is treated with ceftriaxone at 2 weeks of infection. Joint swelling is monitored throughout the experiment, and multiple tissues are collected for borrelia culture, PCR and histology. Also, serum antibody levels are measured.

**Results:** The borrelia strain expressing DbpA and B of Bbss disseminated faster than strains expressing Dbps of Ba or Bg, and was also the only strain to cause joint swelling.

After ceftriaxone treatment, borrelia DNA persisted in the joints of mice infected with the strain expressing Dbps of Bbss for 11 weeks, while no DNA was detected in the joints of mice infected with borrelia lacking DbpA and B adhesins.



**Conclusion:** Dbps of Bbss, Bg and Ba differently contribute to tissue colonization and kinetics of borrelia infection. Expression of Bbss Dbps on borrelia surface leads to high bacterial load in the joint and arthritis development. Expression of Dbps allows borrelia DNA to persist in mouse joints after antibiotic treatment.

**P2047** Apoptosis of Th lymphocytes and expression of Fas receptor and Fas ligand in the culture of peripheral blood mononuclear cells stimulated with *Borrelia burgdorferi* spirochetes

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**Objective:** The elimination of activated lymphocytes by apoptosis is essential in the regulation of inflammatory/immune response to infection. Its overstimulation may lead to an ineffective response and establishment of chronic infection, while decreased apoptosis poses risk of chronic inflammation and autoimmunity. Both long-lasting infection and inflammation continuing after the pathogen elimination has been observed in different clinical forms of Lyme borreliosis (LB). We assessed the influence of *Borrelia burgdorferi* on lymphocyte apoptosis and expression of pro-apoptotic Fas receptor and its ligand.

**Methods:** We have studied cultures of peripheral blood mononuclear cells (PBMC) from 31 patients with LB (early and late neuroborreliosis, late arthritis or acrodermatitis chronica atrophicans) and 16 healthy controls, stimulated for 48 hours with live spirochetes, either *Borrelia burgdorferi* sensu stricto (Bss), *B. garinii* (Bg) or *B. afzelii* (Ba). We have measured a fraction of apoptotic (annexin-V binding) Th lymphocytes and expression of Fas receptor on Th cells cytometrically. Supernatant concentrations of soluble Fas (sFas), soluble FasL (sFasL) and selected cytokines related to activation and/or elimination of Th cells were measured with ELISA.

**Results:** The stimulation increased concentrations of Il-6, Il-10, Il-12, sFas, sFasL and membrane Fas expression both in LB patients and controls, in a fashion dependent on the *B. burgdorferi* species, with the strongest response to Bss. Apoptosis of Th cells was decreased in early neuroborreliosis in the presence of Bss, but not in the patients with late borreliosis or in controls and not with Ba or Bg. Il-10 concentration correlated negatively with the membrane Fas expression and apoptosis under stimulation with Ba and Bg, but not Bss.

**Conclusions:** Expression of Fas/FasL is upregulated in PBMC culture under stimulation with *B. burgdorferi*, together with other features of PBMC activation, but without direct influence on lymphocyte apoptosis. Neither specific pro-apoptotic effect of *B. burgdorferi* on lymphocytes, nor lymphocyte apoptosis abnormalities specific for patients with LB were observed. Decreased apoptosis of lymphocytes in early neuroborreliosis in presence of with Bss may reflect the relatively strongest T cell stimulation and prevalence of pro-survival signals in that setting. Variable responses to different *B. burgdorferi* species suggest differences in the pathogenesis of the infection in vivo.

**P2048** Use of short course intramuscular vs. topical antibiotic treatment for curing experimental *Borrelia burgdorferi* infection in mice

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**Objectives:** It is still unclear what the minimally effective dosage is for the antibiotics recommended for use in curing Lyme disease (LD), and whether these dosages are age dependent. It is also unknown whether topical application of antibiotic at the site of tick attachment can prevent human infection. We hypothesize that by modifying the murine animal model for LD, by using elderly mice, it will be possible to determine minimally effective treatment regimens given topically or by other parenteral routes that could be applicable to geriatric patients who have contracted LD.

**Methods:** Mice were assigned either to an aged (12–18 months of age) group or to a young group of 10–12 weeks of age. Separate groups of C3H mice were infected intradermally (i.d.) with 100 000 culture-grown *Borrelia burgdorferi* (Bb), of human isolate strain BL206. Two-4 weeks after infecting the mice, they received two intramuscular injections (given 18–24 hours apart) of either saline or ceftriaxone (CTX; 50 mg/kg). In other experiments, infected nymphal deer ticks were permitted to feed on the shaven backs of 10–12 week-old C3H mice until the ticks had fed to repletion and had detached. Another group of mice were injected i.d. via syringe with culture-grown BL206. Then, two different types of antibiotics (tetracycline or erythromycin) were applied to the feeding site, beginning at 24–48 hours after tick detachment, or within 5 minutes at the syringe inoculation site. One 4 weeks after treatment, cultures of the urinary bladder and skin tissue were established in BSK media in order to determine the presence or absence of Bb in both treated and untreated control mice.

**Results:** It was found that the two-dosage regimen of CTX was 100% and equally effective in sterilizing the urinary bladders from the Bb-infected mice, irrespective of their ages. In addition, topical application of 2% erythromycin and 3% tetracycline preparations was found to be ineffective in eliminating Bb from the tick bite site or in preventing dissemination to other tissues from the tick bite or syringe inoculation site.

**Conclusions:** These experiments suggest that shorter courses of antibiotics than those currently recommended should be considered for study in patients of all age groups with early uncomplicated LD. Our findings also indicate that the topical application of antibiotic preparations, that are acceptable for use in humans, was unable to prevent tick- or syringe-transmitted infection in a mouse model of human Lyme disease.

**P2049** Application of multiplexing technology for the analysis of the intrathecally released antibodies against *B. burgdorferi* antigens in early neuroborreliosis

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The analysis of the cerebrospinal fluid (CSF) plays crucial role in the diagnostic procedures of neuroborreliosis. Supporting role in diagnosis, plays the quantitative analysis of the intrathecally released antibodies against *B. burgdorferi* antigens, expressed as Antibody Specific Index (ASI), with ELISA being the most commonly used analytical technique. A complex pattern of the antigens, taken together with the time-dependent dynamics of their expression during the course of the disease, calls for more sophisticated methods that would allow more detailed overview of the generation of the corresponding antibodies.

The objectives: the aim of this study was the simultaneous analysis of antibodies (IgG and IgM) against several *B. burgdorferi* antigens in a carefully selected group of patients characterized clinically, serologically and neurochemically.

**Material:** We included 27 clinically defined neuroborreliosis (NB) patients with either IgG or IgM ASI (measured with ELISA) higher than 1.5, and nine patients with excluded neuroborreliosis (with ASI's of both class either below 1.5 or non measureable; Controls).

**Methods:** The multiplexing analyses were performed with the assays from Mikrogen (Neuried, Germany) on the Luminex (Austin, USA) platform.

**Results:** 23/27 NB patients had at least one positive (>1.5) IgG-ASI against either VlsE, p100, p58, p39, p18, or OspC, and none of these patients showed positive OspA-IgG-ASI. In the NB group with IgM-ASI > 1.5, 9/13 patients showed positive IgM-ASI against either VlsE, OspC, p18 (two cases), or p39 (one case), and none of the patients had positive IgM-ASI against p100, p58, or OspA. In the Control group one subject showed very high IgG-ASI against p100, and one case showed positive IgG-ASI against VlsE. In three subjects with ELISA IgG-ASI in the normal range (0.7–1.5), i.e. with the antibodies present in the blood but without their intrathecal release, correspondingly, VlsE, or p100, or p18 ASI's were measurable but normal.

**Conclusions:** The simultaneous analysis of a large panel of antibodies against different epitopes of *B. burgdorferi* makes multiplexing technology a very interesting supplement to the classic ELISA by providing more specific, antigen-related indices to the general, antigen-unspecific ASI's. Whether this additional information proves to be diagnostic relevant will be certainly matter of further studies.

**P2050 Lyme borreliosis in a routine laboratory: patient population and performance of five VlsE containing immunoassays**

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**Objectives:** The aim of the study was to characterize the population for which *Borrelia* serology was requested in two routine laboratories, and to assess sensitivity and specificity of five VlsE-containing assays for diagnosis of Lyme Borreliosis (LB).

**Methods:** From 558 patients, whose sera were sent to our laboratories, clinical information was obtained by telephonic interview. All sera were screened by C6-EIA and if positive, tested by IgG and IgM immunoblot. For comparison of different EIAs, 477 of these sera (including all sera from patients with increased clinical suspicion for LB) were also tested in Diasorin, Serion, Medac and Enzygnost immunoassays, as were 14 other sera from patients with disseminated LB, 92 sera from healthy controls and 94 sera from patients with other diseases. All control sera and 150 clinical sera which were negative in all EIAs were also tested in immunoblot.

**Results:** Of the 558 patients, 31 (5.6%) had clinically been diagnosed with EM. Forty-five (8.0%) had symptoms compatible with disseminated LB, in whom in 12 (2.1%) the diagnosis disseminated LB was confirmed. Fifty-seven (10.2%) had atypical skin rashes; in 18 (3.2%) of these, serology was suggestive of recent LB. Four hundred and twenty-five patients (76.2%) had atypical or no symptoms. We found sensitivities ranging between 57% and 90% for early localized LB and 93% and 100% for early disseminated LB and specificities from 88% to 97% for healthy controls and 62–96% for other diseases. An extended gold serologic standard for sera from patients with atypical symptoms was constructed, in which a serum was considered to be positive if a positive reaction was found in an immunoblot (IgG and/or IgM) or in at least 4/5 EIAs (IgG and/or IgM). Using this standard, the sensitivities ranged from 77% to 90% and specificities from 92% to 98% for detection of antibodies in these patients. No significant differences were found for diagnosis of early LB, although C6, Diasorin and Enzygnost assays performed marginally better. Specificity of C6 and Medac were superior to the other tests. Immunoblots were frequently negative in early LB. Performance of additional immunoblotting in sera non-reactive in EIAs was not useful.

**Conclusion:** Over 70% of sera tested for LB originated from patients with low clinical suspicion for LB. However, 3% came from patients with clinically undiagnosed EM. Sensitivity differences between assays were limited, whereas some tests gave more false-positive results, especially in patients with other diseases.

**P2051 Performance evaluation of the new VIDAS Lyme IgM and IgG assays compared to the previous VIDAS Lyme total antibodies assay on fresh prospective sera**

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**Objective:** Lyme borreliosis (LB) is an infectious disease caused by the spirochete *Borrelia burgdorferi* sensu lato and transmitted through tick bites. Serological methods are the laboratory tools of choice for the medical diagnosis of LB. Currently, the most commonly used methods are the EIA method in combination with the western blot as confirmatory test. Other methods such as PCR are reserved for special cases. A large variety of commercial assays is available for the serological diagnosis of LB. We evaluated the performance of VIDAS Lyme new assays compared to previous VIDAS Lyme assay on fresh prospective samples.

**Materials and methods:** A total of 58 fresh samples were collected for this prospective trial and tested with the first (VIDAS<sup>®</sup> LYT – Detection of total antibodies) and new (VIDAS<sup>®</sup> Lyme IgM (LYM) and Lyme IgG (LYG) – Differential diagnosis of Lyme borreliosis) versions of the VIDAS assay (BioMérieux). A Western Blot (WB) IgM and IgG (Euroimmun) assay was used as a confirmatory test for both positive and negative samples.

**Results and discussion:** Of the 58 samples, 30 were found positive with VIDAS LYT and 24 with VIDAS LYM or LYG. Twenty-four of the LYT positives and 23 of the LYM/LYG positives were confirmed by WB. Out of 32 negative samples (based on WB status), 26 were negative with VIDAS LYT and 29 with VIDAS LYM or LYG.

Based on these results, the overall sensitivity and specificity of the first and new version of the VIDAS Lyme assay vs. WB were calculated and compared. Sensitivity was 92% for LYT and 90% for LYM/LYG. Specificity was 81% for LYT and 93% for LYM/LYG.

Although sensitivity is comparable between both versions of the Lyme assay, specificity is significantly higher with the new version. This improvement is brought by the separate detection of the IgM and IgG antibodies and the new chimeric proteins design based on VISE, DbpA and OspC proteins.

**Conclusion:** The outcome of our study made on fresh specimens shows that the new VIDAS Lyme assays provide additional benefits in many aspects. In particular, specificity is increased thanks to an optimized assay design.

The new VIDAS Lyme IgM and IgG tests are accurate, simple and rapid assays and provide a serological tool that improves the diagnosis of *Borrelia* infections.

**P2052 Correlation between indirect immunofluorescence and Western blot, serological techniques used for the diagnosis of Lyme disease**

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Lyme disease or borreliosis is the most common tick-borne disease in the Northern Hemisphere, cause by at least three species of bacteria belonging to the genus *Borrelia* (*Borrelia sensu stricto*, *Borrelia afzelii*, *Borrelia garinii*). *Borrelia* is transmitted by the bite of an infected tick belonging to a few species of the genus *Ixodes*.

**Objectives and methods:** To define the better strategy to diagnose Lyme disease using the serological tests (indirect immunofluorescence [IIFT] and western blot [WB]). The samples were collected from the patients supposed with Lyme disease, admitted in the Eco-Para-Diagnostic private clinic from Bucharest Romania between October 2010 and November 2011. The tests were performed using commercial kits.

**Results:** We tested a total number of 627 blood samples for specific antibodies against *Borrelia* species, 305 using IIFT and 322 using WB. From the IIFT samples tested (162 for IgG, 143 for IgM), 79 (48.76%) from the IgG samples were positives. We selected 53 from the positive IgG samples, which were also tested using WB technique and we obtained the results as follow: 22 (41.50%) IIFT IgM positive, two (3.77%) WB *Borrelia afzelii* IgM positive, one (1.88%) WB *Borrelia afzelii* IgG positive, one (1.88%) WB *Borrelia garinii* IgM positive,

eight (15.09%) WB *Borrelia garinii* IgG positive, seven (13.20%) WB *Borrelia sensu stricto* IgM positive and four (7.54%) WB *Borrelia sensu stricto* IgG positive. The 53 patients with positive serology for IIFT *Borrelia* species IgG were tested also for co infection with *Bartonella quintana/henselae* IgM and IgG and we found two cases with IgM positive and 10 with IgG positive. From 53 positive samples for IIFT *Borrelia* spp. – 24 (45.28%) were confirmed using WB technique – 10 cases for IgM and 14 for IgG and 29 had WB negative. If the patients have symptoms and the serology is still negative they must be tested again periodically.

**Conclusion:** The indirect immunofluorescence is a good screening test but must be confirmed using the western blot. All the positive samples using IIFT for detecting should be further tested by western blot for confirmation.

#### P2053 Value of the lymphocyte transformation test to determine the acuity of neuroborreliosis

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**Objective:** The lymphocyte transformation test (LTT) is widely used to make assumptions on the acuity of suspected NB. However, to our knowledge, there is a lack of data concerning the reliability of this test.

**Methods:** We applied the LTT (performed with three recombinant antigens; p18, p100, and OspC), CSF PCR, CSF culture, antibody specific index determination for *Borrelia*-IgM and IgG and serology (ELISA and Western-Blot) in a well characterized population of 98 persons divided into the following subgroups; (i) Healthy control persons; (ii) *Borrelia*-serology negative persons with neurocognitive complaints and normal CSF findings; (iii) Patients with other neurological diseases; (iv) *Borrelia*-serology positive patients with neuro-cognitive complaints but without CSF proof for neuroborreliosis (NB); (v) patients with acute neuroborreliosis (NB); (vi) Patients after undergone neuroborreliosis (NB) without any CSF signs of acute neuroborreliosis (NB) at the time of presentation. ANOVA was used to compare all groups with each other; in addition group E was compared to all other groups. A ROC-analysis was performed with the raw values of the three respective LTTs.

**Results:** The groups did not differ with respect to the mentioned antigens used in their LTT results. The areas under the curves of the ROC-analysis were for each LTT below 0.7 with ROC curves coming near to the bisector.

**Conclusion:** LTTs performed with p18, p100 or OspC were not able to provide useful additional information on the acuity of neuroborreliosis.

#### P2054 Methodology in reporting diagnostic accuracy for *Borrelia* specific IgG and IgM antibodies. Tests often differ but performance of the test antigens may be quite similar anyway

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This a study of Diagnostic accuracy of Oxoid IDEIA and Diasorin Liaison IgG and IgM serumantibodies. A straight forward test comparison has been performed using 48 serum samples from patients with Neuroborreliosis and 216 Danish blood donors as healthy controls. Using the instruction provided by the manufacturer the sensitivity of IDEIA/Liaison IgG was 44%/94% ( $p < 0.001$ ), IgM 99%/94% ( $p = 0.02$ ) and for IgG or IgM positive 81%/96% ( $p = 0.02$ ).

**Conclusion 1:** IDEIA is less sensitive in IgG and when IgG or IgM is combined.

The specificity of IDEIA/Liaison IgG was 99%/94% ( $p < 0.001$ ), IgM 99%/96% ( $p = 0.03$ ) and for IgG or IgM positive 98%/89% ( $p < 0.001$ ).

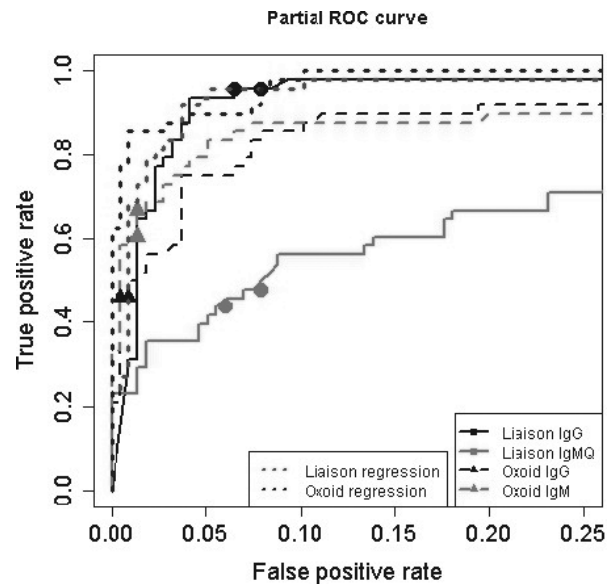
**Conclusion 2:** IDEIA was more specific in both IgG and IgM.

p-values were calculated by the McNemar test.

However conclusions 1 and 2 are inadequate and possibly a misleading interpretation of assay performance. When just counting positive and

negatives samples mainly differences in cutoff policy are measured, not the performance of test antigens.

The ROC curve shows the discriminatory power, allows for comparison of the differences and assessment of proposed cutoffs (see Fig. 1). Visual inspection of the figure is an important first step. It is quite evident that Liaison IgM is both less sensitive and specific compared to the other markers. When adding IgG and IgM together (a logistic regression model was used) the Liaison IgG appears quite similar to the combination of IDEIA IgG + IgM. The IDEIA IgG and IgM combined is slightly more specific and sensitive than Liaison IgG ( $p < 0.01$ , Mann-Whitney Rank sum tests). Liaison IgM does not contribute much diagnostic value as IgM positive samples are also IgG positive. It could be considered to adjust the cutoffs to increase sensitivity of the IDEIA assay and the specificity of the Liaison assay. The proposed intervals for the indeterminate results appear clinically irrelevant.



**Conclusion:** Differences in sensitivity and specificity were mainly related to choice of cutoff not the discriminatory ability of the assays. Reports on diagnostic accuracy for measurement of *Borrelia* specific antibody reactivity should include analysis of the quantitative data. Combination of IgG and IgM and the choice of cutoff should be discussed.

## Experimental treatment (animal model) of progeria bacterial infections

#### P2055 Methicillin-resistant *Staphylococcus aureus* harbouring mecA-LGA251, a new mecA-homologue: limited impact on the outcome of infective endocarditis treated with flucloxacillin in a rat model

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**Background:** *Staphylococcus aureus* remains a leading cause of infective endocarditis worldwide. Penicillin-M is recommended as the antibiotic of choice for the treatment of methicillin susceptible *S. aureus* (MSSA) whilst vancomycin is the antibiotic of choice in case of endocarditis caused by mecA-encoded methicillin resistant *S. aureus* (MRSA). Recently, MRSA harboring a brand new and highly divergent mecA homologue (named mecA-LGA251, located in a novel SCCmec) have been reported. However the clinical impact of this new resistance mechanism on the outcome of clinical infection is unknown. The aim of the present study was to assess the efficiency of penicillinase-resistant betalactam antibiotic in a rat model of *S. aureus* endocarditis using strains harboring the classical mecA gene or the mecA-LGA251 variant.



**Methods:** *Staphylococcus aureus* strain Mu50 was used as reference for mecA-positive strains, and strain NCTC13552 as reference for mecA-LGA251-positive strains (MRlgaSA). Two clinical MRlgaSA isolates were tested, one from a human specimen (SA820) and one from a veterinary sample (SA1100) isolated in France in 2007 and 2008, respectively. The presence of the mecA-LGA251 gene was confirmed by specific PCR. Oxacillin (OXA) MIC (E-test) and OXA population analysis profile (PAP) were determined. Rats with catheter-induced aortic vegetations (-) were treated for 3 days with doses simulating the kinetics after intravenous administration in humans of the standard dose of flucloxacillin (FCX) of 2 g every 6 hour. Animals were killed 8 hour after the end of the last dose and vegetations were cultured.

**Results:** OXA MICs for Mu50, NCTC13552, SA820 and SA1100 were >32, 0.125, 0.38 and 0.5 mg/L, respectively. PAP revealed highly heterogeneous OXA resistance for the three MRlgaSA isolates, without secondary increasing of OXA MICs for the most resistant selected colonies. At sacrifice, all vegetations from untreated animals (n = 5) as well as those infected with Mu50 and treated with FCX (n = 6), were infected. In contrast, FCX treatment successfully cured 6/8 (75%) and 9/9 (100%) vegetations of animals infected with strain SA820 and SA1100, respectively (p < 0.05).

**Conclusion:** Although resistant to cefoxitin and harboring additional PBP, the MRlgaSA isolates responded quite well to a humanized FCX treatment in the rat endocarditis model. Since mecA-LGA251 may be miss-diagnosed, it is not unlikely that cases of MRlgaSA infections, even severe, are currently treated successfully with beta-lactams.

#### P2056 Use of Kineret® to treat Panton-Valentine leukocidin-positive *Staphylococcus aureus* necrotising pneumonia

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**Objectives:** *Staphylococcus aureus* (Sa) is a major human pathogen which virulence is associated to a several secreted toxins. Particularly, Panton-Valentine leukocidin (PVL) contributes to severe necrotizing pneumonia affecting young immunocompetent patients. Despite effective antibiotic treatments, the lethality highlights the need for novel therapies. As uncontrolled inflammation is thought to occur in PVL+ Sa necrotizing pneumonia, the role of PVL in this process was studied. PVL strongly triggers activation of inflammasome in human macrophages, leading to the release of proinflammatory cytokine IL-1b. IL-1b mediates IL-8 and MCP-1 secretion by lung epithelial cells suggesting that inflammasome activation in PVL-intoxicated macrophages may play a key role in the deleterious inflammation observed in a rabbit model of necrotizing pneumonia. We decided to test if the inhibition of IL-1b signalling using Kineret® (IL-1b receptor antagonist, IL-1Ra) may reduce neutrophils infiltration and decrease lung injuries.

**Methods:** The action of Kineret® was investigated both in-vitro and in-vivo. A mixed culture of monocytes-derived macrophages and lung epithelial cells (A549) was treated with recombinant PVL (rPVL – 10 ng/mL) and increasing concentrations of IL-1Ra. IL-8 concentration in the supernatant was determined after 9 hour by ELISA.

USA 300 PVL+ clone (inoculum = 9.5 log CFU/mL) and rPVL (12 µg of LukS-PV and LukF-PV) were used to induce pneumonia in immunocompetent New Zealand rabbits. One group of infected animals was administered Kineret® (10 mg/kg) one hour before and at the time of infection. The concentration of IL-8 in bronchoalveolar lavage (BAL) was determined 6 hour post-infection.

**Results:** In our in-vitro model, PVL triggers the secretion of IL-1b by macrophages which in turn activates lung epithelial cells to produce IL-8. We demonstrated that the addition of IL-1Ra completely abrogated IL-8 secretion in-vitro. In the rabbit model of necrotizing pneumonia, the presence of PVL increased the secretion of IL-1b and IL-8 in BAL while the administration of IL-1Ra did not significantly reduce it.

**Conclusion:** Our in-vitro results suggest that Kineret® might reduce the detrimental PVL-mediated recruitment of neutrophils. Its low activity in our rabbit model of PVL+ Sa necrotizing pneumonia requires

further investigation. Some studies are currently being performed to demonstrate the affinity of the human IL-1Ra for a rabbit receptor as well as its pulmonary penetration.

#### P2057 Comparison of anti-MRSA antibiotics (vancomycin, linezolid, daptomycin, rifampin) and anti Gram-positive fluoroquinolones (moxifloxacin, delafloxacin) against MSSA and MRSA in models of young and mature biofilms

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**Background:** Biofilm-related infections by *S. aureus* are frequent but difficult to treat. Because of their highly bactericidal character and high diffusibility, fluoroquinolones may constitute a useful treatment option. We have developed an in-vitro model of young (6 hour) and mature (24 hour) *S. aureus* biofilm allowing for quantitative evaluation of antibiotic activity on both bacterial survival and matrix production. The aim of this study was to compare the activity of four antibiotics recommended for MRSA therapy with that of two potent anti-Gram (+) fluoroquinolones.

**Methods:** Biofilms were obtained by culturing *S. aureus* ATCC25923 (MSSA) or ATCC33591 (MRSA) in 96-well plates for 6 or 24 hour. Biofilms were exposed to antibiotics at concentrations ranging from 0.5 to 128x the MIC (as determined in broth) for 24 or 48 hour (young or mature biofilms, respectively). Total biofilm mass (matrix + cells) was measured using crystal violet (CV) staining (OD measurement at 660 nm), and viable cells within the matrix using the redox indicator resazurin (reduction to resorufin [Lett. Appl. Microbiol. 2008, 49:249–54]).

**Results:** The table compares the activity of antibiotics at selected concentrations. The MSSA strain produced more biofilm (matrix and viable cells) than the MRSA strain. Against MSSA young biofilms, RIF and DFX fully eradicated viable bacteria and matrix (not shown) at 1 x MIC. All antibiotics (except LZD) cleaned up the matrix and the bacteria at 8 x MIC. Against MRSA young biofilms, RIF, LZD, and DFX were active on viable cells and matrix, whereas VAN and DAP were most active against matrix, but at high concentration. All antibiotics were much less active against mature biofilms, especially those formed by the MSSA strain where no antibiotic showed marked activity on matrix (not shown). For mature biofilms, RIF and DFX were the most effective to reduce MSSA viability, while LZD, DAP, RIF and DFX were most effective against MRSA.

AB	6 h biofilm (24h AB incubation)						24h biofilm (48h AB incubation)					
	MSSA			MRSA			MSSA			MRSA		
	Resorufin Fluorescence (% of control)		Crystal violet Absorbance (% of control)	Resorufin Fluorescence (% of control)		Crystal violet Absorbance (% of control)	Resorufin Fluorescence (% of control)			Resorufin Fluorescence (% of control)		
	1 x MIC	8 x MIC	8 x MIC	1 x MIC	8 x MIC	8 x MIC	2 x MIC	8 x MIC	2 x MIC	8 x MIC	2 x MIC	8 x MIC
VAN	78 ± 17	3 ± 0	5 ± 1	84 ± 7	61 ± 25	8 ± 9	92 ± 13	80 ± 15	69 ± 6	96 ± 10		
LZD	34 ± 7	18 ± 4	18 ± 4	50 ± 10	5 ± 0	17 ± 1	116 ± 21	83 ± 8	29 ± 3	26 ± 1		
DAP	40 ± 6	0	3 ± 3	113 ± 14	28 ± 9	95 ± 70 <sup>b</sup>	116 ± 26	108 ± 23	51 ± 9	29 ± 3		
RIF	7 ± 2	6 ± 1	6 ± 1	14 ± 7	10 ± 7	20 ± 16	64 ± 6	67 ± 7	31 ± 4	29 ± 4		
MXF	25 ± 5	1 ± 0	3 ± 1	98 ± 23	66 ± 4	92 ± 10	124 ± 9	114 ± 21	92 ± 22	78 ± 10		
DFX	2 ± 0	7 ± 0	9 ± 1	45 ± 12	22 ± 3	19 ± 3	67 ± 7	59 ± 8	38 ± 3	33 ± 3		

Control values: resorufin fluorescence: 5405 ± 933 (MSSA, 6h-old biofilm), 3105 ± 924 (MRSA, 6h-old biofilm), 3211 ± 580 (MSSA, 24h-old biofilm), 2856 ± 678 (MRSA, 24h-old biofilm), crystal violet absorbance: 14.30 ± 1.73 (MSSA, 6h-old biofilm), 5.18 ± 1.24 (MRSA, 6h-old biofilm)

\* AB: antibiotic

<sup>b</sup> MIC (mg/L) for MSSA/MRSA: VAN (vancomycin), 1/1; LZD (linezolid), 1/1; DAP (daptomycin), 1/1; RIF (rifampin), 0.03/0.03; MXF (moxifloxacin), 0.03/0.03; DFX (delafloxacin), 0.004/0.004

<sup>c</sup> large variation is due to the sharp decrease of the CV OD taking place between 4 and 16 x MIC with this antibiotic

**Conclusion:** Antibiotic activity is markedly defeated in mature biofilms and seems strain-dependent, probably reflecting differences in the nature, or the physicochemical properties of the biofilm produced. At equipotent concentrations, RIF and DFX were the most constantly effective in this model. Given its very low MICs, DFX may offer a therapeutic advantage and warrants further investigation.

**P2058** AFN-1252 alters in vitro and in vivo *Staphylococcus aureus* gene expression and reduces bacterial counts in a mouse granuloma infection model

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**Objectives:** AFN-1252, a novel antibiotic currently in clinical development for staphylococcal infections, blocks type 2 fatty acid synthesis (FAS II) by inhibiting enoyl-ACP reductase (FabI) in *Staphylococcus aureus*. The current study describes the effects of AFN-1252 treatment on in vitro and in vivo *S. aureus* gene expression, and the pharmacokinetics (PK) and efficacy of AFN-1252 in the *S. aureus* infected mouse granuloma (MG) model are described as well.

**Methods:** Affymetrix gene array and qRT-PCR were used to determine gene expression changes in AFN-1252 treated *S. aureus*. Exponential-phase laboratory cultures of *S. aureus* were treated with either solvent control or AFN-1252 for 15 minutes, and total RNA was extracted from the cells for analysis. The MG model was infected by inoculating *S. aureus* into 5-day-old subcutaneous granuloma pouches, followed by oral administration of AFN-1252 at 2, 26, and/or 50 hours after inoculation. Granuloma fluid was collected at multiple time points over a 24- or 96-hour period following AFN-1252 treatment for CFU counting, mRNA profiling and determining AFN-1252 PK.

**Results:** Exposure of *S. aureus* cultures to AFN-1252 resulted in the anticipated up-regulation of genes involved in the FAS II pathway associated with the FapR regulon and the unpredicted down-regulation of several virulence genes that are controlled by the SaeRS two-component regulator. In the MG infection model, a single oral dose of AFN-1252 at 2 hours post-infection resulted in mean log<sub>10</sub> CFU reductions of 2.9–3.1 in 24–48 hours after dosing. PK analysis of this fluid revealed that the relative exposure (AUC) of AFN-1252 in the granuloma fluid was 85% of the corresponding plasma levels, and qRT-PCR of *S. aureus* RNA extracted from granuloma fluid indicated that fabH expression was up-regulated and virulence factor expression was down-regulated following the single dose of AFN-1252. AFN-1252 was also dosed consecutively (2, 26, 50 hours) in the MG model, which maximally reduced granuloma-associated *S. aureus* counts by 5.3 log<sub>10</sub> CFU within 72 hours of the first dose.

**Conclusion:** AFN-1252 triggered the up-regulation of genes associated with the FASII pathway in *S. aureus*, and it simultaneously down-regulated virulence genes controlled by the SaeRS regulator. AFN-1252 not only altered *S. aureus* gene expression in the granuloma fluid, but it also therapeutically reduced the CFU counts in the fluid as well.

**P2059** Daptomycin vs. vancomycin in the treatment of methicillin-resistant *Staphylococcus aureus* meningitis in experimental rabbit model

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**Objectives:** To compare the antibacterial activity of daptomycin and vancomycin in the treatment of methicillin-resistant *Staphylococcus aureus* (MRSA) meningitis in experimental rabbit meningitis model.

**Methods:** Meningitis was induced by direct inoculation of ATCC 43300 MRSA strain into cisterna magna of New Zealand rabbits. After 16 hours of incubation time, rabbits were separated into three groups as daptomycin (D), vancomycin (V), and control (C) groups. D group received one dose of 15 mg/kg daptomycin, V group received 20 mg/kg vancomycin two times (4 hour apart). C group did not receive any treatment. Quantitative bacterial cultures were performed in CSF samples which were obtained at the beginning and the 8th hour of the treatment. CSF and serum drug levels were measured by bioassay technique in samples obtained at the 8th hour of the treatment. The study was approved by the local ethical committee on animal studies.

**Results:** After 16 hours incubation time, all rabbits fulfilled meningitis criteria. There was no difference between number of bacteria (calculated as log<sub>10</sub>) in three groups at the beginning of the treatment

(C: 3.448 ± 0.318 CFU/mL, D: 3.958 ± 0.568 CFU/mL, V: 3.703 ± 0.666 CFU/mL, p > 0.05). At the 8th hour of the treatment, bacterial count decreased significantly in both treatment groups compared to control group (C: +3.798 ± 0.682 CFU/mL, D: -3.610 ± 0.677 CFU/mL, V: -3.403 ± 0.697 CFU/mL, p < 0.05). Moreover, there was no statistically significant difference between D and V groups. Daptomycin could be measured in all but one rabbit which could not be punctured adequate CSF sample. In D group the CSF/serum ratio was 1.9–4.1%.

**Conclusion:** Our results suggest that the antibacterial activity of daptomycin is similar to vancomycin in the treatment of experimental MRSA meningitis model of rabbits.

**P2060** Assessment of antibiotic lock technique with daptomycin, vancomycin for the treatment of coagulase-negative staphylococci experimental catheter infection

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ALT is an efficient alternative technique for the conservation of tunnelled and implanted catheters in CoNS infections. Vancomycin is the elective antimicrobial in the clinical practice, but one-third of the episodes may not be cured with this. We evaluated the activity of DAP compared to VAN for the conservative treatment of experimental CoNS using the ALT.

**Methods:** A silicone catheter was surgically implanted via jugular vein in 28 New Zealand rabbits C1. The catheter was locked for 18 hour with an inoculum of 10<sup>8</sup> CFU/mL of clinical isolate from a patient with catheter-related septicemia CoNS strain (*S. epidermidis* SE14 or SE94). Thereafter, the inoculum was replaced for 24 hour a sterile saline solution with 100 IU of heparin. Then, catheters were locked for further 24, 48 or 72 hour with 100 IU of heparin plus sterile saline (Control), DAP 50 mg/mL, or VAN 10 mg/mL (animals infected with SE94 were treated only for 24 hour). After treatment, animals were sacrificed and C1 was assessed using sonication techniques of the catheter's tip. X2 or Fisher test and Kruskal–Wallis or Mann–Whitney tests were used to analyse differences in proportion of negative cultures and in mean log<sub>10</sub> CFU as appropriate. A p-value <0.05 was considered significant. \*p < 0.05 vs. control; †p < 0.001 vs. Control and Vancomycin; p < 0.01 vs. Control and Vancomycin; n neg/n T: number negative/Total number animals.

<i>S. epidermidis</i> 14						
Group	24 h		48 h		72 h	
	n neg / n T (%)	mean log cfu per mL (SD)	n neg / n T (%)	mean log cfu per mL (SD)	n neg / n T (%)	mean log cfu per mL (SD)
Control	0/7 (0)	6 (0.8)	0/13 (0)	5.57 (1.1)	0/8	6.1 (0.7)
Dapt 50 mg/ml	6/6 † (100)	0.3 (0) †	14/16 † (87.5)	0.45 (0.42) †	6/7 (85.7)	0.41 (0.3) †
Van 10 mg/ml	0/6 (0)	3.9 (0.8)*	2/17 (11.8)	2.82 (1.5)*	2/9 (22.2)	2.8 (1.5)*

<i>S. epidermidis</i> 94		
Group	n neg / n T (%)	mean log cfu per mL (SD)
Control	0/7 (0)	3.7 (1.2)
Dapt 50 mg/ml	7/7 (100)	0.3 (0) †
Van 10 mg/ml	0/6 (0)	4.4 (1.1)*

**Conclusions:** Daptomycin showed higher activity than Vancomycin for the treatment of experimental Coagulase-negative Staphylococci catheter-related infection with the antibiotic-lock technique. Using this model, daptomycin seems to act faster than vancomycin against in vivo central venous catheter biofilm infections. This observation may be important Daptomycin ALT could be effective for eradicating CoNS central venous catheter biofilm infections.

**P2061** Experimental study of the efficacy of daptomycin in the therapy of high cephalosporin-resistant pneumococcal meningitis

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**Objectives:** The best alternatives to classical therapy with beta lactams in cases of allergy or high resistance in pneumococcal meningitis remain unclear. The aim of this study is to know the efficacy of Daptomycin in this situation.

**Methods:** Using a rabbit model of meningitis we tested the efficacy of daptomycin (D) 15 mg/kg/day compared to those of ceftriaxone (C) 100 mg/kg/day and ceftriaxone + vancomycin (C + V) 30 mg/kg/day. These dosage regimens were selected after pharmacokinetic studies in order to achieve serum concentrations comparable to those observed in humans. Meningitis was induced by intracisternal inoculation of 106 CFU/mL of a highly resistant pneumococcal strain (ATCC 51916) to several groups of rabbits ( $n \geq 8$ /group). MICs (mg/L) were: PEN 0.12, C 32, V 0.25 and D 0.19. After 40 hour inoculation a baseline CSF sample was taken (hour 0) and therapy was started. Antibiotics were given intravenously for 26 hour. Control animals received saline solution. CSF samples were collected during therapy to determine bacterial killing rates, antibiotic concentrations and inflammatory parameters. ANOVA and *t*-test were used to statistical analysis.

**Results:** Bacterial counts and killing rates (KR) in CSF calculated as delta-log<sub>10</sub> CFU/mL are shown in the Table 1. At 6 hour. Seventy-seven percent of D-treated CSF samples vs. 25% of C + V-treated CSF samples were under the level of detection.

	Log UFC/ml h.0	KR at 6h	KR at 24h	KR at 26h
Control	5.146 ± 0.826	0.480 ± 0.756	0.610 ± 1.195	0.560 ± 1.374
C	4.798 ± 0.635	-0.450 ± 1.322	-1.122 ± 1.414	-1.050 ± 1.238
D	5.108 ± 0.852	-4.085 ± 1.06*	-3.705 ± 1.39*	-4.041 ± 1.14*
V+C	4.826 ± 0.848	-2.553 ± 1.403*	-3.839 ± 0.876*	-3.863 ± 0.896*

\* P ≤ 0, 05 vs. Control and C group.

**Conclusions:** In the rabbit model, Daptomycin was as effective and faster than the combination vancomycin + ceftriaxone, and better than ceftriaxone alone in the therapy of highly cephalosporin-resistant pneumococcal meningitis.

**P2062** High efficacy of fosfomycin-rifampin combination against methicillin-resistant *Staphylococcus aureus* in an experimental model of foreign-body infection

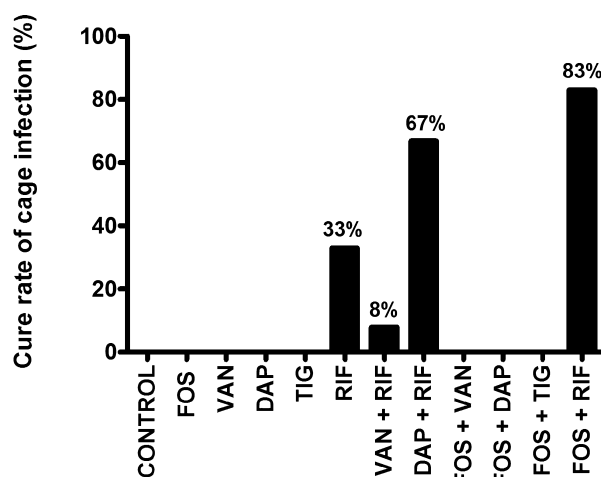
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**Objective:** Increasing antimicrobial resistance limits available options for treatment of methicillin-resistant *Staphylococcus aureus* (MRSA), especially when associated with implants. We evaluated the efficacy of fosfomycin (FOS), vancomycin (VAN), daptomycin (DAP), tigecycline (TIG) and rifampin (RIF), alone and in combinations, against MRSA in a foreign-body infection model.

**Methods:** Teflon cages (32 × 10 mm) were subcutaneously implanted in guinea pigs (weight 450–500 g). Cages were infected by percutaneous injection of  $3 \times 10^6$  CFU MRSA (ATCC 43300). Seventy-two hour after infection, treatment was administered for 4 days by intraperitoneal injection of FOS 150 mg/kg, VAN 15 mg/kg, DAP 50 mg/kg (corresponding to 10 mg/kg in humans), TIG 10 mg/kg, RIF 12.5 mg/kg or their combinations (12 cages per treatment regimen). Antibiotics were administered every 12 hour, except DAP

that was given every 24 hour. Five days after end of therapy, bacteria were counted in aspirated cage fluid and compared to the concentration before treatment to evaluate the antimicrobial effect on planktonic MRSA. Cages were then aseptically explanted and cultured in TSB for 48 hour to determine the eradication of MRSA, expressed as cure rate (percentage of cages without growth of MRSA).

**Results:** The MIC was 1 µg/mL for FOS, 1 µg/mL for VAN, 0.125 µg/mL for DAP, 0.125 µg/mL for TIG and 0.04 µg/mL for RIF. Bacterial counts of  $6.6 \times 10^6$  measured on day 3 after infection were reduced by (median log CFU/mL) 0.3 log with FOS alone, 2.2 log with FOS + VAN, 3.8 log with FOS + DAP, 2.2 log with FOS + TIG, >6.0 log with DAP + RIF and >6.0 with FOS + RIF. Figure 1 shows the cure rate of individual treatment regimens. In untreated animals (controls), no spontaneous cure occurred. Among single-therapy regimens, only RIF showed cure in 4/12 cages (33%). Among combination regimens, only RIF-containing regimens demonstrated cure in 1/12 cages (8%) with VAN + RIF, in 8/12 cages (67%) with DAP + RIF and in 10/12 cages (83%) with FOS + RIF. No emergence of resistance to FOS was observed in failures receiving single or combination treatment regimens (MIC ≤ 16 µg/mL).



**Conclusion:** The highest cure rate of MRSA cage-associated infection was achieved with the combination FOS + RIF (83%), which was superior to other RIF-containing combinations. No emergence of FOS resistance was observed. These data suggest that addition of FOS to RIF might further improve the treatment outcome of MRSA implant-associated infections.

**P2063** A porcine model of haematogenous osteomyelitis: pathogenesis and pathology

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**Objective:** Development and refinement of effective treatment regimes of haematogenous osteomyelitis is depending on the understanding of bacterial induced bone pathology. Therefore, a discriminative porcine model of haematogenous osteomyelitis based on local intraarterial inoculation of *Staphylococcus aureus* (*S. aureus*) was developed. In the model, the pathology of osteomyelitis and the presence of bacteria were elucidated.

**Methods:** Five 12 weeks old female pigs (BW 30 kg) were separated into two groups. Three animals were inoculated into the right femoral artery with *S. aureus* strain S54F9 and the control group of two animals was sham inoculated with saline. Following euthanasia, 11 days after inoculation, the animals were CT scanned and necropsied. Macroscopic bone lesions were recorded and sampled for histopathology. Immunohistochemistry was applied for in situ identification of *S. aureus*.

**Results:** Osteomyelitis lesions were observed by CT scanning and during necropsy in the distal metaphyseal area of the right femoral bone and in the proximal metaphyseal area of the right tibial bone of all infected animals. Sometimes, the inflammation penetrated into and through the cortical bone and periosteum leading to the formation of soft tissue abscesses. Dense, soft and pink granulation tissue surrounded the lesions. Microscopically, the lesions revealed an extensive loss of bone tissue combined with sequestered trabeculae intermingled with colonies of positive *S. aureus* bacteria. Lesions were not observed in control animals.

**Conclusion:** The present model was found to be discriminative of naturally occurring haematogenous osteomyelitis in long bones, usually involving femur and tibia in children. Therefore, the model is suitable for studies focusing on the cellular and molecular mechanisms of disease development. The presence of granulation tissue showed that the osteomyelitis lesions developed into a chronic stage already 11 days after infection. This may explain why antibiotic therapy often fails in patients diagnosed with the disease at an early stage. The model therefore may be an attractive tool for examining new strategies for diagnosing and treatment of chronic bone infections.

**P2064** Efficacy of colistin combination therapy against multidrug-resistant Gram-negative bacteria in mouse lung and thigh infection models

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**Objectives:** Colistin (COL) is being increasingly used for infections caused by multidrug-resistant (MDR) *Pseudomonas aeruginosa* (Pa), *Acinetobacter baumannii* (Ab) and *Klebsiella pneumoniae* (Kp). In vitro data indicate promising activity for COL in combination with doripenem (DOR) and rifampicin (RIF) against Pa, Ab and Kp. Our aim was to examine the efficacy of COL and its combinations with DOR or RIF against MDR Pa, Ab and Kp strains in mouse lung and thigh infection models.

**Methods:** COL-susceptible, -heteroresistant (HR) and -resistant Pa, Ab and Kp strains were used (see Table 1). Lung and thigh infections were established in neutropenic mice. At 2 hour post infection a single dose 20 mg/kg COL was given subcutaneously, either alone (MONO) or together (COMBO) with 100 mg/kg DOR for Pa and Kp infections or with 10 mg/kg RIF for Ab infections; saline-treated mice served as controls. Animals were sacrificed at 0, 3, 6 or 24 hour for viable counts in homogenized lung and thigh.

**Results:** Reductions of log CFU per lung or thigh between control and the most active MONO or the COMBO at 24 hour are presented in the Table 1. COL/DOR COMBO was superior (>1 log) to the most active MONO in both lung and thigh for two of three strains of both Pa and Kp. For the Ab strains, COL/RIF COMBO was more active than MONO for one strain only in the thigh infection.

Test strain	MIC (mg/L)		Lung (n = 4)		Thigh (n = 4)	
	COL	DOR/RIF	MONO*	COMBO	MONO*	COMBO
Pa ATCC 27853	1	0.25	DOR;2.1	6.2	COL;1.1	4.1
Pa FADDI-PA033 (HR)	1	1	DOR;3.6	5.4	DOR;1.5	3.0
Pa FADDI-PA070	>128	0.25	DOR;2.1	1.7	DOR;2.7	2.2
Ab ATCC 19606 (HR)	0.5	(1)	RIF;2.1	1.6	RIF;4.6	5.0
Ab FADDI-AB030	0.5	(64)	COL;0.3	NA	COL;1.3	4.1
Ab FADDI-AB156	16	(16)	COL;0.1	0.5	COL;3.2	3.9
Kp ATCC 13883 (HR)	1	<0.125	DOR;0.6	1.1	COL;2.4	3.4
Kp FADDI-KP032	1	8	DOR;2.5	3.6	COL;0.9	1.6
Kp FADDI-KP027	>128	<0.125	NA	2.6	DOR;0.9	2.7

NA, not active; \*most active monotherapy

**Conclusion:** The in vivo efficacy of COL combinations was promising and strain dependent. These combinations are undergoing further evaluation in multi-dose regimens designed to optimize PK/PD exposure over a 24 hour treatment period.

**P2065** Efficacy of ceftaroline fosamil in combination with the non-beta-lactam beta-lactamase inhibitor avibactam against AmpC-producing *Enterobacter cloacae*: comparative study with doripenem in a rabbit experimental endocarditis model

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**Objectives:** Avibactam (AVI) is a new non-beta-lactam beta-lactamase inhibitor that has little intrinsic antimicrobial activity, but has the ability to inhibit class A and class C beta-lactamases, including extended-spectrum beta-lactamases (ESBLs) and some class D enzymes. This study compared the in vivo activity of ceftaroline fosamil (CPT fosamil) alone, CPT fosamil in combination with AVI (CXL), and doripenem (DOR) against an AmpC-producing *E. cloacae* strain using a rabbit model of endocarditis.

**Methods:** Minimum inhibitory concentrations for CPT (the active form of CPT fosamil), CPA (the combination of CPT and AVI), and DOR were 128, 1, and 0.064 mg/L, respectively. Rabbits infected with *E. cloacae* were randomly assigned to: no treatment (controls), CPT fosamil (computer-controlled infusion syringe pump simulating a human-equivalent [HE] dose of 600 mg three times daily [tid]), CXL (simulating a HE dose of 600 mg tid), and DOR (simulating a HE dose of 500 mg tid). Bacterial counts in cardiac vegetations were determined at the end of a 4-day treatment.

**Results:** Results are presented in the Table 1.

Treatment (n)	Mean ± SD log <sub>10</sub> CFU/g of vegetation
Control (12)	8.66 ± 0.84
CPT fosamil (HE 600 mg tid) (6)	8.39 ± 0.25
CXL (HE 600 mg tid) (8)	5.11 ± 1.41*
DOR (HE 500 mg tid) (8)	5.13 ± 2.44*

CFU = colony-forming units; n = number of animals; SD = standard deviation.  
\* P<0.001 versus control animals and CPT fosamil-treated animals.

**Conclusions:** As expected, CPT fosamil was ineffective against an AmpC-producing *E. cloacae* strain. CXL showed bactericidal activity after 4 days of treatment with a simulated HE dosage in this experimental model. CXL showed efficacy equivalent to that of the carbapenem, DOR, in terms of reducing bacterial load in vegetations. These data demonstrate the ability of the beta-lactamase inhibitor, AVI, to improve the in vivo activity of CPT fosamil against otherwise nonsusceptible pathogens, and strongly support the addition of AVI to CPT fosamil as an effective strategy against AmpC-producing isolates. This combination may provide a therapeutic alternative to carbapenems for the treatment of infections caused by multidrug-resistant Gram-negative bacteria.

**P2066** Biofilm formation by *Staphylococcus aureus* on orthopaedic implants in a novel murine infection model

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**Objectives:** Biofilm formation on orthopedic implants constitutes a severe illness with a high rate of treatment failure. Development of new treatments is hindered partly due to the lack of animal models for implant-associated biofilm infections. The objective of this study was to develop a novel murine model, in which it is possible to establish a *S. aureus* biofilm infection on the surface of a transcortical tibia implant. This infection should result in an osteomyelitis (OM), without systemic dissemination of bacteria, and without affecting the overall health of the mice.

**Methods:** Two strains of *S. aureus*, one reference strain (Xen29) and one MRSA (Xen30), were used in C57Bl/6 mice. Implant-associated OM was established by a transcortical implantation of a stainless steel pin through the tibia. A biofilm was established on the pins prior to implantation by conditioning the pins for 24 hours in 25% murine serum followed by 24 hour incubation in Müller-Hinton bouillon with 10<sup>6</sup> CFU/mL bacteria and 3.5% NaCl. At five defined time points, five animals from each group were sacrificed, and bacteria were quantified by CFU counts from tibia and implant samples. Bacteria were extracted

from the tibia by tissue homogenization and from the implants by ultrasonication. Biofilm formation on the implant was assessed by DAPI staining and epifluorescence microscopy. Regular weighing as well as monitoring of fluid intake and activity levels assessed the overall health of the test animals. Blood cultures were done after sacrifice.

**Results:** A local, clinical infection was developed in all animals. Epifluorescence microscopy confirmed the presence of a biofilm by day 4 after the implantation of the infected pins. Bacterial load by day 4 on the implants was 4.2 log CFU per implant, 95% CI [3.5; 5.0] for Xen29 and 4.7 log CFU per implant, 95% CI [4.0; 5.3] for Xen30. The infection in the adjacent bone was also measured: 6.4 log CFU per implant, 95% CI [6.1;6.8] for Xen29 and 4.7 log CFU per implant, 95% CI [4.0;5.3] for Xen30. All blood cultures were negative and the animals experienced no significant loss in weight during the study.

**Conclusion:** We have established a novel animal model in which it is possible to induce an osteomyelitis originating from a *S. aureus* biofilm on an orthopedic implant. This infection is localized to the tibia and without bacterial dissemination.

**P2067** Efficacy of micafungin solved in ethanol in the treatment of experimental *Candida parapsilosis* catheter infection using the antifungal-lock technique

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**Background:** *Candida* species have the ability to form biofilms, what impairs the treatment of infected catheters. When infection involves a highly needed catheter might not be an option. Our aim was to evaluate the efficacy of liposomal amphotericin B (L-AmB) or micafungin (MICA) in the treatment of *C. parapsilosis* (CP) catheter infection (CI) using the antifungal lock technique (ALT).

**Methods:** New Zealand rabbits were surgically implanted with a silicone catheter through the jugular vein. After insertion the catheter was locked with Antibiotic Medium norm;3 (Oxoid) supplemented with 7.5% dextrosa and 100 IU of heparina/mL containing 10<sup>7</sup> CFU/mL of *Candida parapsilosis*. Forty-eight hour later the catheters were filled with antifungal solution for further 48 hour. Treatment groups: Control L-AmB 5 mg/mL, MICA 10 mg/mL, Ethanol 20%, Ethanol 40%, MICA solved in Eth 20% and MICA solved in Eth 40%. At the end of the treatment period animals were sacrificed and CI was assessed using sonication culture techniques. The total number of CFU recovered from each catheter tip was recorded. Differences in % negative catheters and log CFU were analyzed using Fisher and Mann-Whitney tests; samples with no growth were assumed to be log = 0.3. A  $p < 0.05$  was considered significant.

	Control	L-AmB	Ethanol 20%	Ethanol 40%	MICA	MICA + Eth 20%	MICA + Eth 40%
n negative/n total (%)	0/12 (0)	4/13 (31)	0/10 (0)	1/3 (33)	2/13 (15)	1/5 (17)	4/7 (58)*
log total cfu (SD)	4.12 (1.6)	2.34 (1.5)†	3.48 (1.1)*	2.57 (1.99)*	2.1 (1.4) †	1.9 (1.5)*	0.4 (0.1) †

\* $p < .05$  vs Control; †  $p < .01$  vs Control; ‡  $p < .05$  vs other groups of treatment; \*  $p < .05$  vs Control.

**Results: Conclusions:** Ethanol at 40% increases the efficacy of Micafungin in the treatment of Experimental *Candida parapsilosis* Catheter Infection Using the Antifungal-Lock Technique.

## New aspects on helminth infections and intestinal protozoans

**P2068** Molecular and phylogenetic characterisation of *Echinococcus granulosus* samples from Iberian peninsula

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**Background:** Hydatidosis is a worldwide zoonosis caused by the larval stage of the tapeworm *Echinococcus granulosus*. The taxonomy

of the genus *Echinococcus* had been a controversial issue for several years, recently reviewed based on gene sequencing data and now widely accepted. The aim of this work was to characterize and perform a phylogenetic analysis of Portuguese and Spanish samples from different hosts.

**Methods:** A total of 100 isolates (55 Portuguese and 45 Spanish) obtained from sheep (53), goats (1), cattle (17), horses (4), pigs (12) and humans (13) were characterized by sequencing regions of the mitochondrial COI and NDI genes. Sequences were aligned, including against homologous sequences from GenBank, and phylogenetic trees were generated using Neighbour-Joining (from Kimura 2-parameter correction distances) and Bayesian analyses.

**Results:** Preliminary results showed the presence of different *E. granulosus* genotypes as G1, G3, G4 (*E. equinus*) and G1. Humans presented mainly G1.

**Discussion:** The phylogenetic analyses using the preliminary results showed some degree of variance between isolates of *E. granulosus sensu stricto* (G1-G3 cluster) and a significant degree of variance between the other *Echinococcus* sp. The Iberian Peninsula isolates belonged mainly to the cluster of *E. granulosus sensu stricto* (G1 and G3 genotypes), but also included *E. equinus* (G4) and *E. granulosus* G7, in pigs, samples, in Spain. No geographical clusters were identified between the two countries.

**P2069** Management of cystic echinococcosis in pregnancy

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The frequency of Cystic Echinococcosis (CE) in pregnancy is low, with approximately 1/20 000–30 000 new births in endemic areas. Consequently, experience in managing this condition during pregnancy is limited. In the current literature, some women are treated with surgery, others with percutaneous treatment or chemotherapy. We report our experience with six pregnant patients with CE seen in our centre from 1990 to 2011. This was an observational, retrospective-prospective cohort study and the inclusion criteria were: (i) to be pregnant (ii) to harbour one or more echinococcal cysts exclusively in the liver. The mean age was 27 (range:17–39). The cysts were all transitional and inactive (WHO IWGE standardized ultrasound classification); there were three CE3b, two CE4 and one CE5. We chose the “watch and wait” approach (expectant management) for each patient as the cysts were not complicated and they were completely asymptomatic. All patients were monitored by ultrasound and serology and completed their pregnancies without significant complications between the 36th and the 40th week. Two patients delivered by caesarean section, one due to podalic presentation and the other as a cautionary measure against the risk of cyst rupture despite the fact that the cyst had remained unchanged during the course of pregnancy as happened with all the other women in the series. All newborns were healthy and have remained so to date.

We conclude that the “watch and wait” approach for uncomplicated transitional and inactive CE of the liver is a viable option for pregnant women harbouring liver cysts and because the cysts are not at risk of rupture during delivery, C-section can be avoided.

**P2070** Twenty-year seroepidemiological study of echinococcosis in Northwestern Greece

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**Objectives:** Cystic echinococcosis is one of the most widespread parasitosis in the Mediterranean region. The disease is most commonly due to *Echinococcus granulosus* and may occur in any organ or tissue. The aim of this study was to determine the seroprevalence of *Echinococcus granulosus* infection in North-Western Greece.

**Methods:** Serum samples from 1787 suspected patients (adults and children) were tested in the University Hospital of Ioannina during the

period of two decades (1992–2011). The presence of antibodies against *Echinococcus granulosus* was determined using an indirect haemagglutination assay (IHA, Cellognost-Echinococcosis, DadeBehring, Germany). According to the manufacturer's interpretation data a titre of 1:16 or higher was accepted as positive.

**Results:** Seroepidemiological study showed that in 168 of 1787 patients (9.4%) with suspected echinococcosis, antibodies to *Echinococcus* with low to high titres were detected by IHA. Antibody seroprevalence increased significantly with age (85.7% were adults) but no significant differences were found between the positive patients in regard to their sex. *Echinococcus* infection was clinically diagnosed in all seropositive patients. Out of IHA-positive patients that were confirmed as echinococcosis cases, 85 were positive in low titres 1:16–1:64.

**Conclusion:** Seroprevalence of *Echinococcus granulosus* infection in North-Western Greece is still high. Thus, it is suggested that clinicians and health authorities, should pay greater attention to this major public health issue.

**P2071 Immunohistological staining with the monoclonal antibody Em2G11 is highly specific and sensitive for *Echinococcus multilocularis* larvae in human tissue**

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Alveolar echinococcosis (AE) and cystic echinococcosis (CE) are two parasitic diseases in humans caused by the metacestode stages of *Echinococcus multilocularis* and *Echinococcus granulosus*, respectively. Differential diagnosis is fundamental for the choice of specific therapy strategies and prognosis.

We have analyzed 96 archived formalin-fixed, paraffin-embedded tissue samples, including five cutting needle biopsies and three fine needle aspirates from patients with AE or CE with the monoclonal antibody MAbG11 (Peter Deplazes, Zürich), specific for the antigen Em2G11 in the laminated layer of the metacestode of *E. multilocularis*.

We show that, in human tissue, staining with MAbG11 is highly specific for the laminated layer and the calcareous corpuscles of the *E. multilocularis* metacestode while no staining was observed in the metacestode stage of *E. granulosus*. Furthermore, the antibody marks small particles of *E. multilocularis* (Spems) of <1 µm in the necrotic tissue surrounding the main lesion. Spems are also detected in the liver sinusoids and lymphatic tissue outside the main lesion, most probably due to shedding from the laminated layer.

In six of 96 samples, the conventional histological diagnosis based on haematoxylin and eosin and PAS stainings alone had to be adjusted when revised by immunohistology with MAbG11. The specific staining was proved on bioptic material fixed for more than 60 years in formalin.

We conclude that the MAbG11-antibody is a highly specific tool in the diagnostic algorithm of AE also on long-time archived formalin-fixed or paraffin-embedded tissue. The staining modality with small particles outside the main lesion of *E. multilocularis* sheds new light on the interaction of the parasite with the human host and suggests a systemic effect.

**P2072 Occurrence of cysticerci serology and risk factors for neurocysticercosis in eastern Nepal**

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**Objective:** Neurocysticercosis (NCC), caused by infestation of the human central nervous system (CNS) with the tissue cyst of *Taenia solium*, is the most common parasitic disease of the human nervous system. Seizures are by far the most common clinical manifestation and occur in 70–90% of cases.

**Methods:** During March 2008–May 2009, the serological test was carried out in a series of 90 cases of seizure disorders aged more than

14 years presented to B. P. Koirala Institute of Health Sciences, Dharan, Nepal using an enzyme linked immunosorbent assay for circulating antigen (Ag-ELISA).

**Results:** *Taenia solium* antigen were detected in 13 (15%) of the patients. The diagnosis of neurocysticercosis was made in 35% cases (26 out of 75) based on clinical and neuroimaging findings. Neuroimaging was not done in 15 cases. Seropositivity was associated with neuroimaging studies consistent with NCC (OR = 13.2 95% CI 1.43–305.79, p = 0.014). It is significant for multiple ring enhancing lesions (p = 0.00935 for CT Head and 0.00274 for MRI Head). Living in KACHCHA house, family members >5 and age >60 years have higher odds ratio for positive serology of cysticercosis although there was no statistical significance.

**Conclusion:** On the basis of clinical and imaging findings, the serological test was found to have sensitivity of 41.5% and specificity of 98.4% with the positive predictive value of 92.3% and negative predictive value of 81.8%. So, in the settings where imaging facilities are not available a positive serological test could qualify to start the treatment for NCC. In conditions of negative serological test but if NCC is suspected on clinical grounds then neuroimaging is advocated and could be the reason for referral for neuroimaging.

**P2073 Has improved water and sanitation changed the prevalence of schistosomiasis and soil transmitted helminths amongst female primary school aged children in Ugu District of KwaZulu-Natal, southern Africa?**

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**Background:** Despite efforts to improve sanitation and provision of clean water; schistosomiasis and geohelminths are still prevalent in South Africa with an estimated 4.5 million people infected with schistosomiasis and a larger but unknown number infected with geohelminths.

**Aim:** To determine the prevalence and intensity of schistosomiasis and geohelminths and association with reported dysuria, "red urine" and water contact in an area that has had water and sanitation intervention.

**Methods:** This study was conducted in the southern coastal region of Ugu district amongst female primary school girls 10–12 years (n = 1057) randomly selected from 18 schools at altitude below 300 m. Geohelminths i.e. *Ascaris lumbricoides*, *Trichuris trichiura*, hookworm and *Taenia solium* and *Schistosomiasis mansoni* and *Schistosomiasis haematobium* were investigated. Permission was obtained from schools and written informed consent from parents and assent from school girls. One stool sample and three urines on consecutive days were obtained per subject and analysed using Kato-Katz and urine centrifugation methods respectively. Demographic information, girls' knowledge about schistosomiasis (isichenene in Zulu), water contact and experience of red urine were investigated.

**Results:** The prevalence of *S. haematobium* was 28.0% (mean intensity 28 eggs/10 mL; n = 954), *A. lumbricoides* 25.0% (mean intensity 24.5 epg; n = 853) and *T. trichiura* 26.0% (mean intensity of 25.91 epg; n = 853). No hookworm, *Taenia* and *S. mansoni* were found. Of the pupils, 13.5% had Ascariasis and Trichuris double-infection, 7.0% had urinary schistosomiasis and Trichuris double-infections, 6.4% had urinary schistosomiasis and Ascariasis, and 3.7% had triple infection. When questioned, 63.1% reported risk water contact, 40.1% knew about schistosomiasis, 21.4% had dysuria and 17.8% had had red urine in the past week. Significant associations were found between prevalence of urinary schistosomiasis and dysuria (p = 0.039), red urine (p < 0.005) and water contact (p < 0.005). A previous survey in Ugu District had reported prevalences in 1998 of 20.2%, 63.0% and 59.3%, for urinary schistosomiasis, ascariasis, and trichuriasis respectively, and in 2000, subsequent to treatment, 12.1%, 33.1% and 47.4%.

**Conclusion:** With a quarter of the primary school girls (10–12 years) infected with soil and water transmitted helminth (STH) infections there is an urgent need to improve access to treatment, reduce water contact and improve sanitation.

**P2074** A case with relapsed schistosomiasis

T. Yildirmak\*, F. Simsek, A. Kantürk, H. Gedik (Istanbul, TR)

**Objective:** A case with relapsed Schistosomiasis was presented in this study.

**Methods:** A 31-year-old male patient was admitted with complaints of bloody urine and groin pain. His complaints partially recovered but pain increased with bloody urine with clots after antibiotic therapy. He had history of trip to lake Malawi five months ago. Abnormal laboratory findings were AST:75 U/L, ALT:118 U/L, eosinophilia in peripheral blood examination, as well as erythrocyte with 57/HPF and leukocyte 11/HPF in urine analysis. Computed tomography revealed diffuse bladder wall thickening (10 mm) and non-hodgking lymphoma was diagnosed with bladder biopsy. Second biopsy was taken due to the fact that positron emission tomography – computed tomography (PET-CT) revealed no pathologic sign. Granulomatous inflammation and parasite eggs with eosinophil leukocytes were examined in the second biopsy by a different pathologist.

**Results:** Praziquantel was given one day as 60 mg/kg. The patient's symptoms disappeared after treatment, laboratory findings recovered. He had no complaint third month of follow-up. Again 21 months after initial treatment, patients was suffering from bloody urine. Hyperemic mucosa and edematous lesions were examined with cystoscopy. Bladder biopsy revealed a few live miracidium without dysplasia or neoplasm. Patient was hospitalised with relapsed schistosomiasis. He had no history of trip to abroad after first treatment. Abnormal laboratory findings were erythrocyte with 184/HPF and leukocyte 8/HPF in urine analysis, and eosinophilia in peripheral blood 10.96%. Praziquantel was given four days as 20 mg/kg/bid. During the visit 15 days after the completion of the treatment, the patient had reported that macroscopic hematuria was persistent until 3 days ago but disuria was still persisting. Laboratory findings were erythrocyte with 13/HPF and leukocyte 7/HPF in urine analysis, eosinophilia in peripheral blood 5.5%.

**Conclusion:** In Turkey, official and updated reports regarding Schistosomiasis could not be reached. A few cases had been reported from villages close to Syria in the previous years. Some sporadic cases, who were reported in recent years, had history of trip to abroad all. Three months after treatment, existence of live eggs in urine or tissue means to failure of treatment. Patients should be followed up at least six months due to probability of recurrence that could be in two years and chronic inflammation that leads to development of malignancy.

**P2075** Prevalence and risk factors for acquiring *Strongyloides stercoralis* infection among patients attending a tertiary hospital in Thailand

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**Objectives:** *Strongyloides stercoralis* causes persistent and fatal disseminated infections in immunocompromised hosts. In this study, we aimed to determine the prevalence and risk factors for acquiring chronic strongyloidiasis and the associated morbidity in adult patients attending Siriraj Hospital, a tertiary hospital in Thailand.

**Methods:** A case-control study was carried out at Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand between July 2008 and April 2010. Case and control were identified from 6029 patients whom results of fecal examination were available. Case was patient who had *S. stercoralis* larva detected from fecal examination. Control was randomly selected from patients who did not have *S. stercoralis* larvae detected from at least three consecutive fecal examinations. The proportion of control: case was approximately 2:1. Demographic and clinical data for the day of diagnosis and retrospectively up to 15 days preceding the date of fecal examination were reviewed from their medical records.

**Results:** Overall 149 (2.47%) patients had *S. stercoralis* larva identified from the fecal examination. There were 105 males (70.5%), with the mean (SD) age of 53.9 (17.2) years old. Four hundred and thirty-nine controls were selected. Male (OR = 3.22, 95% CI 2.14–

4.85), occupational associated with high exposure of soil and water (OR 3.09, 95% CI [1.73–5.49]), HIV infection (OR = 4.01, 95% CI [1.87–8.55]), and eosinophilia (OR = 3.96, 95% CI [2.48–6.36]) were found to be risk factors associated with chronic strongyloidiasis in this setting. Corticosteroid or other immunosuppressive treatment, other concomitant illnesses such as diabetes mellitus, SLE were not associated with increased risk of chronic strongyloidiasis.

**Conclusion:** In this setting, strongyloidiasis was seen more often in patients with eosinophilia and with HIV infection. Prevention of fatal complication caused by *S. stercoralis* by regular fecal examination, for early detection and treatment of latent strongyloidiasis, is recommended in these high risk patients.

**P2076** Myositis due to the novel species of *Microsporidium* and review of the literature

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**Objectives:** At least 15 species of microsporidia have been implicated in human infections. It has increasingly been documented in immunocompromised patients other than those with HIV infection and even in immunocompetent individuals. To our knowledge, this is the first report of microsporidial myositis caused by the suspected new species of microsporidium in an otherwise healthy individual. In addition, 11 patients with microsporidial myositis reported in the literature is also reviewed.

**Methods and results:** We describe a case of microsporidial myositis in a healthy Thai male living in Lopburi, central Thailand. He presented with a 15-month course of fever as well as progressive and generalized muscle weakness. Bone marrow biopsy specimens revealed increased plasma cells and histiocytes and focal aggregation of microsporidial spores with characteristic belt-like stripe. The small subunit ribosomal RNA sequence of this microsporidium is novel, and has a close phylogenetic relationship with the Endoreticulatus, a genus of lepidopteran microsporidia. To our knowledge, 11 patients with microsporidial myositis have been reported. Trachipleistophora species is the most common causative agent, followed by Brachiola, Pleistophora, and Microsporidium.

**Conclusion:** The true prevalence of microsporidiosis in humans may be underestimated due to unawareness and diagnosis difficulties. Only 11 patients with microsporidial myositis have been reported. The infection routes are uncertain, but the species that can infect humans have been identified in water and several kinds of animals. Due to closely related phylogenetic relationship with lepidopteran microsporidia, we postulate that these non-blood sucking insects could be its natural hosts.

**P2077** Performance of an enzyme immunoassay for the diagnosis of strongyloidiasis

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**Objectives:** Strongyloidiasis is caused by the parasite *Strongyloides stercoralis*. This is an intestinal nematode with worldwide distribution, but is especially common in tropical and subtropical areas. The disease usually manifests as intestinal symptoms (diarrhea). *S. stercoralis* infected patients are particularly at risk for severe complications if they are also immunocompromised. Observation of larvae in the stool of infected patients is the diagnostic method most frequently used. Our study aims to evaluate the effectiveness of a commercial EIA in the diagnosis of Strongyloides.

**Methods:** From July to October 2011, serum and stool samples from patients with eosinophilia remitted from the Tropical Disease Unit of our hospital were studied. Serum samples were tested by a commercial enzyme immunoassay (EIA) (DRG® Strongyloides IgG) and stool samples were tested by microscopic examination of stool issued on three consecutive days and by blood-agar culture.

**Results:** A total of 175 serum samples and 175 stool samples corresponding to 175 patients were included. Sixty-six percent (116) were women and 33% (59) were men. The mean age of the study population was 35.9 (1–81) years and 10.8% (19) of patients were children.

Twenty-six (14.9%) serum samples were positive for *Strongyloides*. Five (2.9%) stool samples were positive by microscopic examination and one (0.6%) by blood-agar culture. The geographic distribution of the five patients confirmed by microscopic examination was: two from Guinea Ecuatorial (one corresponding to an eight year old child), one R. Dominicana, one Perú and one Bolivia. The prevalence of *Strongyloides*' infection was higher in males (60%). Sensitivity of EIA using microscopic examination as the gold standard was 100%.

**Conclusion:** Due to the poor and intermittent elimination of *Strongyloides* larvae in stool, fecal microbiological culture has low sensitivity. Microscopic observation of three stool samples increases the sensitivity considerably. Serological diagnosis has high sensitivity but low specificity. It would be useful in the diagnosis of *S. stercoralis* to perform serological screening, subsequently confirmed with microscopic observation of larvae in stool samples. Additional studies are needed with larger number of samples, in order to draw more conclusions about the performance of this EIA in the diagnosis of strongyloidiasis.

#### **P2078** Utility of a screening program of strongyloidiasis in immigrant population

A. Rodriguez-Guardado\*, M. Rodriguez, F. Perez, V. Carcaba, J. Carton (Oviedo, ES)

**Objective:** Strongyloidiasis is an infection caused by the nematode *Strongyloides stercoralis*. Populations with high risk include immigrants from tropical countries. The goals of this study were: (i) to determine the frequency of imported strongyloidiasis; (ii) to describe epidemiological, laboratorial and clinical features of imported strongyloidiasis.

**Methods:** During 2008–2011 we conducted a prospective screening program of chronic strongyloidiasis in all immigrants patients attending in Tropical Medicine Unit. Combined examination of three concentrated stool samples, culture in blood agar and Enzyme-linked immunosorbent assay for serum anti-*S. stercoralis* antibodies was used as screening. We considered that infection exists if the microscopic visualization of larvae in stool sample and/or the ELISA was positive. In positive patients was discarded the presence of other nematodes. We realized an epidemiological questionnaire that included: risk factors to have the disease and presence of symptoms. Eosinophilia in blood test was studied. All positive patients were treated with ivermectin. Quantitative variables were analyzed with the Student t test or the Mann–Whitney test when appropriate. Qualitative variables were analyzed with the chi square test or Fischer's exact test when necessary. All p-values of 0.05 or less were considered statistically significant.

**Results:** We screened 570 patients. The most frequent countries of origin were: Equatorial Guinea (26%), Senegal (20%), Ecuador (13%), and Bolivia (7%). Sixty-five patients (16%) had positive serological test and in four patients the microscopic visualization was positive. The countries of origin were: Equatorial Guinea (34%), Ecuador (20%), Senegal (11%), Nigeria (10%). Strongyloidiasis was more frequent in subsaharian patients (51 vs. 14,  $p = 0.052$ ). Mean time in Spain was 936 days (31–2987). The most frequent symptoms are abdominal pain (60%), eosinophilia [28% 14 patients (21.5%)] were asymptomatics. Two patients have HIV infection and one patient HTLV-I infection. Thirty patients have eosinophilia in blood (mean 2552 cells/mm<sup>3</sup>). The eosinophilia in blood were significantly higher in subsaharian immigrants (2.262 vs. 1.462,  $p = 0.014$ ).

**Conclusions:** The presence of infection for *S. stercoralis* is frequent although without symptoms. To prevent potentially fatal hyperinfection syndrome, it is necessary realized screening with several stool examinations and serologic testing in immigrant population, and in infected instituted the treatment.

#### **P2079** Efficacy and safety of combined therapy with albendazol and ivermectin in chronic strongyloidiasis: observational study

A. Rodriguez-Guardado\*, M. Rodriguez, F. Pérez, M. Martinez, N. Morán, V. Carcaba, J. Carton (Oviedo, ES)

**Objective:** Strongyloidiasis remains an important health problem due to autoinfection, which may result in hyperinfection. Ivermectin and albendazole are effective. However, the most effective dosing regimen are to be determined. A observational study carried out in Spain describing the efficacy of combined therapy with ivermectin and albendazole for treatment of strongyloidiasis.

**Methods:** A prospectively observational study was conducted in which all patients with chronic strongyloidiasis attending in Tropical Medicine Unit of Hospital Central de Asturias were treated with a combined therapy: albendazole (400 mg/12 hour/7 days) and ivermectin 200 µg/kg/day 2 days. Combination of repeated examination of three concentrated stool samples, culture in blood agar and enzyme-linked immunosorbent assay for serum anti-*S. stercoralis* antibodies was used as diagnostic assay. Infection exists if the microscopic visualization of larvae in stool sample and/or the Elisa was positive. The presence of other nematodes or filarias were discarded. Patients were followed-up with 2 weeks after initiation of treatment, then 1, 3, 6, 9 months and 1 year after treatment with serological and parasitological screening in each follow-up visit. The disease was cured if two consecutive test were negative. The primary endpoint was clearance of strongyloides larvae from feces or negativization of serological test after treatment and at one year follow-up.

**Results:** Eighty-one patients were included in the analysis. The most frequent countries of origin were: Equatorial Guinea (32%) and Ecuador (18.5%). All patients showed positive serological test and three patients had stool test positive. 80% of patients were immigrants and the rest long-time traveller. The median range of follow up were 38 (4–74) weeks. The most frequent symptoms are abdominal pain (60%) and eosinophilia (28%). Eighteen are asymptomatic. Thirteen patients have eosinophilia in blood (mean 2552 cells/mm<sup>3</sup>). All patients were treated with ivermectin plus albendazole. In all patients except two the diagnostic test became negative one month before the treatment and in the follow up. Cure rate were 97.5%. Eosinophilia became negative one month after the treatment. Two patients leave the follow-up and the rest are yet to follow. No patients recidived. No severe side effects were recorded.

**Conclusions:** Combined therapy with ivermectin and albendazole provides a safe and a highly effective treatment for *S. stercoralis*. No severe side effects were recorded.

#### **P2080** High prevalence of dientamoebiasis in patients attending a tertiary-care hospital in northern Italy: a 6-year study

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**Objectives:** *Dientamoeba fragilis* still remains neglected as a cause of intestinal complaints probably due to the misconceptions that it is uncommon and non-pathogenic.

The aim of this study, partly retrospective and partly prospective, was to determine the proportion of *D. fragilis* infection among the infections by other intestinal parasites, in order to obtain a picture of the epidemiological situation in a population of patients suspected of having an intestinal parasitosis.

**Methods:** Conventional diagnosis of intestinal parasitoses (microscopic examination of fresh/concentrated faeces and cultivation in Robinson's medium) was performed on 1143 faecal samples belonging to 651 patients in a period of five years and ten months. The DNA extracted from the same samples was used in a real-time PCR assay targeting the 5.8S rRNA gene of *D. fragilis*.

**Results:** Real-time PCR revealed the presence of *D. fragilis* in 255 samples of 162 patients. In 61 of these cases the dientamoebiasis was diagnosed by PCR alone having conventional methods detected *D.*



*fragilis* in 101 patients. *D. fragilis* infection was detected in 24.9% of the patients, second in frequency among the diagnosed intestinal parasitoses (among protozoa infections, before *Giardia intestinalis* and after *Blastocystis hominis*).

**Conclusion:** Though a number of studies from many parts of the world have reported patients infected by *D. fragilis* whose gastrointestinal symptoms solved only after therapeutic intervention, few laboratories routinely test for *D. fragilis* and few prevalence data, probably underestimated, are available, also due to the difficulties in detecting the parasite by conventional parasitological techniques.

In this study the proportion of *D. fragilis* infected patients evidenced a remarkably high prevalence of dientamoebiasis in the analysed population (both Italian and foreigners). All the patients with *D. fragilis* infection presented with gastrointestinal symptoms and most of them were co-infected with either non-pathogenic protozoa (like *Entamoeba coli* and *Entamoeba dispar*) or protozoa whose pathogenicity is controversial (*B. hominis*), or harboured no protozoa other than *D. fragilis* or no other enteropathogenic agents (bacteria, viruses). A targeted therapy administered to most of the patients with dientamoebiasis solved gastrointestinal complaints, strengthening the accumulating evidence for the pathogenicity of *D. fragilis* being it the possible source of symptoms in such patients.

**P2081 Faecal parasitology: concentration methodology really does matter!**

M. Manser\*, A. Saez, P. Chiodini (London, UK)

**Objectives:** In response to concerns by UK NEQAS Parasitology regarding participants failing to report parasites in UK NEQAS faecal parasitology specimens and also noting that they were recovering lower numbers of ova and cysts than those seen by UK NEQAS in the pre-distribution examination of the specimens, a questionnaire was sent to all participants to establish the routine method they used to examine for parasite stages in faecal samples.

**Methods:** Two hundred laboratories responded to the questionnaire. All laboratories used a concentration method based on the Modified Ridley-Allen (1) technique but with variations in the method namely centrifugal force, centrifugal time, pore size of the sieve and use of a solvent. Therefore faecal concentrations using the Parasep® faecal concentration method were done in UK NEQAS on specimens containing a range of ova and cysts incorporating these variations.

**Results:** Recovery of all parasites was affected by the centrifugal force, the centrifugal time and whether or not a solvent was used (2). The number of parasite stages seen increased with centrifugal force and centrifugal time. However, higher centrifugal force and time resulted in an increase in the amount of debris making the deposit more difficult to examine.

**Conclusion:** All laboratories should use the recommended centrifugal force (1000 g) and time (3 minutes) when using this technique to optimise diagnosis by microscopy.

**References:** (1) Allen A. V. H. and Ridley D. S. (1970): Further observations on the formol-ether concentration technique for faecal parasites. J. Clin. Pathol. 23, 545 - 546. (2) Comparison between the Midi Parasep and Midi Parasep Solvent Free (SF) faecal parasite concentrators; A C Saez M M Manser, P L Chiodini.; J Clin Pathol online June 16, 2011.

**P2082 A multiplex real-time PCR for intestinal protozoa in a paediatric population**

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**Objectives:** To describe clinical and microbiological characteristics of intestinal protozoal infections in a prospective study using a multiplex RT-PCR in a paediatric population.

**Methods:** During a 12 month period, children (0–17 years) presenting to their general practitioner or paediatrician with gastro-intestinal

symptoms, for whom the physician requested a PCR intestinal protozoa on faeces, were eligible for inclusion. A multiplex RT-PCR was performed for *Blastocystis hominis*, *Dientamoeba fragilis*, *Giardia lamblia*, *Cryptosporidium* sp. and *Entamoeba* sp. Questionnaires (on characteristics of gastro-intestinal symptoms and the degree of abdominal pain, scored on a paediatric visual analogue scale (VAS)) were taken at time of inclusion (T = 0) and after 6 weeks (T = 6). Complaints at inclusion and after 6 weeks, treatment and microbiological outcome were analysed, using SPSS package.

**Results:** One hundred and forty-three children (mean age 8 year, 53% girls) were included. The most common symptom was abdominal pain (78%), followed by nausea (29%) and change in stool the routine (29%). Ninety-eight children (69%) had a positive PCR: *D. fragilis* was detected most frequently (Table). In 42 children (29%) >1 protozoa was detected, mostly *D. fragilis* and *B. hominis* (n = 37). In 85 children (87%) with a positive PCR, treatment was started (clioquinol, 65%, metronidazol, 29%, or paromomycin, 2% (in 4% drug of choice was unknown)). After treatment a significant reduction in abdominal pain (mean VAS score from 5.6 to 2.2, p < 0.001) was measured. For 52 children (36%) a second PCR at a mean of 42 days after treatment was performed: this was positive in 27 cases (52%). PCR-outcome did not seem to be influenced by choice of drug.

**Table**

	PCR (T=0) (N=143)	PCR (T=6) (N=52)
<i>Blastocystis hominis</i>	42 (29%)	14 (27%)
<i>Dientamoeba fragilis</i>	88 (62%)	18 (35%)
<i>Giardia lamblia</i>	9 (6%)	0
<i>Entamoeba</i> species	0	0
<i>Cryptosporidium</i> species	2 (1%)	0

**Conclusion:** Protozoal infections in children with gastro-intestinal complaints are frequently detected by PCR. Double infections are common, mostly by *D. fragilis* and *B. hominis*. After treatment abdominal pain was significantly reduced, however PCR remained positive in more than half of the treated cases.

**P2083 A novel ELISA-based diagnostic test may replace the traditional microscopy in detection of *Blastocystis* spp. in human stool specimens**

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**Objectives:** *Blastocystis* is an enteric protozoan parasite highly prevalent in humans and animals. It is worldwide associated with non-specific symptoms, i.e. diarrhea, abdominal pain, anal itching, excess gas, and irritable bowel disease, and therefore under-diagnosed. Detection of *Blastocystis* is routinely performed by microscopy, culture, and formol-ether concentration technique (FECT). Yet, these methods are laborious, require special skilled personnel, and time consuming. Since *Blastocystis* has several morphological forms (vacuolar, cyst, amoeboid, granular, multivacuolar, and avacuolar), microscopy is difficult. FECT destroys some of the forms during stool processing, therefore is unreliable. Culture requires 2–3 days for diagnosis and may allow preferential growth of specific strains while eliminating others. ELISA-based test for detection of *Blastocystis* antigens in fresh and preserved stool samples was recently launched and evaluated (CoproELISA *Blastocystis*, Savyon, Israel). The aim of this work is to demonstrate the usefulness of the newly developed test, as a proper alternative to currently used methods, especially the microscopy.

**Methods:** A mixture of the most abundant human infecting strains was used to prepare polyclonal anti-*Blastocystis* antibodies, which compose the ELISA. A cohort of 251 fresh/frozen samples was tested by the newly developed ELISA, microscopy examination of Lugol's iodine staining, culture and staining with fluorescent (FITC) anti-*Blastocystis* antibodies (Antibodies Inc, USA). The culture and fluorescent antibodies results were considered as consensus for reference purposes.

**Results:** Considering the consensus results as reference, the ELISA performance demonstrates 82% sensitivity, 80% specificity, 81% accuracy, 82% PPV and 80% NPV. The sensitivity of Lugol staining microscopy was 24%. The ELISA detects the most prevalent subtypes in humans (1, 2, 3, 4, 5, and 7), and most of the known morphological forms.

**Conclusions:** This work presents a unique ELISA that provides superior performance compared to microscopy, the currently most widely used method. The ELISA enables high throughput screening, adaptation to automatic procedures and is overall cost-effective. In addition it is expeditious in providing reliable results and efficient requiring no special skilled personnel. Taken these considerations, the ELISA is expected to be the method of choice for diagnosis of *Blastocystis* in the common laboratory.

#### **P2084** Role of *Dientamoeba fragilis* and *Blastocystis* spp. in irritable bowel syndrome

I. Mumcuoglu\*, C. Gungor, T. Purnak, N. Aksu (Ankara, TR)

**Objectives:** This study was designed to investigate prevalence of *Dientamoeba fragilis* and *Blastocystis* spp. in irritable bowel syndrome (IBS) patients and evaluate whether there was a possible link between IBS and parasitic infections.

**Methods:** Stool specimens collected from 55 IBS patients, 80 patients with gastroenteritis (Control group 1) and 50 healthy volunteers (Control group 2) were included to the study. Fresh stool specimens were cultured in Robinson medium for *Dientamoeba fragilis* and in Ringer's solution containing 10% horse serum and 0.05% asparagine for *Blastocystis* spp. at 37°C. All stool samples were also evaluated with native-lugol, trichrome and Kinyoun's acid fast-staining methods.

**Results:** *Blastocystis* spp. was found 29.1% of IBS group by direct examination and 32.7% by trichrome staining and culture method. In control group 1, *Blastocystis* spp. was found 7.5% of patients by direct microscopy, 10.0% by trichrome staining and 18.8% by culture method. *Blastocystis* spp. were identified in 2.0% of patients with direct microscopy and trichrome staining and 6% by culture method in control group 2. There was no significant difference in prevalence of *Blastocystis* spp. between IBS patients and control group 1 ( $p > 0.05$ ). Statistically significant difference was found between IBS patients and the control group 2 ( $p < 0.05$ ). Patients with IBS were significantly have five or more *Blastocystis* spp. per field than control groups. Direct microscopy, staining method and stool culture were found to have same reliability in IBS group ( $p > 0.05$ ), whereas stool culture method was found more sensitive than microscopy ( $p < 0.05$ ) in control groups. Eighteen patients in IBS group who had *Blastocystis* spp. infection treated with metranidazole and parasite eradication was ensured in 12 (67%) patients, remaining six patients (33%) were cured by trimethoprim-sulfamethoxazole. After eradication all symptoms were cured in four patients (22.2%), there were only constipation problem left in eleven patients (61.1%) and there were no changes in clinical findings in 3 (16.7%) patients.

**Conclusion:** *D. fragilis* was not found in any of the patients. The reason could be there is already low infection rate of *D. fragilis* in our country. However, significantly having five or more *Blastocystis* spp. per field (X40) in IBS patient and reversion of IBS symptoms after the treatment in most of the patients was thought there could be possible link between IBS and *Blastocystis* spp. infections.

#### **P2085** Molecular analysis of *Giardia* assemblages and clinical outcome in the Scottish population

E. Ritchie, K.G. Pollock, B. Jones, C.L. Alexander\* (Glasgow, UK)

*Giardia lamblia*, an intestinal parasite, exists as assemblages where A and B infect humans predominantly causing gastrointestinal symptoms. Transmission can be via contaminated food and/or water. There have been clusters of *Giardia* cases within Scotland in the past two years of unknown origin. This study characterises *Giardia* assemblages and determines their influence on clinical outcome to gain a fuller

understanding of the pathogenicity of *Giardia* and to identify public health issues.

Scottish stool samples which are either microscopy positive for *Giardia* cysts or microscopy negative but deemed to be at "high risk" of giardiasis are included in this on-going study (October 2011–September 2012). DNA is extracted from anonymised, consented samples using the QIAamp DNA Stool Mini Kit. DNA is subjected to a semi-nested PCR assay targeting the beta-Giardin gene. PCR positive samples are sequenced (Applied Biosystems3500XL) to identify assemblage and sub-assemblages and this data will be supported by PCR-RFLP analysis. To date, patient consent from 19 individuals has been received. The median age is 38 with an 11:8 male to female ratio. Previous medical histories include Irritable Bowel Syndrome or Inflammatory Bowel Disease ( $n = 3$ ). The most common clinical symptom is chronic diarrhoea ( $n = 17$ ) followed by abdominal pain ( $n = 7$ ), tiredness ( $n = 6$ ), intermittent diarrhoea ( $n = 6$ ), weight loss ( $n = 5$ ) and nausea ( $n = 4$ ). Foreign travel is indicated in 17 cases preceding the onset of symptoms (Europe  $n = 6$ ; Africa  $n = 5$ ; Asia  $n = 2$ ). One traveller visited three continents (Asia, Europe and Africa). Recreational water contact is implicated in five cases. Of the 19 cases examined, nine were microscopy positive for *Giardia* cysts which correlated with PCR positivity. In addition, three samples were also shown to be positive by PCR but not by microscopy. Assemblage analysis has been performed on seven of PCR positive samples; four Assemblage A, two Assemblage B and one mixed (i.e. A and B).

Ninety percent of the submitted cases have a pre-travel history to destinations out-with the UK where diarrhoea is the predominant symptom. This supports the requirement for good pre-travel advice to ensure travellers are aware of the known risk factors. PCR appears more sensitive at detecting *Giardia* from stools and there are a variety of assemblages within the Scottish population. Correlations between clinical symptoms and specific assemblages will be assessed over the duration of this study with the increase in sample numbers.

#### **P2086** Point-of-care tools for the characterisation of *Cryptosporidium* isolates from children in Tanzania

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**Objectives:** *Cryptosporidium* spp. are a major cause of diarrhea in depressed areas mainly affecting children and HIV-infected individuals. The infection is self-limiting in immunocompetent hosts, but can be severe in immunocompromised and malnourished persons. Treatment is less than optimal and solving drugs are not available.

**Methods:** From September 2011 to October 2011, stool samples were collected from 112 HIV-positive children residing at the Mission of Children's Hospital and Research Institute "Bambino Gesù" (OBG) of Rome, "Villaggio della Speranza", Dodoma, Tanzania. In situ each stool sample, after washing and filtering, was concentrated by centrifugation and then submitted to microscopic examination. Furthermore, after a further centrifugation an aliquot of the resulting pellet was spotted onto a FTA ELUTE Micro Card. At OBG, DNA from dried fecal spots (DFS) was extracted, after punching two 5-mm discs, with automatic extraction on a Biorobot EZ1 DSP (Qiagen). Also an aliquot of concentrated wet feces was submitted to DNA Manual Extraction (DME) by QIAamp DNA stool mini kit. A GP60 gene fragment was amplified, sequenced and probed against BLASTN algorithm.

**Results:** At microscopic examination 85/112 (76%) samples resulted negative, 21/112 (19%) resulted positive and 6/112 resulted uncertainly positive (5%). GP60 amplification yielded single products of expected size for 18/35 DME samples, and for 4/4 DFS samples, analyzed to date. Seven amplicones were successfully subgenotyped revealed subgenotypes IIAA15G2R1 and IIAA16G2R1.

**Conclusion:** These preliminary molecular DME tools showed that 51% of the entire sample set was positive for *Cryptosporidium* spp. consistently with the four DFS samples, while the microscopic analysis showed only a 21% of positivity. Therefore, the molecular method herein described might represent a point of care in the diagnosis of

HIV-related cryptosporidiosis cases. Moreover, this approach represents a useful survey tool to subgenotype lineages and to elucidate genetic richness of *Cryptosporidium* pediatric isolates especially in these depressed areas.

**P2087** Determining species and sub-genotypes of *Cryptosporidium* infecting St. Kilda Soay sheep

L. Connelly\*, B. Jones, C.L Alexander (Glasgow, UK)

**Objectives:** The objectives of this study were to assess the genetic diversity of *Cryptosporidium* species isolated from a population of wild sheep in St. Kilda which was the selected for sampling due to minimum exposure by humans. The species/genotypes/sub-genotypes of *Cryptosporidium* infecting feral Soay sheep (*Ovis aries*) on Hirta in St Kilda was examined over a 2 year period of varying host population density.

**Methods:** Two sampling procedures were performed to collect stools during a one month period; (i) stools collected on capture of sheep or on observing defecation, (ii) post mortem samples. In the first instance, a 1 mL emulsion of stool in water was prepared and a small aliquot added to a slide. Slides were stained using auramine phenol and examined for the presence of oocysts using a fluorescence microscope. For all *Cryptosporidium* microscopy positive samples, immunomagnetic separation (IMS) was required to concentrate oocysts from stool samples. Once concentrated, the *Cryptosporidium* DNA was extracted from the oocysts by repeated rounds of freeze-thawing the IMS bead-oocyst complexes. Species/genotypes were identified using two nested-PCR assays which amplified two loci of the *Cryptosporidium* 18S rRNA. Amplicons were subjected to RFLP analysis and/or sequencing. All *C. parvum* positive isolates were subtyped using two nested-PCR assays that amplify the *Cryptosporidium* GP60 gene.

**Results:** Of the 276 samples collected, 246 were *Cryptosporidium* oocyst positive using microscopy and after processing for molecular analysis, 28.9% were *C. parvum*, 11.1% *C. ubiquitum* (*Cryptosporidium* cervine genotype), 11.4% *C. bovis*, 2% *C. ryanae* (mixture with *C. bovis*) and possibly *C. andersoni* (4.2%). This is the first report demonstrating the presence of *C. ryanae* in sheep. In addition, this study highlights the genetic variation in the Hirta *C. parvum* population as GP60 heterogeneity was observed. Sixteen of the 52 *C. parvum* positive isolates were GP60 positive however, only 12 could be sub-genotyped from single PCR products. The 12 *C. parvum* isolates were sub-genotyped into family IIa (n = 1) and two new *C. parvum* (II) sub-genotypes (n = 11).

**Conclusion:** The study demonstrates a high prevalence and diversity of *Cryptosporidium* species/genotypes present in the isolated Soay sheep population on the Island of St. Kilda.

**P2088** Temporal, seasonal and geographical distribution of *Cryptosporidium* in Scottish waters

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**Objectives:** The objective of this study was to employ molecular methods to gain an insight into the diversity of *Cryptosporidium* species in Scottish raw water (RW) and final water (FW). It would also provide further information on the geographical and seasonal occurrence of this parasite to assist in the management of potential outbreaks.

**Methods:** One thousand forty-two *Cryptosporidium* microscopy positive samples were analysed in Scottish RW (43.8%) and FW (56.2%) over a 12 month period from nine regions in Scotland; North West (NW), North East (NE), North Central (NC), South West (SW), South East (SE), Central West (CW), South Central (SC), Central East (CE) and Central Central (CC). DNA was extracted from oocysts in slide preparations of water concentrates and amplified by PCR using two 18S rRNA gene loci followed by RFLP analysis and/or sequencing.

**Results:** (i) Human-associated *Cryptosporidium* species detected were; *C. parvum* (11.2% RW, 4.3% FW), *C. hominis* (1.5% RW, 1.3% FW) and *C. ubiquitum* (7.2% RW, 12.6% FW). (ii) Seasonal distribution; In

RW, *C. parvum* was present throughout the year (except January and April) (frequencies ranging from 1.9% to 65.6%). *C. hominis* was present during August-November at low frequencies (2.4–7.1%). *C. ubiquitum* was identified every month except for December, (5.3–31.8%). In FW *C. parvum* was present except in March and July (frequencies of 6.2–30%). *C. hominis* occurred during Aug-Oct, (1.4–17%). *C. ubiquitum* was found all year (frequencies 10.5–57.1%). (iii) Geographical distribution; *C. parvum* was isolated in the NW and NE regions of Scotland (21.4% and 15.3% respectively) and in the CE region (17.1%) and CW region (20.6%). *C. hominis* was found less frequently in NW (6.5%), NE (6.1%), SC (2.1%) and CE (4.9%) regions. *C. ubiquitum* was detected in all regions but was more frequently found in the NW (45.9%) compared to NE (7%) region.

**Conclusions:** Three *Cryptosporidium* species associated with human disease were detected in both Scottish raw and final waters, namely *C. parvum*, *C. hominis* and *C. ubiquitum*.

*C. parvum* frequencies were significantly higher in the summer in RW yet lower during a similar period in FW. *C. hominis* was only present in the summer and autumn whereas *C. ubiquitum* was detected throughout the year in RW and peaked in FW during autumn. *C. parvum* and *C. hominis* were localised to specific regions of Scotland whereas *C. ubiquitum* was found in all regions examined.

**P2089** Genetic diversity of *Cryptosporidium* isolates from humans in North India

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**Background:** Many species/genotypes of *Cryptosporidium* infect a wide range of hosts including humans, domestic and wild animals. These are one of the most important pathogens causing diarrhoea in immunocompromised hosts. Morphological differentiation of species is not possible, thus molecular tools have been used for the same.

**Objectives:** To differentiate species or genotypes of *Cryptosporidium* isolates from human faecal samples by SSU rRNA based PCR-RFLP and to subtype *C. hominis* and *C. parvum* by sequence analysis of GP60 region.

**Methods:** *Cryptosporidium* oocysts were detected in stool samples by bright field microscopy (100x) of Ziehl-Neelsen stained faecal smears. DNA was extracted with Qiagen Kit and all the samples were genotyped by SSU rRNA based PCR-RFLP tool which differentiates all known *Cryptosporidium* species/genotypes causing infection in humans. With this technique ~850 bp fragment was amplified by nested PCR and species or genotypes were differentiated by banding patterns in restriction analysis of secondary PCR products with enzymes SspI and VspI. *C. hominis* and *C. parvum* isolates were sub-typed by sequence analysis of nested PCR amplified GP60 gene. For subtype families, sequences were searched for similarity by BLAST analysis and aligned with reference sequences retrieved from GeneBank by multiple sequence alignment (ClustalW, BioEdit7.0.5.3). Subtypes within each subtype family were named by manual counting of tandem repeats in the poly-serine tract of GP60 gene.

Table GP60 sequence based analysis of *Cryptosporidium* isolates.

Species	Subtype family	Total isolates	Subtypes
<i>C. hominis</i>	Ia	7	IaA18R3(1), IaA19R3(2), IaA21R3(2)*, IaA26R3(1), IaA27R3(1)
	Id	3	IdA15G1(2), IdA16G1(1)
	Ic	10	IcA11G3T2(1), IcA11G3T3(9)*#
	If	5	IfA13G1
<i>C. parvum</i>	Ile	3	IleA5G3*
	Ild	3	IldA14G1(1), IldA15G1(2)
	Ile	1	IleA7G1(1)
<i>C. meleagridis</i> #		3	

\* Mixed infection (1 sample)

# Mixed infection (3 samples)

Numbers in brackets indicate number of isolates

**Results:** Thirty faecal samples were found to be microscopically positive for *Cryptosporidium* oocysts. RFLP analysis revealed 24 isolates as *C. hominis* and six as *C. parvum*. GP60 based sequencing of *C. hominis* and *C. parvum* divided them into seven subtype families and 14 subtypes. Sequencing could detect four cases of mixed infections with *C. hominis* and *C. parvum*/*C. meleagridis* (Table).

**Conclusion:** SSU based PCR-RFLP tool discriminated *Cryptosporidium* into two species and *C. hominis* was more prevalent in this area. GP60 sequence based subtyping has revealed the coexistence of *C. hominis* with *C. meleagridis*/*C. parvum* and also *C. hominis* subtypes. These species were missed by SSU rRNA based RFLP analysis which detected only *C. hominis* in concurrent infections.

## Viral infections in the compromised host

### P2090 CMV active infection monitoring by real-time PCR in allogeneic stem cell transplant recipients

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The objective of this study was to evaluate real-time PCR (rt-PCR) Affigene® CMV trender in the monitoring CMV active infection and treatment viral response in allogeneic stem cell transplant recipients (allo-SCT) patients.

**Methods:** In a prospective study, 638 plasma samples correspond to 34 allo-SCT performed between May 2010 and September 2011 were studied, with a median follow-up of 128 days. All the patients received antiviral prophylaxis High-dose Acyclovir (ACV-HD) and were monitored post-SCT with antigenemia pp65 CINApool® Argene (AG) and real time PCR Affigene® CMV trender after automatic DNA extraction with NucliSENS® easyMAG®, BioMérieux (rtPCR). A positive sample was defined by AG > or = 2/4 × 10<sup>5</sup> PMNs and/or rtPCR > or = 57 copies/mL. An episode was defined as the period between the first positive sample by AG and/or rt-PCR, until the first negative sample by both techniques.

**Results:** Thirty episodes of active CMV infection were detected at median 34 days post SCT in 19 patients (56%). Plasma samples were positive in 8.5% and 22.6% by AG and rt-PCR, respectively (S = 39.5% E = 98.5% VPP = 90.7% VPN = 81.7%). A total of 174 plasma samples (27.3%) were not evaluable for AG due to the lack of white blood cells in these samples, 10.9% showed a positive result for rt-PCR. Ninety-four plasma samples (14.7%) were only positive by rt-PCR, and five samples were positive only by AG (0.78%). Twenty episodes (66%) were detected by both techniques and ten episodes (33%) only by rt-PCR assay. For episode detection the first positive technique was AG in 10 episodes (33%) and rt-PCR in 27 episodes (90%); the rt-PCR was positive 6 days before AG in 50% of episodes compared with 15% for AG (median = 6 days). Seventeen out of 19 patients received one or more course of preemptive therapy upon positive AG and/or rt-PCR results and none developed CMV end-organ disease.

Twenty-five CMV episodes were treated. In the monitoring of the response to antiviral treatment AG became negative earlier than rt-PCR: median days after initiation of treatment was 10 days for AG vs. 17 days for rt-PCR. Only in two cases (11.8%) AG and rt-PCR became negative at the same time. In the rest of episodes (88.2%) while AG was negative, rt-PCR continued positive (median 7 days).

**Conclusions:** Rt-PCR Affigene CMV trender seem to be a more sensitive, rapid, and accurate diagnostic tool of CMV reactivation infection in SCT recipients, allowing an earlier preemptive therapy and a better monitoring of the treatment viral response.

### P2091 Incidence and severity of cytomegalovirus infection and disease in high-risk solid organ transplant recipients

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**Objectives:** To describe the incidence and severity of CMV disease in high risk solid organ transplant (SOT) recipients.

**Methods:** We performed an analysis of all SOT recipients with CMV donor/recipient mismatch (D+/R-) from July 2003 until December 2009. Prophylaxis, development of CMV disease and outcomes were analyzed.

**Results:** We performed 1536 solid organ transplants. CMV D+/R- was present in 11%. Median time of follow-up was 1475 days [IQR 848–2232]. Drugs used for prophylaxis were: valganciclovir 83 (49.4%), ganciclovir 12 (7.1%), ganciclovir initially and switched to valganciclovir 34 (20.2%), no prophylaxis 13 (7.7%) and in 26 cases (15.5%) prophylaxis was unknown. Median time of prophylaxis was 90 days. Seventy-three high-risk patients (43.5%) presented CMV infection, while CMV infection incidence in the global cohort was 10.4% (p < 0.001). By type of transplant, all of our heart D+/R- developed CMV infection. We didn't find differences in CMV infection incidence according to the type of immunosuppression or induction therapy. Twenty-nine patients (40%) had asymptomatic CMV replication, 32 (44%) CMV viral syndrome and 12 (16%) CMV invasive disease (four hepatitis, three duodenitis, two gastritis, one probable pneumonia and two patients had more than one affected organ). The median time between the transplant and CMV infection was 128 days [IQR 80–163]. Forty percent of patients with CMV infection had negative values for antigenemia at diagnosis, independently on the type of CMV infection (38% in case of asymptomatic replication, 44% in viral syndrome and 33% in visceral disease). We didn't find ganciclovir resistant strains. Twenty patients (27%) had bacterial and five (7%) fungal coinfection. There were no differences in the incidence of acute rejection according to the development of CMV infection (p = 0.5). Fourteen patients (8%) died during follow-up. Although there were no CMV-related deaths, mortality was higher in patients that presented CMV infection comparing to high-risk patients that didn't present the infection (p = 0.006).

**Conclusions:** Heart mismatch recipients presented higher incidence of CMV infection. A high proportion of patients with CMV infection had negative antigenemia, being DNA detection more sensitive for diagnosis. Mortality is higher in patients with CMV infection in the post-transplant.

### P2092 Chromosomally integrated HHV-6 in healthy donor and patients treated for haematological malignancy

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**Objectives:** Chromosomal integration of Human herpesvirus 6 (CI-HHV-6) is a biological phenomenon in which HHV-6 viral DNA integrates into telomeric part of the chromosomes and can be vertically inherited from parent to child. Therefore viral DNA is present in every cell of the organism and the ratio of human and viral DNA is about 1:1.

**Methods:** We aimed to find the frequency of CI-HHV-6 in a samples from 1019 patients treated for malignant haematological disease (ALL, AML, Hodgkin Lymphoma) in a Czech centres between the years 1995–2010. Samples were primarily used for detection of minimal residual disease or viral detection. To confirm the frequency also in a general population, we have tested DNA samples from 420 healthy donors. In the samples, HHV-6 DNA was detected by RQ-PCR. Viral DNA load was normalised to 100 000 human genome equivalents assessed by quantification of albumin gene. CI-HHV-6 was confirmed by detection of viral DNA in the nail samples. Same approach was used for screening of the nails obtained from the donors from general population too. DNA was extracted by using Qiagen DNA Blood Mini and Qiagen DNA Micro Kit according to the manufacturer's instruction. IgM and IgG antibody levels were detected in sera samples from 148 patients at time of diagnosis and CI-HHV-6 carriers. Antibody detection was performed using PanBio ELISA kits.

**Results:** CI-HHV-6 was detected in 13 patients (1.3%) and in five healthy donors (1.2%). Vertical transmission of CI-HHV-6 in the relatives was confirmed by family studies. Both variants of the virus were detected in patients and healthy donors respectively – A (9 + 3) and B (4 + 2). We have detected common reactivation HHV-6 B DNA

in six patients with CI-HHV-6 A treated with chemotherapy. Three patients underwent allogeneic HSCT and one was transplanted from the donor with CI-HHV-6. We did not observe any HHV-6 related complications in the patients. We did not detect a difference in an antibody levels in the patients with and without CI-HHV-6.

**Conclusions:** Similar frequency of CI-HHV-6 does not suggest any risk of this phenomenon on the development of haematological malignancies. Same antibody profile and HHV-6 B reactivation suggest the similar behaving of the HHV-6 infection in CI-HHV-6 carriers and in general population. Our experience suggests the CI-HHV-6 to be important only with potentially harmful virostatic treatment. Supported by Grant of Ministry of Health of the Czech Republic NS 9907-4.

**P2093 Epstein-Barr virus DNAemia is an early surrogate marker for later development of immunosuppression-related adverse events in solid organ transplant recipients**

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**Background:** Epstein-Barr virus (EBV) DNAemia (EBVd) has been suggested as a surrogate marker of immunosuppression following SOT. Monitoring EBVd might help to identify patients at highest risk for late immunosuppression-related adverse events.

**Methods:** We analyzed a cohort of SOT recipients at our institution (2003–2005) who survived more than 180 days after transplant. Patients were followed until March, 2010. EBVd determined in whole blood by real-time PCR assay Abbott EBV PCR KIT (Abbott Diagnostics, Chicago, IL, USA) was systematically performed during the first six months. We evaluated the duration and intensity of EBVd, according to three variables: (i) high grade EBVd (higher than 1000 copies/mL); (ii) persistent EBVd (longer than 30 days). These parameters were evaluated as potential predictors of late adverse events (occurring after the sixth month): graft dysfunction (GD), graft loss (GL), death and immunosuppression-related adverse events (IRAE), defined as at least one of the following: major neoplasia, and/or opportunistic or severe infections. A Cox proportional hazard model was used to estimate the effect of early EBV replication on these late events along the study period.

**Results:** The cohort consisted of 81 patients: 53 renal, 21 liver, and seven cardiac transplants. A median of 10 samples were screened per patient (range 1–15). EBVd was detected in 71/81 patients (87.7%) and within the first week after transplant in 42.9%. Persistent EBVd was found in 39 patients (48%) and high EBVd in 43 (53%). Patients with early persistent EBVd had significantly higher rates of IRAE (38.5% vs. 9.5%;  $p = 0.004$ ) and a trend towards higher late mortality (25.6% vs. 12%;  $p = 0.2$ ). High EBVd was also related with a higher rate of late IRAE (37.2% vs. 7.9%;  $p = 0.004$ ). We did not find a higher incidence of GD nor GL neither in patients with persistent or high EBV infection. Multivariate Cox proportional hazard model demonstrated that early persistent EBVd (Hazard Ratio [HR]: 6.35, Confidential Interval 95% [CI]: 1.85–21.7) and high grade EBVd (HR: 8.3; CI: 2.1–32.48) were independently related with IRAE.

**Conclusions:** EBVd is detectable in the majority of SOT along the first 6 months after Tx. High and persistent EBVd during the first six months are surrogate markers of increased risk of immunosuppression-related late adverse events.

**P2094 Clinical impact of adenovirus in paediatric haematopoietic stem cell transplant recipients**

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**Objectives:** Viral infections/reactivations are one of the main relevant complications in allogeneic hematopoietic stem cell transplant (HSCT)

recipients. Pediatric patients are at particularly high risk of primary adenovirus infection with different clinical manifestations and poor outcome, given the paucity of therapeutic options. Routine monitoring is usually not performed, although it is indicated in high risk patients. In symptomatic patients, adenovirus can be detected in infection sites and whole blood. Herein, the occurrence and clinical impact of Adenovirus in pediatric HSCT recipients were studied.

**Methods:** Over a 1-year period, 603 specimens (472 whole blood, 82 swabs, 17 urine, six cerebrospinal fluid, six bone marrow, five liver biopsies, and 15 other samples) from 181 allogeneic HSCT recipients (106M/75F; age, range 3–16) were tested for Adenovirus by real-time PCR. Routine virological monitoring included CMV and EBV, while other herpes viruses were searched only on the basis of clinical indications. Clinical features and outcome were evaluated.

**Results:** Adenovirus resulted positive in 115/603 (19.1%) specimens from 19/181 (10.5%) patients; six individuals were positive on different types of specimens (with positivity on other specimens usually preceding that on whole blood). As regards whole blood, Adenovirus was positive in 60/472 (12.7%) specimens from eight (4.4%) patients. Viral load ranged from  $<1200$  to  $>5 \times 10^6$  copies/mL whole blood. A viral load  $>10^5$  was persistently found in five patients, three of which eventually died, despite treatment with cidofovir.

**Conclusion:** Adenovirus can be detected in up to 10% of pediatric HSCT patients and can be associated to multiple site or systemic involvement. Although no cut-off is usually adopted, the finding of poor outcome in patients with higher viral load suggests the opportunity to evaluate the administration of pre-emptive therapy in patients with persistent positivity or systemic involvement.

**P2095 Screening of haematopoietic stem cell transplant patients by adenovirus PCR on blood: results of an 18-month survey**

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**Objectives:** Adenovirus infections are potentially life-threatening complications after hematopoietic stem cell transplantation (HSCT). In a retrospective study, we evaluated the value of quantitative adenovirus PCR on blood samples for the early diagnosis of severe invasive and disseminated adenovirus infection.

**Methods:** We conducted a retrospective analysis of all adenovirus PCR assays performed on EDTA blood samples of a cohort of HSCT patients between February 2010 and August 2011. Pediatric HSCT patients were screened weekly until day 100 or immune reconstitution. Adult patients were screened weekly only in case of a high risk HSCT. A quantitative in-house developed real-time Taqman<sup>®</sup> PCR on the Applied Biosystems 7900-analyzer was used, following automated DNA extraction on the m2000sp analyzer (Abbott). Primers and probes were chosen by aligning 104 sequences of the 53 different adenovirus serotypes that were known at the time of implementation of this test.

**Results:** We received 935 blood samples from 111 unique patients, 87 adults and 24 children. Adenovirus was detected in 50 samples from 15 patients (overall incidence 13.5%). We noted a higher incidence in children than adults (25.0% vs. 10.3%). Median time between transplantation and first detection of adenoviral DNA in blood was 50 days in children (range 6–223 days) and 69 days in adults (range 40–343 days). Eighty percent of patients, who developed adenoviremia, did so within 100 days after transplantation. Three patients had transient low viral loads ( $<2.7$  log copies/mL) without clinical significance. Nine patients had moderate viral loads (2.8–4.7 log copies/mL) with only signs of localized infection. Three patients presented with signs and symptoms of disseminated disease. They all had an increase in viral load with  $>1$  log copies/mL over a period of 2 weeks, resulting in maximum viral loads of  $>6.7$  log copies/mL. The overall case fatality rate was 13.3%, increasing to a 66.6% fatality rate when the viral load exceeded 5 log copies/mL.

**Conclusion:** Adenoviremia is a frequent complication after HSCT. In our population, we found an overall incidence of 13.5%. 80% of

infections occurred within 100 days after transplantation. A 1 log increase in viral load over a period of two weeks was predictive for evolution to severe invasive and disseminated disease. Viral loads exceeding 5 log copies/mL were associated with a high mortality rate.

**P2096** Value of adenovirus PCR on stool in paediatric haematopoietic stem cell transplant recipients

*K. De Vreese\*, I. Meyts, K. Beuselinck, K. Lagrou (Leuven, BE)*

**Objectives:** We compared real-time adenovirus PCR on stool samples from 16 pediatric hematopoietic stem cell transplant (HSCT) patients with conventional diagnostic techniques for the detection of gastrointestinal adenovirus infection. We also evaluated the value of adenovirus PCR on stool samples in predicting invasive and disseminated disease.

**Methods:** Between February 2010 and April 2011, 67 stool samples and 255 EDTA blood samples were collected from 16 pediatric HSCT patients. Blood samples were analyzed within 2 days by quantitative adenovirus PCR. Stool samples were frozen at  $-20^{\circ}\text{C}$  pending batch processing. Semi-automatic DNA extraction was performed on approximately 40 mg of stool using the BioMérieux EasyMAG analyzer, followed by an in-house developed real-time PCR, detecting 53 serotypes, on the Applied Biosystems 7900-analyzer. The results of the PCR on stool were compared with those of conventional viral cell culture on HeLa cell lines and adenovirus serogroup F antigen testing. Samples from seven patients were available for genotyping.

**Results:** Adenoviral DNA was present in the stool of 11/16 patients (69%), whereas stool culture was positive in only six patients (37.5%). Positive viral cell culture was associated with a low cycle threshold ( $C_t$ ) and thus a higher viral load. Four out of six patients with positive stool PCR and positive stool culture developed invasive and/or disseminated disease. In all four patients, PCR on stool was strongly positive with  $C_t$  values  $<20$ . Adenovirus could be detected prior to or simultaneously with the development of adenoviremia. Of the remaining two patients, one had asymptomatic shedding with transient adenoviremia and the other presented with gastrointestinal complaints attributed to Graft-versus-Host Disease (GvHD).

Out of five patients with positive PCR and negative culture, three presented with gastrointestinal symptoms originally attributed to GvHD, but nonresponsive to steroid therapy. The remaining two patients had no gastrointestinal complaints. Adenovirus serogroup F antigen testing was negative in all patients. Genotyping in seven patients showed that none of the adenoviridae belonged to serogroup F; three patients had serogroup A, three had serogroup C, one had serogroups A and C.

**Conclusion:** Adenovirus PCR on stool samples allows early and sensitive detection of infectious origin of diarrhea in pediatric HSCT patients.  $C_t$  values  $< 20$  were predictive for evolution to invasive and/or disseminated disease.

**P2097** Investigation of the intracellular expression of interferon- $\gamma$  in cells in response to BK viral peptide stimulation in haematopoietic stem cell transplant recipients

*R.J. Drew\*, J. Dunne, B. Crowley (Dublin, IE)*

**Objectives:** The objective of this study was to investigate BKV-specific T-cell immune reconstitution in patients with resolved BKV haemorrhagic cystitis infections (BKV-HC) following haematopoietic stem cell transplantation (HSCT). This was examined by investigating CD4+ and CD8+ intracellular expression of interferon- $\gamma$  in patients with resolved BKV-HC infections when stimulated with BK viral peptides, and comparing the response to that of healthy volunteers and HSCT controls.

**Methods:** Eleven healthy volunteers, four HSCT controls and five HSCT recipients with resolved BKV-HC infections were included in the study. Peripheral blood mononuclear cells (PBMCs) were isolated from blood samples. The PBMCs were stimulated with the BKV

peptides VP1 and LT-ag, separately and also in combination, for 6 hours. The PBMCs were then washed and stained with CD3-APC, CD4 PE/Dy647, CD8-PE and intracellularly with anti-human interferon- $\gamma$  FITC. The percentage of cells expressing interferon- $\gamma$  was measured by flow cytometry and a new commercial ELISpot kit, TRANSspot<sup>TM</sup>.

**Results:** Resolved BKV-HC cases showed a higher median percentage of CD8+ T cells expressing interferon- $\gamma$  in response to VP1, LT-ag and the combination of VP1 and LT-ag compared to healthy control volunteers and transplant controls (Table 1). There was a statistically significant difference in the percentage of CD8+ T-cells expressing interferon- $\gamma$  in the resolved BKV-HC cases than healthy volunteers in response to the combination of VP1 and LT-ag. However, when the CD4+ cell response was examined, healthy volunteers had a higher median expression of interferon- $\gamma$  but this was not statistically significant. None of the study participants had a positive response to BKV detected by the TRANSspot assay, however 2/4 HSCT controls and 1/5 BKV-HC cases had a response to CMV antigens as measured by this kit.

**Conclusion:** This study has shown that patients with resolved BKV-HC infection have an increased percentage of CD8+ T cells that express interferon- $\gamma$  in response to single and combined BKV peptides, than HSCT controls and healthy volunteers. Healthy volunteers have a higher percentage of BKV-specific CD4+ T cells that produce interferon- $\gamma$  in response to BKV peptides, and this is essential in maintaining suppression of latent viruses such as BKV.

**P2098** Hepatitis B serological changes following allogeneic bone marrow transplantation

*B. Teh\*, M.A. Slavin, J. Szer, J. Sasadeusz (Melbourne, AU)*

**Objectives:** There have been increasing reported cases of Hepatitis B (HBV) reverse seroconversion (RS) in immunocompromised patients with serological evidence of past HBV infection [Hepatitis B surface antigen (HBsAg) negative, core antibody (antiHBcAb) positive] following allogeneic haematopoietic stem cell transplant (allo-HSCT).

**Methods:** We performed a retrospective review of serial HBV serological testing in patients who had undergone allo-HSCT at our centre between 2000 and 2006 supplemented by a review of HSCT donor and blood bank records as appropriate.

**Results:** We detected 12 patients with serological evidence of past HBV, with one case of RS post transplantation (8.3%). The patient with RS had a pre allo-HSCT HBV surface antibody (antiHBsAb) of 2.5 IU/mL and received HBV screened (HBsAg, antiHBcAb) negative donor stem cells. Seven of the 12 patients remained serologically unchanged following transplantation although six patients had declining levels of Hepatitis B surface antibodies, with 2 to below 10 IU/mL. The remaining four patients with past HBV had loss of antiHBcAb whilst remaining HBsAg negative.

An additional 14 patients who had no markers of HBV infection pre allo-HSCT developed isolated antiHBcAb post allo-HSCT in the setting of known HBV screened negative donor stem cells. All 14 received multiple units of blood and plasma derived products post transplant which were screened HBsAg negative and none received HBV prophylaxis pre or post allo-HSCT. Eleven of the 14 patients remained consistently antiHBcAb positive up to 5 years post allo-HSCT. Patients with a change serological status had serial serological follow up for a median of 24.0 months (range 3.0–61.5 months) and routine testing of patients and donors with HBV DNA levels were not performed.

**Conclusions:** Monitoring of HBV serological markers (including antiHBsAb) and HBV DNA levels pre and post-allo HSCT in recipients and donors would allow early detection and treatment of RS or new acquisition of HBV. Vaccination of donors has a potential protective role for HBV in the allo-HSCT recipient.

## Internet and electronic resources

### P2099 Bibliometric analysis of European publications in infectious diseases and clinical microbiology areas in 2010

O. Sipahi, H. Sipahi, T. Yamazhan\*, M. Tasbakan, S. Ulusoy (Izmir, TR)

**Objectives:** In this study it was aimed to make the bibliometric analysis of 2010 European publications related to infectious diseases and clinical microbiology areas.

**Methods:** Bibliometric data related to 2010 were retrieved from SCImago journal and country rank web site (<http://www.scimagojr.com>). Data related to infectious diseases and clinical microbiology were accessed by using the infectious diseases subcategory and clinical microbiology subcategory of the database. Top 10 European countries according to citable documents (articles + reviews) were detailed with total citations, citation per paper and H index.

**Results:** When evaluated according to total number of citable documents (articles and reviews) United Kingdom, France and Netherlands are the top three countries in the infectious diseases area. In the medical microbiology area Spain, Germany and United Kingdom are top three countries. United Kingdom which is European number one in infectious diseases, is globally the second after United States. Spain which is the European number one in medical microbiology, is the fifth after United States, India, China and Japan) (data not shown). Turkey is the 9th in infectious diseases area and the 6th in clinical microbiology area.

Table 1: Top 10 European countries in terms of publications in infectious disease area.

Country	Citable documents	Citations	Self-Citations	Citations per Document	H index
United Kingdom	834	1,260	469	1,28	101
France	516	309	201	1,00	75
Netherlands	397	361	99	0,96	66
Germany	336	419	117	1,08	73
Spain	277	233	61	0,73	56
Italy	239	237	74	0,83	59
Switzerland	239	390	121	1,46	73
Belgium	208	314	94	1,34	56
Turkey	185	73	34	0,33	30
Sweden	104	201	18	1,79	45

Table 2: Top 10 European countries in terms of publications in clinical microbiology area.

Country	Citable documents	Citations	Self-Citations	Citations per Document	H index
Spain	302	241	96	0,55	53
Germany	246	472	160	1,28	77
United Kingdom	240	356	116	1,23	76
France	225	313	104	1,13	69
Italy	173	248	68	1,12	50
Turkey	188	64	22	0,32	25
Greece	77	80	19	0,84	31
Switzerland	75	127	37	1,37	48
Czech Republic	70	14	8	0,18	24
Sweden	72	87	15	1,13	49

**Conclusion:** Although at least some of the European countries are quite competitive both in infectious diseases and medical microbiology areas, further progress is needed.

### P2100 Imperial antimicrobial prescribing policy: harnessing smartphone technology to develop a clinical decision support application for the antimicrobial prescribing policy of a multicentre university teaching hospital

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**Objectives:** Provision of local policy and guidelines has been shown to be an effective means of promoting prudent antimicrobial stewardship. At Imperial College Healthcare NHS Trust (ICHNT) the local antimicrobial prescribing policy is available to clinicians in many formats including a pocket guide, poster and on the intranet. We report here on the development, dissemination and evaluation of a smartphone

application, the iAPP (Imperial Antimicrobial Prescribing Policy) of the local antimicrobial prescribing policy. The iAPP was evaluated (i) on whether it is an effective means of diffusion of policy at the point of care; (ii) the extent to which the target group (clinicians) accessed and engaged with it; (iii) clinician attitudes on the convenience and appropriateness of its use in clinical settings.

**Methods:** Pre- post intervention surveys were used as part of the evaluation. The subjects for the surveys were junior doctors via the post-graduate medical centres and pharmacists. Focus groups informed the different stages of development. As part of a multi-modal dissemination strategy the iAPP was promoted via (i) pharmacy teaching sessions for junior doctors; (ii) emails sent to all new doctors; (iii) the front page of the Trust intranet; (iv) a news item in the weekly paper of the Trust.

**Results:** Ninety-three completed the pre-intervention questionnaire. Of these 76% used a smartphone at work. The iAPP was launched on the 1st of August 2011 to coincide with the new intake of doctors, 40% (376) of whom downloaded the iAPP within the first month of release, registering 3204 individual sessions. Post-intervention (n = 48), 83% found the iAPP easy to use, 85% found that it added to their knowledge base regarding antimicrobial prescribing, 96% found that it influenced their antimicrobial prescribing practice, with 70% stating that the inbuilt calculators for creatinine clearance, ideal body and obese weight dosing influenced their antimicrobial prescribing practice at the point of care.

**Conclusion:** This work provides insights into mobile technology adoption and implementation process within the context of an AHSC, with implications for wider healthcare settings. Mobile technology, in particular the smartphone platform, offers point of care access to clinical information, data and resources, and complements the more traditional platforms of antimicrobial policy dissemination. Further work on evaluation of mobile technology as a clinical decision support system is required.

### P2101 A web-based genome library for surveillance, detection, characterisation and drug resistance monitoring of influenza virus infection in the Philippines

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**Objectives:** Currently, influenza surveillance in the Philippines is largely paper-based making it difficult to make use of the data. This study aimed to design and construct a web-based interactive genome library for the surveillance, detection, and characterization of influenza virus infection in the Philippines.

**Methods:** There were three major development tools used in creating the website. The first is the Liferay portal which was used as the container of the portlets and handles its deployment and customization. Following the desktop metaphor, a portal is like a user's desktop and the portlets like files and applications placed upon the desktop. Liferay has a control panel for user account administration, user restriction, and page and portlet privacy control. Secondly, MySQL was used to host the database schemas being used by the portal and its portlets. Data stored include user account information, clinical data, DNA sequences, portal and portlet settings. Lastly, the Netbeans IDE (Integrated Development Environment) was used to test, debug, and to develop the portlets that will be integrated in the portal.

**Results:** The website was designed to assist in the national Influenza-Like Illness (ILI) Surveillance Network of the Department of Health – National Epidemiology Centre (DOH-NEC). We have converted the ILI report forms to an electronic format that can be accessed online by health workers from the regional sentinel sites and that can also be accessed by designated personnel from DOH-NEC. Clinical data entered by regional health workers, as well as, local influenza DNA sequences will be stored in the database. The database will also integrate drug-resistance results for influenza specimens. A key feature of this website is the Interactive Map of the Philippines where a graphical summary of the ILI-related cases and deaths by gender and sex for each desired region will be displayed. Although some of the

information will need to be kept confidential, independent researchers will have access to some of the data.



**Conclusion:** The developed website/database can be a very useful tool in the national ILI surveillance network in the Philippines. Linking of clinical data with sample sequence data is in progress, and will be invaluable from an epidemiologic standpoint. Eventually, this database could be extended to more diseases in the Philippines and can be used as a model for other countries that are moving towards electronic surveillance databases.

#### **P2102** RipSeq Single, a bacterial reference database through social curation

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**Objectives:** Public DNA sequence databases like GenBank are often used for the identification of bacteria from clinical specimens. While GenBank contains large numbers of current references, it also contains many uninformative or even erroneous references that can hinder accurate identification. Our objective was to minimize the effects of these uninformative references by creating online software named RipSeq Single (Isentio, Norway) that allows users to create a more medically relevant reference database. Users can annotate, reject and approve references through personalized methods, and choose to share information with a community of users.

**Methods:** All GenBank bacterial references were placed into a SQL database and a pipeline was created to add new references daily. Algorithms were created to allow additional annotation of references such as valid nomenclature, publication status, and type strain status. References were separated into BLASTable subsets based on these annotations. Other features of the software include sample management, automated chromatogram trimming and consensus generation, batch analysis of files, automated selection of the best reference(s) based on user settable guidelines. Social curation was performed by five laboratories and by internal personnel. References were annotated with collaborative edits (rejections and approvals) and the addition of educational content. Editorial oversight of public content is continuously performed by internal personnel at Isentio.

**Results:** To date, >3 700 000 public bacterial references are in our database. Of these, 503 880 had valid species names and 24 025 were derived from type strains. With approximately 10 000 known bacterial species, 8596 distinct species were represented in GenBank and 6555 distinct species were from type strains. With community curation, over 2000 sequences were queried against the database and resulted in the rejection of 172 reference sequences and the addition of 583 annotated notes.

**Conclusion:** RipSeq Single provides an interface for a community of users to create a dynamic bacterial sequence database that can improve the quality of and add value to the end user's experience in interpreting

sequence data. Genomic information rapidly changes and annotation of references by social curation optimizes knowledge sharing in a fast-paced manner. Further social curation of the database will continue as part of this dynamic process and editorial oversight is ongoing.

#### **P2103** Formulating the microbiology content of the National Laboratory Medicine Catalogue in England

J.K. Sutton\* (Southampton, UK)

**Objectives:** The goal of the National Laboratory Medicine Catalogue (NLMC) is to create a single standardised list of legitimate pathology test requests for use within the NHS in England. Benefits of this ambitious project include defining common terminology for test names and facilitating unambiguous test interpretation at a national level, hence contributing to patient safety. The governance process for assuring the quality of NLMC content is hosted by the Royal College of Pathologists (London) on behalf of the NHS. The Department of Health owns the NLMC and its content. It is proposed that all providers of clinical diagnostic pathology services in Microbiology, Biochemistry, Haematology, Histopathology and Immunology in England will be required to use the NLMC, though individual local providers will be free to determine which tests from the catalogue they make directly available to clinicians.

**Methods:** As NLMC Specialty Lead for Microbiology I have consulted with a range of colleagues to devise a simple, comprehensive and robust system for consistent naming of microbiology investigations.

**Results:** This system is being used to standardise microbiology test request names on a web-based test editing tool with anticipated release of the catalogue in 2012. Each request is comprised of a series of discrete information (data) elements which, when combined as part of a relational database, define the relevant investigation. The information elements defining all pathology test are: test request name, collected specimen type, collection method, topography (anatomical location), morphology and laterality. In addition, it will be recommended that in local implementation of the NLMC, there should be the facility to enter additional clinical and/or test-specific information to enable comprehensive and safe test requesting. Test names are independent of precise assay methodology and protocols, but broad categories including culture based and nucleic acid detection methodologies are separately represented within the catalogue.

**Conclusion:** The NLMC will have significant impact in standardising pathology requesting within the NHS. Moving forward, the NLMC will require prospective review and revision and stakeholders will be invited to help refine and improve its content. The NLMC project's remit now includes reporting of pathology investigations, and generic standards of reporting including units of measure will be incorporated into the catalogue.

#### **P2104** Antifungal drug interactions database

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**Objectives:** Antifungal drugs are often taken for a long period of time and are capable of interacting with a wide range of other medications e.g. the azole group of antifungals are a substrate and inhibitor of cytochrome P450 (CYP) which is important for the elimination of many other drugs. These interactions can in some cases cause serious alterations in the effective dose of either the antifungal drug or the concomitant medication.

Doctors and pharmacists experience with oral or IV antifungal drugs can be limited so information provided to the patient on possible interactions runs the risk of being incomplete. In addition several antifungals have not been in use for a long time and new interactions are still being discovered. There is a need for a single central resource to hold all interaction data for this class of drugs.

**Method:** The Aspergillus Website is one of the best used and most highly ranked websites that provide information on the treatment of



aspergillosis and support to patients. The website is run by a team closely associated with the National Aspergillosis Centre (NAC), Manchester, UK. It therefore has ready access to relevant clinical advice and is already established as a well used route of communication for medical information and support for aspergillosis. We set out to provide a database in which all currently known interactions with antifungal drugs are recorded.

A four level colour system is used and designed for patients; white - no interaction, green - minor interaction, orange - moderate interaction, needs medical input and red - significant interaction, needs urgent medical assessment. The text related to each interaction explains the reason for the interaction and suggests ways of circumventing it.

Addition of new information is a regular ongoing process using new scientific papers and our own clinical experience at the NAC as a guide.

**Results:** We launched the Antifungal Drug Interaction database ([www.aspergillus.org.uk/nac/interactions/patientchoosegeneric.php](http://www.aspergillus.org.uk/nac/interactions/patientchoosegeneric.php)) in July 2011 with 708 concomitant drugs and six antifungals covered; itraconazole, voriconazole, posaconazole, micafungin, amphotericin B and caspofungin.

In its first three months of operation the database was used over 1300 times.

**Conclusions:** The Antifungal Drug Interactions database has been well received, suggesting that it is useful and easy to use, and that the information is a valuable addition to the support we offer patients and doctors.

#### **P2105** Microbiological alert system in hospital intranet (Hygeia) to produce quick automatic information in microbiological important positive test

T. Alarcon\*, J. Guillem, G. Fernandez, M. Lopez-Brea (Madrid, Barcelona, ES)

**Objective:** The aim of this study was to evaluate the use of a computerized microbiological alert system in Hospital Intranet (Hygeia).

**Methods:** Three Alert types were defined:

1 A test with a viral positive result: Flu Real Time PCR, CMV Antigenemia.

2 A test with a blood culture positive result: Blood culture positive result (each bottle was considered, with a maximum of six per patient) and results of Gram staining (when the same bacteria type was observed in the different bottle only one was alerted).

3 Isolation of a multi resistant bacteria ESBL producing *E. coli*, *P. mirabilis* and *K. pneumoniae*, multi-resistant *P. aeruginosa*, *A. baumannii*, and *S. maltophilia*.

The defined Alert was shown in the Hospital Intranet once validated in the Microbiology Computer System. Alert was activated with provisional or definitive validation. The Alert was shown in red color during 48 hours and in blue color afterwards and whilst the patient is hospitalized. The result of the test or the name of multi-resistant bacteria as well as the date of specimen reception and date and hour of validation was shown in the alert.

The Microbiology Computer System used was Servolab (Siemens Healthcare Diagnostic). The Hospital Intranet was developed by the Clinical Information Unit and the Computer Department of the Hospital Universitario de la Princesa.

**Results:** A total of 3015 Alerts were activated from March 2009 to January 2011.

The number and results with alerts corresponding to a positive test are shown in the Table. The number and results with alerts corresponding to a multi-resistant bacteria are also shown in the Table. Test chosen as alert are those which usually are communicated by telephone call. The computerized alerts do not substitute the telephone call but was used simultaneously. Computerized alert allows distribute information not only to the clinician attending the patient but to all clinicians involved in patient care (doctors on duty, other specialties doctors).

**Conclusion:** The Microbiological Computerized Alert System is a very useful method to give a quick result of important tests, avoiding

Test	Defined Alert	Alert Num.	Alerted Result	Num
pp65 CMV/ANTIGENEMIA	Positive	270	Number PMNs/10 <sup>5</sup>	270
FLU REAL TIME PCR	Positive	246	Positive. Flu AH1N1 Positive. Flu B	245 1
BLOOD CULTURE	POSITIVE	1389	POSITIVE	1389
GRAM STAIN OF BLOOD CULTURE	We observe	287	- Gram (-) rods - Gram (+) rods, type cocciforms. - Gram (+) cocciln groups. - Gram (+) diplococci and cocciln chains - yeast	122 10 111 37 7
Group of Bacteria	Alerted Microorganism		Num. Of alerts	
Gram positive	Staphylococcus aureus resistente a meticilina		314	
Enterobacteriae	Escherichia coli portador de BLEE		391	
	Klebsiella pneumoniae portador de BLEE		105	
	Proteus mirabilis portador de BLEE		1	
Non fermenters	Acinetobacter baumannii		6	
	Pseudomonas aeruginosa Multi-resistente		2	
	Stenotrophomonas maltophilia		4	

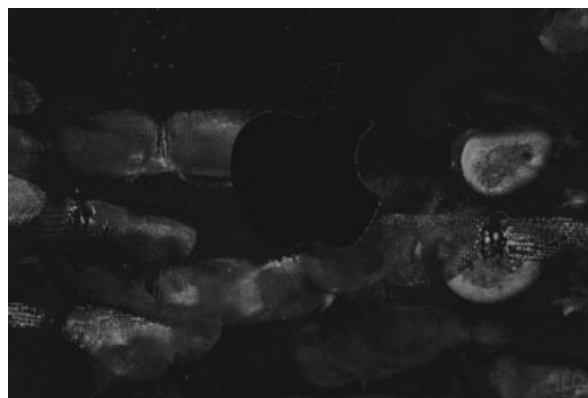
misinterpretation that sometimes could happen with telephonic information. Positive Blood cultures were the most alerted test. ESBL producing *E. coli* and Methicillin resistant *S. aureus* were the multi-resistant bacteria most alerted.

#### **P2106** deBac-App – first standardised hygiene application for disinfecting the iPad®

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**Objectives:** As seen in previous studies, the bacterial contamination of mobile phones of hospital employees is a potential threat. Newly introduced, highly mobile tools such as Tablet PCs also contribute to the debate about “clean hands” and instruments in health care settings. Recently, iPads were introduced in several clinical departments and wards at Hannover Medical School. To facilitate the use of these devices in a clinical setting, we proposed specifically adapted hygienic standards. To enable the users to easily follow the standard cleaning procedure, to track the cleaning efforts and to help the staff with documenting the whole process, we developed a free-of-charge application called “deBac-app”.

**Methods:** In a cooperative project of the Institute for Medical Microbiology and Hospital Epidemiology, the P.L. Reichertz Institute for Medical Informatics, both based at Hannover Medical School (MHH), and Frobose GmbH (Hannover, Germany), an interactive and easy to use disinfecting guide “deBac-app” for the iPad® was developed. In six iterative steps, the app guides the user through the standardized cleaning procedure. During each step, the user is asked to interact with the device: “deBac-app” tracks the various parts of the cleaning process and logs the results using its internal protocol function. Later, the protocol may be emailed to any interested recipient. A timer can be set to remind the user to clean the device on a daily basis.



**Results:** “deBac-app” is in use in several departments and clinical wards at Hannover Medical School. The application was downloaded by 569 users from 38 nations in the first 2 weeks after publication. At Hannover Medical School, several trials are conducted to test the usability, acceptance and efficiency of the cleaning guide.

**Conclusion:** As for institutional requirements for using Tablet PCs in a clinical setting, the hygiene of the device has to be standardized and traceable. “deBac-app” easily satisfies these requirements, thus supporting the users regarding the device’s hygiene and reducing the risk of transmitting nosocomial infections. The application is broadly used in several clinical departments and on wards at Hannover Medical School. “deBac-app” is the first hygiene-app for the iPad® and can be downloaded free-of-charge from the Appstore. Both English and German languages are provided. In a forthcoming study, the efficiency regarding hygiene when using or not using the app will be compared.

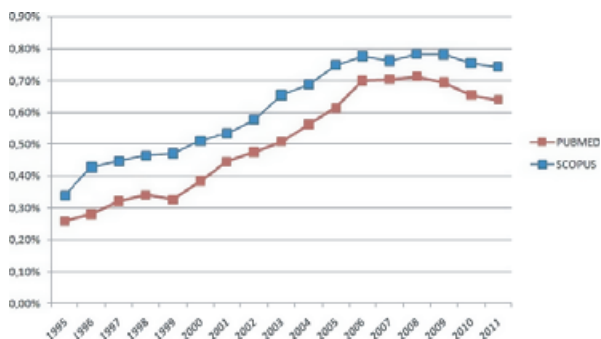
#### **P2107** Biomedical research productivity in Greece: effect of the financial crisis

*M. Falagas\*, G. Samonis, V. Bardakas, F. Abel, M. Mavros (Athens, Heraklion, GR)*

**Objectives:** Greece has been suffering a major financial crisis since 2009. We hypothesized that the financial crisis has also affected the productivity of biomedical research conducted in Greece.

**Methods:** We searched the PubMed and Scopus databases for journal articles with a Greek affiliation and compared them with the respective global values for the period 1995 to 2011 (until November 10).

**Results:** Analysis of the PubMed database revealed a stable increase in biomedical research productivity in Greece from 1995 to 2006 (from 0.26% to 0.70% of global productivity;  $p < 0.05$  for most comparisons of successive yearly periods), which was followed by 4 years of stable relative productivity ( $0.70 \pm 0.01\%$ ). This was succeeded by a considerable decline in 2010 (0.65%;  $p < 0.001$ ) and 2011 (0.64%). The analysis was also performed using data from Scopus database, which yielded similar results. Our findings suggest that the plateau phase of relative biomedical research productivity in Greece in 2006–2009 was interrupted by an abrupt decline in 2010, extending throughout 2011. This decline in relative biomedical research productivity coincided with the financial crisis that started in Greece in 2009 (with a 1-year lag period).



**Conclusion:** Although the impact of the current financial crisis on the observed decrease in relative biomedical research productivity in Greece in 2010–2011 cannot be precisely defined, one may at least postulate that it has played a considerable role. It may be expected that the direct effects of the decrease in research funds will be more evident in the subsequent years. The observed decline in research productivity in 2010–2011 may also be a result of the considerable psychological stress that accompanies a financial crisis and also affects researchers. In conclusion, a decline in relative biomedical research productivity in Greece was observed, starting shortly after the initiation of the financial crisis, the precise contribution of which, however, is unclear.

#### **P2108** Development of a web-based antimicrobial resource to improve antimicrobial prescribing – 5-year review

*J.A.T. Sandoe\*, P. Howard, A. Olusoga, J. Dunne, K. Warburton (Leeds, UK)*

**Introduction:** In 2007 our Trust was performing poorly for Clostridium difficile infection (CDI) and meticillin resistant *Staphylococcus aureus* (MRSA) bacteraemia. No antimicrobial stewardship programme existed, so the Improving Antimicrobial Prescribing Group was established.

**Aims:** The project aimed to build a web-based infection management resource with:

- 1 Evidence-based, local consensus infection management and prophylaxis guidelines.
- 2 Recommendations for clinical diagnosis, investigation, treatment (empiric and directed therapy), allergy, IV to oral switch, duration of therapy, referral criteria.
- 3 On-line peer review system to enable stakeholder involvement.
- 4 Monitor on-line use of guidelines.
- 5 Educational resource for prescribers.
- 6 Audit resource, with plans, tools and results.
- 7 Links to British National Formulary (BNF) and Electronic Medicines Compendium (eMC).

**Results:** 1 One hundred and five guidelines have been developed.

2 Guidelines have been modified and updated continuously in response to new evidence.

3 Modification allowed on-line comments once the guideline was in use.

4 All guidelines link to eBNF/eMC, drug dosing tools and/or restrictive supply processes.

**Between 2008 and 2011:** 1 Guideline “hits” increased from 1500 to >10 000 hits/month.

2 Prevalence of patients on antimicrobials decreased from 35% to 27%.

3 Documentation of indication on prescription chart increased from 80% to 93%.

4 Documentation of duration on prescription chart increased from 62% to 92%.

5 CDI decreased from 75 cases per month to 17.

6 MRSA bacteraemia decreased from 11 to 1/month.

7 Many audits show improved compliance with guidelines.

**Discussion:** Many hospitals use paper-based antimicrobial guides. These go out of date. We developed pathways that focus on accurate diagnosis and appropriate investigation of infection, with antimicrobials recommended only if necessary. The development process promotes stakeholder ownership and therefore subsequent usage, confirmed by hits data. Monthly point prevalence has shown a drop in patients on antimicrobials. Changes in antimicrobial prescribing may have helped reduce CDI/MRSA. Web-based, evidenced-based, local consensus antimicrobial guidelines are an effective method to support prescribers in their diagnosis and treatment of infection. Links to external and internal information resources such as eBNF, eMC and dose calculators improves patient safety. Feedback processes ensure guidelines are up to date. Guidelines designed and delivered in this manner are used clinically.



## New clinical data on infections in compromised hosts

### **P2109** Results from the European Cubicin® Outcomes Registry and Experience: daptomycin is effective as first-line treatment for Gram-positive infections in patients with haematological malignancies

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**Objectives:** Cancer patients with haematological malignancies are at high risk for serious Gram-positive (G+) infections. In comparison to other anti-staphylococci drugs (linezolid, tigecycline, vancomycin, teicoplanin and penicillins) Daptomycin (DAP) has the most potent in-vitro activity against G+ pathogens. Clinical data for DAP in the treatment of patients with G+ infections and haematological cancer is limited. Here we report the clinical experience with first-line DAP therapy against serious infections in cancer patients with haematological malignancies.

**Methods:** Data were collected from European Cubicin® Outcomes Registry and Experience (EU-CORE), a non-interventional, multicentre study between Jan 2006 and Jun 2011. Patients with haematological malignancies who received at least one dose of DAP for the treatment of bacterial infections were included in this analysis. Treatment success (cured, improved, failure or non-evaluable) was assessed in haematological cancer patients with or without neutropenia, following DAP therapy. Safety data was collected up to 30 days after end of the therapy.

**Results:** Amongst 267 patients with haematological malignancies, 83 received DAP as first-line therapy. In this cohort, 24% of patients were ≥65 years old, 61% were male and 57 (69%) had neutropenia. The most frequent initial dose of DAP (40%) was 6 mg/kg and the median duration of therapy was 10 days (range: 1–47 days). The most frequent infections were bacteraemia (40%) and SSTIs (21%) and the most common pathogens were coagulase negative staphylococci (CoNS 30%: 17% *S. epidermidis*, 13% others) *S. aureus* (12%) and enterococci (7%). High clinical success rates were achieved with DAP given either as first-line or second-line therapy in patients with haematological cancers (80% and 72%, respectively). Within the cohort of patients who received DAP as first-line therapy, similar clinical success rates were seen in subgroups with neutropenia regardless of severity of this condition (severe 79%; moderate 75%; mild 69%) or without neutropenia (80%). DAP was also effective against CoNS (82%), *S. aureus* (80%) and enterococci (100%). Adverse events (AEs) possibly related to DAP were reported in 3 (4%) cases; two patients (2%) discontinued DAP due to AEs regardless of drug relationship. Four deaths and four SAEs were reported but were not suspected to be related to DAP.

**Conclusions:** DAP was well-tolerated and highly effective as first-line therapy in the treatment of G+ infections in patients with haematological cancer with or without neutropenia.

### **P2110** Daptomycin as first-line therapy for infections in patients with solid tumours: clinical experience from the European Registry

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**Objectives:** The use of bactericidal agents is preferable in patients with immunosuppression, including cancer patients. Clinical data for daptomycin in the treatment of cancer patients with Gram-positive infections in randomized trials are limited. This analysis evaluated the

efficacy and safety of daptomycin as first-line therapy for infections in patients with solid tumours in a “real world” setting.

**Methods:** Patients with solid tumours were identified from the retrospective multicentre, non-interventional registry, European Cubicin® Outcomes Registry and Experience (EU-CORE) (Jan 2006–Jun 2011). Patients were assessed for clinical outcome (cured, improvement, failure, non evaluable) following daptomycin therapy. Safety assessments were conducted up to 30 days after the last administered dose of daptomycin.

**Results:** Amongst a total of 387 patients with solid tumours, a cohort of 120 cases received daptomycin as first-line therapy. In this cohort, 57% patients were male; 55% were ≥65 years of age and 8% were neutropenic. The most frequently used initial dose of daptomycin was 6 mg/kg (55%) and the median duration of therapy was 13 days (range: 1–33 days). The most common primary infections were SSTI (42%) and bacteraemia (29%). The most common pathogens were coagulase-negative staphylococci (23%) and *S. aureus* (15%). Aminoglycoside, carbapenem and penicillin were the most commonly used concomitant antibiotics (23%, 19% and 15%, respectively). High success rates were achieved for diverse pathogens with daptomycin therapy administered either as first-line or second-line antibiotic (78% and 83% respectively). Possibly daptomycin-related adverse events (AEs) and serious AEs were reported in 3% and 2% of patients, respectively, and only 1% cases permanently discontinued study drug due to AEs.

**Conclusion:** The advantages of daptomycin concerning its in vitro potency, as compared to other anti-Gram-positive drugs, have been well documented. The presented results indicate that daptomycin is well tolerated and effective in patients with solid tumors, as first line therapy.

### **P2111** Characteristics of initial vs. breakthrough bacterial infections among hospitalised haematological cancer patients

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**Objectives:** Infection is the most important preventable cause of death among hematological cancer patients. We sought to analyze pathogen distribution and antibiotic susceptibilities for infections occurring initially, at the onset of fever with neutropenia, vs. breakthrough infections, diagnosed with persistent or recurrent fever following broad-spectrum antibiotic.

**Methods:** We prospectively monitored infections at the Hematology and Bone Marrow Transplant Units at Davidoff's Cancer Center in Israel between 2007 and 2011. We included all consecutive hospitalized patients with fever, clinically and/or microbiologically documented infections (MDI) during neutropenia, after intensive chemotherapy or after hematopoietic cell transplantations. Infections were defined using consensus criteria. We compared initial vs. breakthrough episodes of bacterial MDIs.

**Results:** Throughout the study period, there were 162 initial and 405 breakthrough febrile episodes, of which 104/162 (64%) and 75/405 (19%) were MDIs or bacteremias. Invasive fungal infections (proven or probable) were documented in 7/162 (4%) of initial and 13/405 (3%) of breakthrough episodes. In both initial and breakthrough MDIs, Gram-negative bacteria predominated (64% and 71%, respectively) and *E. coli* were the most common bacteria. *Staphylococcus aureus* was the most common Gram-positive bacteria in patients with an initial episode (13%), while *Enterococcus* spp. were most common with a breakthrough episode (16%).

Breakthrough Gram-negative MDIs were significantly more resistant than initial Gram-negative MDIs (ceftriaxone 71% vs. 29%, ceftazidime 80% vs. 45%, piperacillin-tazobactam 86% vs. 40%, gentamicin 78% vs. 52% and imipenem 95% vs. 76%, respectively),  $p < 0.01$  for all. Gram-positive MDIs showed similar trends in susceptibilities to antibiotics when comparing initial to breakthrough episodes (penicillin 43% vs. 24% and oxacillin 56% vs. 33%, respectively). Appropriate empirical antibiotic treatment was administered in 71/97 (73%) of initial episodes vs. 40/74 (54%) of breakthrough episodes,  $p = 0.009$ .

**Conclusion:** We show that there are two distinct epidemiological profiles during the course of febrile neutropenia with regard to bacterial infections. Reporting of pooled antibiotic resistance patterns might be misleading and non-informative for clinical practice. Guidelines for the management of febrile neutropenia should better address these separate time points with regard to empirical antibiotic treatment.

**P2112** First and recurrent episodes of bloodstream infections caused by Enterobacteriaceae producing extended-spectrum beta-lactamases in haematological patients

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**Objectives:** Recently a shift forwards gram-negatives bloodstream infections (BSI) and an increasing rate of Enterobacteriaceae producing Extended Spectrum Beta-Lactamases (ESBLs) have been reported in hematological patients (pts). The aim of this study was to evaluate the outcomes of ESBLs producing Enterobacteriaceae BSI and the recurrent episodes of BSI in pts with hematological malignancies (HM) after chemotherapy (CMT).

**Methods:** Episodes of BSI due to Enterobacteriaceae producing ESBLs in pts with HM after course of CMT were included in this study. Recurrent ESBLs BSI was evaluated in the same pts. We defined as recurrent episodes those occurring after consecutive course of CMT.

**Results:** During the study period (2007–2010), we had first episode of BSI due to Enterobacteriaceae producing ESBLs in 73 pts (17–78 years, median 41 years); of these, 68 (88%) pts were in neutropenia. Underlying diseases were acute leukemia in 19 (26%) pts, lymphoma in 36 (50%) pts and other HM in 18 (24%) pts. The majority of episodes of BSI were in induction phase of de novo HM (40%) and in consolidation (26%) therapy. Etiology agents were *E. coli* (47%), *K. pneumoniae* (44%), *E. cloacae* (7.5%), *P. mirabilis* (1.5%). At the time of ESBLs BSI onset fluoroquinolones prophylaxis and antibiotics treatment were in 5% and in 18% pts, respectively. Carbapenem was administered to 49 (67%) pts (1st line to 10 pts, 2nd line to 39 pts). Twenty-one pts (29%) received other beta-lactams by therapy. Overall mortality was in 18 (25%) pts; of these, 5 (7%) pts had ESBLs BSI as the cause of death and 13 (18%) pts had resistant HM and infection as the cause of death.

Consecutive course of CMT was conducted in 37 (50%) pts in 39 days (by median interval). Febrile neutropenia developed in 23 (62%) pts; of these 7 (30%) pts had recurrent episode of BSI due to Enterobacteriaceae producing ESBLs (*E. coli* in five pts and *K. pneumoniae* in two pts). Carbapenem was administered to 8 (35%) pts (in two pts as 1st line, in six pts in 2nd line). Other 15 (65%) pts received other beta-lactams. One patient (4%) died (*K. pneumoniae*).

**Conclusion:** BSI by ESBL-producing Enterobacteriaceae associated with high mortality in resistant HM. Only half pts with preceding ESBLs BSI had fever after the consecutive course of CMT and the recurrence of ESBLs were in 30% of pts with febrile neutropenia. Necessity of carbapenem administration to pts with preceding ESBLs BSI not exceeded 35% in these cohort pts (67% vs. 35%,  $p = 0.007$ ).

**P2113** Prospective evaluation of SeptiFast test in patients undergoing allogeneic stem cell transplantation

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**Introduction:** A new commercially available kit (SeptiFast test) to detect DNA from 20 clinically relevant pathogens (bacteria and fungi) has recently been evaluated in cohorts of haematological patients with promising results. We evaluated the usefulness of the SeptiFast test in a cohort of patients undergoing allo-BMT in comparison to blood culture results for monitoring and diagnosing bacterial and fungal infections.

**Material and methods:** After approval from the local ethics committee and written informed consent, data from the first 50 patients from an ongoing prospective clinical study, treated at two bone

marrow transplant (BMT) units of the Charité Medical Center (RA, LU) were analyzed during March 2011 to October 2011. Patient had mostly acute leukaemia (62%) with a mean age of 52 year (54% female). Patients were monitored twice weekly for bacterial and/or fungal infections from admission until discharge from BMT unit (mean length of stay 36 day) and were evaluated according to results of blood cultures (BC) and SeptiFast PCR test results. EDTA samples were analyzed routinely twice weekly as well as when fever (FUO) occurred. In addition to the blood samples for SeptiFast test, blood cultures (10 mL) were taken when patient had fever and analysed conventionally using BACTEC 9420 system. Preparation of DNA and PCR testing from 3 mL EDTA-blood with SeptiFast was performed as recommended by the manufacturer.

**Results:** Overall 337 blood samples were analyzed (mean seven samples/patient). 30% of patients had a history of IFD and 13% a history of sepsis. Two hundred and fifty-six samples (76%) were taken during surveillance and 76 samples (22%) during fever. Seven of 69 BC's (=10%) were positive (3× *E. coli*, 4× coagulase-neg. *Staphylococci*; CoNS). SeptiFast was positive in 35 samples (9× *A. fumigatus*, 1× *C. albicans*, 1× *Enterobacter aerogenes*, 3× *Enterobacter cloacae*, 2× *Enterococcus faecalis*, 1× *Enterococcus faecium*, 3× *E. coli*, 1× *Klebsiella oxytoca*, 2× *Klebsiella pneumoniae*, 7× CoNS, 2× *Pseudomonas aeruginosa*, 1× *S. aureus*). All CoNS and *E. coli* detected in conventional blood cultures were identified by SeptiFast as well. However, the majority of other pathogens (mostly gram-negative bacteria and fungi) could only be detected by SeptiFast. Patients with presumed fungal pneumonia were positive in SeptiFast test (mostly *A. fumigatus*) but not in blood cultures.

**P2114** Dynamics of intestinal colonisation with Enterobacteriaceae with ESBL or resistant to fluoroquinolones in neutropenic oncohaematological patients undergoing levofloxacin prophylaxis

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**Objectives:** The aim of fluoroquinolone extended prophylaxis (FP) in neutropenic patients is to prevent endogenous infections. However, its use is controversial because of the risk of selecting resistant organisms. We performed a prospective follow-up study to assess potential selection of ESBL-producing (ESBL-E) and/or fluoroquinolone resistant (FQR-E) Enterobacteriaceae in the intestinal compartment.

**Methods:** We studied 25 neutropenic patients (July-2009 to December-2010) with acute leukaemia and lymphoma treated with chemotherapy. Fecal samples were obtained before starting the prophylaxis (at admission) and twice weekly during and after prophylaxis suppression. Samples were plated on MacConkey agar + cefotaxime (1 mg/L) for ESBLs-E isolation and/or carbapenemases and MacConkey + ciprofloxacin (0.1 mg/L) for the detection of FQR-E. A follow-up of the clinical samples, including blood cultures was done. Clonal diversity was studied by PFGE-XbaI and ESBLs characterized by PCR and sequencing. The presence of FQR qnr and aac(6)-Ib-cr genes was investigated by PCR.

**Results:** Two hundred and eighty-two faecal samples from 25 patients (mean age 45 years; 17 women) corresponding with 37 episodes of neutropenia were processed. Patients received an average of 18 days of FP per episode. Seventy-one resistant Enterobacteriaceae isolates were recovered from 13 patients (52%) [49 FQR-*E. coli*, 17 ESBL-*E. coli* (10/17 strains presented co-FQR), Four ESBL-*K. pneumoniae* and 1 ESBL-*K. oxytoca* also FQR]. No carbapenemase producers were isolated. Before the start of FP, 20% (5/25) of patients were colonized with FQR-E and 4% (1/25) by ESBLs-E. During prophylaxis, 32% (8/25) were colonized by FQR-E while ESBL-E colonization was not detected. After prophylaxis, the patients colonized by FR-E increased to 44% (11/25) and ESBL-E were detected in 24% of patients (6/25). By PFGE, 29 different *E. coli*, three *K. pneumoniae* and one *K. oxytoca* strains were detected. The ESBL type most frequently identified was CTX-M-9 (59%). qnr gene was detected in two strains, while non of them harboured the aac(6)-Ib-cr gene. Five FQR-*E. coli* isolates were recovered from blood cultures of two

patients, one with an indistinguishable PFGE-pattern with that obtained from the corresponding fecal isolate.

**Conclusions:** After FP a high percentage of patients were colonized by FQR-E exhibiting a high clonal diversity. Selection of resistant Enterobacteriaceae in the intestinal compartment increase the risk of bacteremia with FQR isolates.

**P2115 Long-term impact of the discontinuation of levofloxacin use for antimicrobial prophylaxis in neutropenic patients**

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**Background:** In Dec 2009 due to rising Enterobacteriaceae resistance to quinolones (QNL) in hematological patients, use of levofloxacin (LVF) for antibacterial prophylaxis was discontinued at our center.

**Objectives:** To assess the long-term impact of discontinuation of LVF as antibacterial prophylaxis in neutropenic patients.

**Methods:** Aggregated yearly data on global and specific antimicrobial consumption, gram-negative bloodstream infection and QNL resistance rates among gram-negative bacteria isolated in blood cultures were retrospectively analyzed (01/2007 to 09/2011). To control whether changes in LVF consumption were influenced by changes in the density of neutropenic patients we considered trimethoprim/sulfamethoxazole (TMP/SMX) consumption as an internal control.

**Results:** Yearly data on antimicrobial consumption, frequency of bloodstream infection caused by gram-negative bacteria and QNL resistance among gramnegatives are presented in Table 1. Overall, a 46.8% decrease in LVF consumption was observed after the decision to discontinue QNL prophylaxis among neutropenic patients (16.7 vs. 8.9 DDD/100 patient-days;  $p < 0.001$ ). During the same period, TMP/SMX consumption was reduced by 10.5% (12.3 vs. 11.1 DDD/100 patient-days;  $p = 0.047$ ). The rate of bacteremia caused by gramnegative microorganisms did not significantly increase after discontinuation of antibacterial prophylaxis with QNL (0.36 vs. 0.41 episodes/100 patient-days;  $p = 0.708$ ). The rate of QNL-resistance among gram-negative isolates obtained in blood cultures decreased from 51.1% to 36.5% after cessation of prophylaxis with LVF, although difference was not significant ( $p = 0.170$ ). Overall antibacterial consumption slightly decreased after discontinuation of prophylaxis with QNL (170.2 vs. 157.8 DD/100 patient-days;  $p < 0.001$ ), although an increase in consumption of antipseudomonal agents was observed (67.3 vs. 74.4 DDD/100 patient-days;  $p < 0.001$ ), mainly due to an increase in cefepime use (8.3 vs. 19.9 DDD/100 patient-days;  $p < 0.001$ ).

ANTIMICROBIAL	CONSUME	2007	2008	2009	2010	2011	BEFORE	AFTER
Patient-day	Total	8178	8082	8429	7320	8436		
Levofloxacin	DDD/100 patient-day	18.37	10.52	11.57	10.18	7.88	18.34	8.90
Trimethoprim	DDD/100 patient-day	15.99	12.63	11.43	11.21	10.61	12.30	11.11
Antibacterial	DDD/100 patient-day	182.09	158.76	168.91	158.11	157.81	170.25	157.86
Group 3	DDD/100 patient-day	66.53	58.93	70.67	69.72	79.21	67.38	74.48
Meropenem	DDD/100 patient-day	41.24	38.32	41.52	39.88	33.69	42.36	38.79
Cefepime	DDD/100 patient-day	7.37	3.11	14.04	13.40	26.40	8.31	19.90
MICROBIOLOGY		2007	2008	2009	2010	2011	BEFORE	AFTER
GNB bloodstream infection	Total	29/100	27/100	37/100	29/100	23/100		
	100 patient-day	0.35	0.37	0.44	0.40	0.42	0.36	0.41
Enterobacteria bloodstream infection	Total	20/100	19/100	20/100	24/100	17/100		
	100 patient-day	0.24	0.22	0.24	0.33	0.31	0.23	0.32
Pseudomonas bloodstream infection	Total	5/100	3/100	11/100	4/100	3/100		
	100 patient-day	0.06	0.36	0.12	0.49	0.35	0.08	0.34
GNB Quinolone resistance	Total	12/100	10/100	22/100	10/100	9/100	14/87	9/30
	%	41.4%	45.5%	59.5%	34.5%	36.1%	48.8%	36.3%

TABLE 1. YEARLY AGGREGATED DATA ON ANTIMICROBIAL CONSUMPTION AND MICROBIOLOGICAL EVENTS. Group 3= antipseudomonal agents. GNB: Gramnegative Bacilli

**Conclusions:** Discontinuation of prophylaxis with LVF among neutropenic in a hematological ward was associated with a trend to decline in the rate of QNL-resistance among clinical isolates of gram-negative microorganisms, without significant changes in the rate of bacteremia caused by these microorganisms. Policies of antibacterial prophylaxis with QNL should be adopted according to its expected benefit given the local epidemiological background.

**P2116 Evaluation of complications related to central venous catheters in patients with haematological disease in a single centre: a 3-year experience**

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**Objectives:** In haematology divisions using central venous catheters (CVC) for the administration of chemotherapy is become indispensable however they are associated with a range of complications. We want to verify the incidence of complications both for the short-term CVC (Certofix type) and for tunnelled CVC (Hickman type).

**Methods:** A retrospective study was conducted on a series of 220 consecutive patients hospitalized in the Division of Adult Hematology and Bone Marrow Transplant of San Gerardo Hospital in Monza during the period between January 2008 and February 2011 with hematological diseases undergoing to intensified cycles of chemotherapy.

**Results:** Patient characteristics and CVC are shown in Table 1.

The total time of catheterisation was 9836 days for Certofix CVC (CC) and 16 270 days for Hickman lines (HC). 95 (19.6%) CVC were removed for infectious diseases (76.8% CC and 20% HC). The removal rate of each type of CVC for an infective cause was 21.2% for CC and 16.4% for HC.

In 58 cases it was possible to demonstrate microbiologically a CVC-related infection (CRI). The remaining CVC were removed by clinically proven infections.

The main diagnostic criteria for CRI was the differential time (more than 2 hours) from positive CVC-blood culture (BC) and peripheral BC (33 cases). In eight cases the infections were demonstrated thanks to a quantitative BC and in 20 cases thanks to CVC-tip culture when the line was removed. CRI were caused by 31 gram positive (22 Staphylococcus Epidermidis), 25 gram negative (three Escherichia Coli, seven Pseudomonas Aeruginosa) and two Candidae.

We have calculated the rate of CRI in our population: CC infections rate for 1000 days of catheterization is 4.3 instead HC infections rate is 0.9 (including only the microbiologically proven CVC-related sepsis).

These rates increased if were included clinically suspected infections (respectively 7.5 and 1.2 for 1000 days).

Gender:	
Male	120
Female	100
Age:	
< 65 years	156
> 65 years	64
Disease:	
Acute myeloid leukemia	159
Lymphoproliferative disease	48
Others	13
CVCs type:	
Single lumen Certofix line	1
Bilumen Certofix line	345
Triple lumen Certofix line	17
Single lumen Hickman line	116
Bilumen Hickman line	1
Peripherally inserted central catheter (PICC)	3
Porth-a-cath	3
CVCs duration of positioning (days):	
bilumen Certofix line	27,6
Triple lumen Certofix line	20,5
Single lumen Hickman line	178,9
PICC	16
Porth-a-cath	352,7

There was 16 insertion site infections of CC and three tunnel infection in HC (respectively 4.4% and 2.6%).

There were nine mechanical complications and 14 thrombotic complications.

**Conclusions:** This work shows that the positioning of a CVC in an haematological patient is a feasible and safe procedure. The main complication remains CRI (20% CVC was removed because of infection in our series). Even if our infections rate is much lower than what is reported in literature the relatively high infectious rate highlights the need to improve CVC management, vital part of care for this population.

#### **P2117** A case series of *Nocardia* brain abscesses: review of treatment and outcomes

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**Objectives:** *Nocardia* species are soil borne actinomycetes, and are responsible for a wide spectrum of diseases including brain abscesses. *Nocardia* brain abscesses may be associated with a high mortality rate, especially in immunocompromised patients. The choice and duration of antibiotic treatment are often not standardised.

We aim to describe cases of *Nocardia* brain abscesses, and review their treatment and outcomes.

**Methods:** *Nocardia* brain abscess is defined as clinical presentation consistent with a brain abscess with radiological confirmation, and microbiological diagnosis of *Nocardia*, with *Nocardia* species isolated from either direct (brain abscess) or indirect specimens (respiratory specimens and others) by culture and/or 16S rRNA gene sequencing. We identified retrospectively all cases of *Nocardia* brain abscesses from 2003 to 2010 from the laboratory systems of three hospitals in New South Wales, Australia—Nepean Hospital, Liverpool Hospital, and John Hunter Hospital. Case records were reviewed. Information was obtained including patient demographics, clinical features, treatment received, radiological and survival outcome.

**Results:** There were 17 cases identified from the three hospitals in this case series. The age of the patients ranged from 43 to 86. Nine of the 17 patients were male. Thirteen number of patients (76%) were immunosuppressed prior to acquiring infection. None of the patients were known to have positive HIV serology.

The median duration of neurological symptoms at the time of diagnosis was 21 days. The area of the brain most commonly affected was the parietal lobe, in 11 (65%) of the 17 patients. Pulmonary involvement was seen in 13 cases (76%).

The most common isolate was *Nocardia asteroides* complex, found in nine (53%) cases.

The average time to procuring antibiogram was 12 days. 11 (65%) patients were given an antibiotic regimen with meropenem and trimethoprim-sulfamethoxazole.

Eight (47%) patients had drainage and/or excision surgery, whereas 4 (24%) had stereotactic biopsy and/or aspiration, and 5 (29%) had no surgical intervention. Abscess resolution was seen in 8 (47%) patients on follow-up imaging. Survival rates were 88% at 30 days and 76% at 12 months.

**Conclusion:** The clinical outcome and therapy for *Nocardia* brain abscesses may be dependent on the extent of the disease, the species of *Nocardia*, and the underlying host factors.

#### **P2118** Infectious complications in paediatric solid organ transplant recipients

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**Objectives:** Infectious complications are associated with increased morbidity and mortality in solid organ transplant recipients. Few studies focused on the paediatric population. Aim of our study was to analyze the characteristics and risk factors of post-transplant infections

in a single center-cohort of paediatric heart or kidney transplant recipients.

**Methods:** Medical records of children who underwent heart or kidney transplantation in Turin, Italy, between 2000 and 2010 were retrospectively analysed. Patients with a follow-up <6 months were excluded from the study. Statistical analysis was performed using SPSS 15 for windows.

**Results:** Fifty-three patients (13 heart-s and 40 kidney-recipients) were included. Mean age at time of transplantation was  $4.4 \pm 4.46$  years and  $10.4 \pm 5.56$  years, respectively in heart- and in kidney-recipients ( $p < 0.0001$ ). Overall, 130 infections were documented. The majority (56.9%) of them occurred during the third period (>180 days) after transplantation. Respectively 15.4% and 27.7% of infectious complications were recorded in the first (<30 days) and in the second (between 30 and 180 days) period. Bacterial complications were predominant (58.8%), followed by viral infections (36.2%). Fungal infections were rare (3.8%). Specific localizations included systemic infections (25.5%), lower respiratory tract (23.3%), urinary tract (15.3%), digestive tract (10.9%) and ear, nose and throat (10.2%). The most frequently encountered pathogen was CMV (13.8%), followed by *E. coli* (7.7%), EBV and *Staphylococcus* spp. (4.6% each). The number of infections per patient was significantly higher in heart-recipients considering either global infections (5.46 vs. 1.55,  $p < 0.0001$ ) or viral (1.92 vs. 0.53,  $p = 0.002$ ) and bacterial infections (2.75 vs. 0.9,  $p = 0.002$ ). Survival rates at 1, 3 and 5 years were significantly lower in the heart-transplant group (70% vs. 97%;  $p = 0.0007$ ). By univariate analysis, risk factors for infection included younger age ( $p < 0.001$ ), surgery prior to transplantation ( $p = 0.05$ ), positive donor CMV serology with negative recipient results ( $p = 0.038$ ), and heart-transplantation vs. renal-transplantation procedure ( $p < 0.0001$ ).

**Conclusions:** Our study confirms the high rate of infectious complications in paediatric transplant recipients, particularly in heart-recipients. This finding underlines the crucial role of pre-transplant infectiological screening and of post-transplant prophylaxis.

#### **P2119** Delayed sternal closure after heart transplantation and nosocomial infection

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**Objectives:** Delayed sternal closure (DSC) is an useful surgical technique in the early management of the hemodynamic instability related to the closure of the sternotomy after cardiac surgery. There are few data about the risk of developing infections derived from this technique, and they are not data in the case of heart transplant surgery. The aim of this study was to analyze the risk of infection in heart transplant recipients who underwent DSC.

**Material and methods:** Prospective cohort study of all patients who underwent heart transplant surgery that required DSC from 1st of January of 1993 to 31st of December of 2010. Data about epidemiological characteristics of patients, surgery and complications that occurred in the postoperative period were collected.

**Results:** There were a total of seven patients: 6 (85.7%) males, with a mean age of 61.4(SD 6.5)years. Four patients (57.1%) had a Charlson index = 4. ASA index was >4 in all cases. Two (28.6%) patients were diabetic. Heart transplantation was done because of severe coronary ischaemia (71.4%) and dilated miocardiopathy (28.6%). Mean duration of surgery was 426.6 (74.3)minutes. All patients received preoperative antibiotic prophylaxis with cefazolin, 43% of them received prolonged prophylaxis during more than 2 days. Duration of ventilatory support was 10.7 (5.1) days. Indications of DSC were uncontrollable bleeding in 4 (57%) patients and cardiac compression in 3 (43%) patients. The average time that sternotomy remained opened was 1.5 (0.9) days. One patient developed mediastinitis due to *S. epidermidis* and *C. glabrata*. There were no cases of superficial or deep surgical site infections. Other infections recorded were: 5(71.5%) low respiratory tract infections and 1 (14.3%) catheter-related bacteraemia. Mean duration of DSC was 2.3 (1.7)days. The average hospital stay was

29.7 (29.7)days. Mortality was 71.4%, occurred at 1.6 (0.9)days after heart transplant. Two patients died without completing the sternal closure. The causes of death were: cardiogenic shock in 3 (60%) patients, septic shock in 1 (20%) patient and combination of both causes in 1 (20%) patient.

**Conclusions:** Heart transplant patients who required DSC are a high-risk group of patients (Charlson index >4 in 57.1% patients). Patients underwent DSC have most often respiratory tract infections (71.4%). However, risk of surgical site infections in patients underwent DSC after heart transplant is low (14.3%). Mortality after DSC (71.4%) was mainly due to non infectious complications.

**P2120 Microbiology from bronchoscopy in haematologic patients: comparison of stem cell recipients with non-recipients**

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**Objectives:** We investigated microbiologic results of bronchoscopy (BRS) in haematologic patients with pneumonia and compared microbiology from haematopoietic stem cell recipients (HSCRs) with that from non-recipients (non-HSCRs).

**Methods:** We retrospectively reviewed medical records of consecutive haematologic patients who experienced BRS from APR 2009 to AUG 2011 at the Catholic haematopoietic stem cell transplantation centre in Korea.

**Results:** A total of 176 cases were identified. Mean age was  $47.7 \pm 15.4$  years, and 122 patients (69.3%) were men. Underlying diseases were acute myelogenous leukemia (n = 65, 36.9%), acute lymphoblastic leukemia (n = 24, 13.6%), myelodysplastic syndrome (n = 26, 14.8%), etc. HSCR group accounted for 48.9% (n = 86), of which 87.2% (n = 75) were allo-HSCR. The number of culture-positive bacterial, fungal, and tuberculosis (TB) cases from bronchoalveolar lavage (BAL) were 44 (25.0%), 5 (2.8%), and 11 (6.3%), respectively. *Acinetobacter baumannii* (n = 10) was the most common bacterial isolate followed by methicillin-resistant *Staphylococcus aureus* (n = 9) and coagulase negative staphylococci (n = 6). Four *Aspergillus*, one *Rhizopus* and five non-TB mycobacterial cases were identified. *Pneumocystis jiroveci* polymerase chain reaction (PCR) was positive in 32 cases (18.2%). Multiplex PCR for respiratory viruses were performed in 147 cases, of which 21.1% (31/147) were positive. Respiratory syncytial virus (n = 11) was the most common virus followed by human rhinovirus (n = 10), parainfluenza virus (n = 9), influenza A (n = 4) and metapneumovirus (n = 1). In four cases, two different viruses were positive. One hundred thirty one cases were

Characteristics	HSCRs (n=86)	Non-recipients (n=90)	OR (95% CI)	P value
Mean age $\pm$ SD	45.5 $\pm$ 15.4	49.9 $\pm$ 15.2	-	0.055
Male gender	60 (69.8)	62 (68.9)	1.04 (0.55-1.98)	0.899
Underlying disease				
Acute myelogenous leukemia	39 (45.3)	26 (28.9)	2.04 (1.10-3.81)	0.024
Acute lymphoblastic leukemia	12 (14.0)	12 (13.3)	1.05 (0.45-2.49)	0.905
Myelodysplastic leukemia	11 (12.8)	15 (16.7)	0.73 (0.32-1.70)	0.528
Lymphoma	4 (9.3)	9 (10.0)	0.92 (0.34-2.31)	0.876
Multiple myeloma	6 (7.0)	6 (6.7)	1.05 (0.33-3.39)	0.935
Aplastic anemia	3 (3.5)	9 (10.0)	0.33 (0.09-1.25)	0.133
Chronic myelogenous leukemia	0 (0)	5 (5.6)	1.06 (1.01-1.11)	0.059
Others	7 (8.1)	8 (8.9)	0.81 (0.32-2.62)	0.859
No. of cases with positive cultures				
Bacterial culture	21 (24.4)	23 (25.6)	0.94 (0.48-1.86)	0.862
TB culture	6 (7.0)	5 (5.6)	1.28 (0.37-4.34)	0.697
Fungal culture	2 (2.3)	3 (3.3)	0.69 (0.11-4.24)	1.000
PCR results*				
<i>Pneumocystis jiroveci</i> (n=146)	15 (20.3)	17 (23.6)	0.82 (0.38-1.80)	0.626
RV multiplex PCR (n=147)	19 (25.3)	11 (15.3)		0.130
Cases with all test results* (n=131)				
Negative for all tests	25 (37.3)	25 (39.1)	0.93 (0.46-1.88)	0.087
Positive for more than one test	10 (14.9)	12 (18.8)	0.76 (0.30-1.91)	0.558

\* Some patients were excluded.

Abbreviations: HSCR, hematopoietic stem cell recipient; OR, odds ratio; CI, confidence interval; SD, standard deviation; PCR, polymerase chain reaction; RV, respiratory virus

performed for all aforementioned tests, among them, 38.2% (n = 50) were negative for all tests and 17.6% (n = 23) revealed more than one isolate. Comparison of the microbiologic results from between HSCRs and non-HSCRs is presented in the Table 1. Acute myelogenous leukemia was more common in the HSCR group (45.3% vs. 28.9%, odds ratio 2.04, 95% confidence interval 1.10-3.81, p-value 0.024). Microbiologic results were not significantly different between the two groups.

**Conclusion:** Various organisms were found from BAL in haematologic patients with lung infiltration. There were no significant microbiologic differences between HSCRs and non-HSCRs.

**P2121 MRSA screening among pre- and post- liver transplanted patients: direct detection of MRSA using PCR method comparing with conventional culture**

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**Objective:** MRSA is an important cause of infection among liver transplanted patients. Rapid detection of methicillin-resistant *Staphylococcus aureus* (MRSA) colonization can be a useful tool to control MRSA nosocomial infections. The aim of this study was to compare the use of polymerase chain reaction (PCR) screening method with standard microbiologic culture for the detection MRSA colonization in pre- and post- liver transplantation patients.

**Methods:** The study was performed in a university hospital located in Brazil from August 2010 to July 2011. A total 106 patients with liver disease and 40 liver transplanted patients treated at Hospital das Clinicas of University of Sao Paulo were evaluated. Nasal and groin swabs were collected and submitted to standard culture and PCR methods. The swabs were collected in pairs, with one swab was for microbiological culture and the other one for specific genes detection by PCR. The culture was performed with selective medium and PCR reactions were done according protocols previously described. PCR of 16S rRNA were performed before the specific PCR with the aim of determine if DNA extraction was successful. A multiplex PCR assay was performed for the identification of major types of staphylococcal cassette chromosome mec (SCCmec).

**Results:** A total of 520 swabs were collected (292 nasal and 228 groin swabs) from all 146 patients. For culture method we used 146 nasal swabs and 114 groin swabs. The others swabs were used to perform PCR technique. Of the total of 146 nasal swabs, 21 (14.4%) were MRSA culture positive and 100 (68.5%) had PCR amplification for coA and mecA genes. Of the 114 groin swabs, only 13 (8.9%) showed positivity for MRSA culture and 88 swabs (60.3%) were positive for both genes by PCR technique. MRSA colonization was seen in 68.5% patients by PCR method but only 30 (20.5%) patients had positive results by conventional culture method. Among 71 coA/mecA positive nasal swabs from pre-liver transplanted patients, 18 (25.4%) were defined as SCCmec type II, 12 (16.9%) SCCmec type I, 10 (14.1%) SCCmec type III, 7 (9.9%) SCCmec type IVa and only two (2.8%) SCCmec type IVb.

**Conclusion:** PCR is a better screening method than culture, culture of groins did not increase the positivity of surveillance and SCCmec type II was the most frequently detected. Clinicians may be able to use the nasal swabs using PCR results to prevent and control MRSA infection in this population of patients.

**P2122 Frequency and genotypic characterisation of microsporidia among patients with renal transplantation, human immunodeficiency virus and haematological malignancy**

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**Background:** Microsporidium, an opportunistic protozoan, infects immunocompromised hosts. Though patients with renal

transplantation (RT) and hematological malignancy (HM) are immunocompromised, data on microsporidiosis among them and studies on comparative utility of its various diagnostic methods are scanty. Accordingly, we aimed to study, (i) frequency of microsporidia among patients with HIV, RT and HM, (ii) Comparative evaluation of calcoflour white stain and polymerase chain reaction (PCR) taking modified trichrome stain as gold standard, (iii) genetic characterization of Microsporidia.

**Methods:** Five hundred and fifty stool samples from 182 patients with HIV, RT, HM were examined from April 2010 to August 2011 for microsporidia by stool microscopy [modified trichrome and calcoflour white stains (n = 182)], PCR was done in all stool samples positive by calcoflour white stain [n = 70]. Species identification by restriction fragment length polymorphism (RFLP) and confirmed by sequencing.

**Results:** Eighteen of 182 (10%) patients (12/18, 66.7% male) and none of 70 healthy subjects were positive for *Microsporidium* (P = 0.003) by modified trichrome stain. 70/182 (38.5%) patients were positive by calcoflour white stain. Microsporidia was significantly detected among patients with RT and HIV as compared with healthy subjects using modified trichrome stain (12/121, 10% vs. 0/70; p = 0.003 and 4/35, 11.4% vs. 0/70; p = 0.01). Microsporidia were more often detected in patients with diarrhea than those without diarrhea (15/103, 14.6% vs. 3/79, 3.8%, p = 0.05). 11/14 patients had CD4 count <200 cells/ $\mu$ L. Sensitivity and specificity of calcoflour white stain and PCR were 100%, 68.3% and 88.9%, 100%, respectively. 16/18 (88.9%) were positive by PCR and *Enterocytozoon bienersi* (*E. bienersi*) was identified in all of them which was confirmed by sequencing.

**Conclusions:** Microsporidia is an important cause of diarrhea in patients with HIV and RT. Stool PCR has high specificity than calcoflour white stain to diagnose Microsporidia. *E. bienersi* is the common species causing intestinal microsporidiosis in India.

#### P2123 Feasibility of active surveillance cultures in paediatric patients with haemato/oncologic diseases

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**Objectives:** Active surveillance cultures (ASC) in critically ill patients are used to predict possible causative pathogens of subsequent blood stream infections (BSI). Feasibility is under discussion. Paediatric data are scarce. The objective of the study was to assess congruence of isolated pathogens in ASC and in subsequent BSI in paediatric haemato-/oncologic patients.

**Methods:** At the Division of Paediatric Haematology and Oncology of the Medical University Graz, ASC are routinely taken before and during prolonged episodes of neutropenia. In febrile patients, blood cultures are drawn before initiation of empiric antimicrobial therapy. In a retrospective analysis of the years 1999 through 2010, we compared isolates causing bacteraemia with the results of ASC obtained in the respective patients within 4 weeks prior BSI. Bacteria known as typical contaminants (e.g. coagulase-negative *Staphylococci*, corynebacteria) were considered as contamination unless they were isolated in at least 2 blood cultures within the same episode.

**Results:** Out of 3376 blood cultures drawn during the analysed period, an organism was isolated in 190 (5.6%). These isolates were assigned to 66 BSI episodes in 57 patients.

In 26 episodes, no ASC had been performed within 4 weeks prior to BSI. In the remaining 40 episodes, underlying diseases were haematologic malignancies (n = 24), non malignant haematologic diseases (n = 5), and solid tumors (n = 11). Thirteen episodes occurred after allogeneic (n = 8) or autologous (n = 5) SCT. Median age at BSI was 8.1 years (5 months–28.8 years).

In 31/40 (77.5%) episodes the causative agent had been isolated by ASC. Details see Table 1.

**Conclusion:** In the majority of cases, the causative agent of a subsequent BSI had been isolated in advance by means of ASC.

	BSI	Congruence of ASC prior to BSI	
		pos. (%)	neg.
<i>Staphylococcus aureus</i>	2	1 (50)	1
other gram-positive cocci	10	8 (80)	2
gram-positive rods	2	0 (0)	2
<i>Pseudomonas aeruginosa</i>	8	8 (100)	0
<i>Enterobacteriaceae</i>	14	12 (86)	2
yeasts	3	2 (67)	1
others	1	0 (0)	1
<b>total</b>	<b>40</b>	<b>31 (77.5)</b>	<b>9</b>

Table 1. Congruence of isolated pathogens in blood stream infections (BSI) and active surveillance cultures (ASC)

Therefore, ASC might assist in the choice of the empiric antimicrobial treatment in febrile paediatric haemato-/oncological patients.

## AIDS and HIV infection

### P2124 Time to initiation of antiretroviral therapy between 4 and 12 weeks of tuberculosis treatment in HIV-1 infected patients. Results from the TIME Study

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**Background:** Optimal timing for initiation of antiretroviral therapy (ART) among HIV-1 infected patients with tuberculosis (TB) is limited in the setting of early ART at CD4 count of <350 cells/mm<sup>3</sup> in the middle income countries.

**Methods:** Thai HIV/TB co-infected patients who had CD4 count of <350 cells/mm<sup>3</sup> and diagnosed TB were randomized to initiate a once daily regimen of tenofovir/lamivudine/efavirenz at 4 weeks (group A) vs. 12 weeks (group B) of TB treatment between 2009 and 2011. The primary endpoints were all-cause mortality and hospitalization according to an intent-to-treat analysis.

**Results:** Of 156 patients, 79 were in group A and 77 patients were in group B. Overall mean  $\pm$  SD age was 38  $\pm$  9 years; median (IQR) CD4 was 43 (47–106) cells/mm<sup>3</sup>; and median (IQR) HIV-1 RNA was 5.8 (5.4–6.3) log copies/mL. Eighty-three (53%) patients were diagnosed extra-pulmonary or disseminated TB. Eleven (7%) mortalities occurred in a totaling of 137 patient-years of follow-up. Seven percent (6/79, 8.76 per 100 patient-years) mortalities were in group A and 6% (5/77, 7.25 per 100 person-years) mortalities were in group B (OR = 0.845, 95%CI = 0.247–2.893, p > 0.99). The same trends were found in the subgroup of patients with baseline CD4 count <100 cells/mm<sup>3</sup> (9% vs. 13%, RR = 1.591, 95%CI = 0.396–6.397, p = 0.725) and <50 cells/mm<sup>3</sup> (8% vs. 10%, RR = 1.239, 95%CI = 0.338–4.542, p = 0.753). Twenty-eight (35%) patients in group A and 25 (32%) patients in group B were hospitalized (OR = 1.142, 95%CI = 0.588–2.217, p = 0.737). Grade 2–4 adverse events related to ART and TB treatment were 39% (31/79) in group A and 34% (26/77) in group B (OR = 1.267, 95%CI = 0.659–2.435, p = 0.509). In multivariate analysis adjusting for timing to initiating ART, 'low albumin' (OR = 3.717, 95%CI = 1.529–9.009, p = 0.004) and 'low baseline CD4 count' (OR = 1.014, 95%CI = 0.999–1.029, p = 0.061) were the independent predictors of all-cause mortality. Immune reconstitution inflammatory syndrome was more frequent in group A with an incidence of 8.86 vs. 5.02 per 100 person-months in group B over the first 6 months of ART (p = 0.069).

**Conclusions:** In this study which conducted in a middle income country with early ART at CD4 count of <350 cells/mm<sup>3</sup>, survival advantage associated with very early initiating ART in HIV-infected patients with active TB was not found in any CD4 stratum. However, patients with low albumin and low baseline CD4 count were associated with higher risk of death.



**P2125 Association between Vitamin D and viral load (HIVRNA) among HIV-infected patients**

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**Objectives:** (i) Quantify the prevalence of low 25-OH vitamin D (vitD) levels among HIV patients, (ii) Identify the vitD breakpoint associated with an increased risk of HIVRNA > 400 copies/mL, (iii) Determine if vitD is independently associated with HIVRNA.

**Methods:** A cross-sectional study was performed among patients at the Albany Medical Center between January 2007 and July 2011. Inclusion criteria were: (i) age ≥18 years, (ii) HIV-infection, (iii) availability of ≥1 vitD level in the medical chart and (iv) ≥1 HIVRNA laboratory result within the study period. The following data were extracted from the patients' medical records: demographics, co-morbid conditions, serum creatinine, CD4 count, HIVRNA, and medication histories. The Institute of Medicine classification system was used to characterize low vitD and was defined as <20 ng/mL. Classification and Regression Tree (CART) software was used to identify the breakpoint in vitD associated with HIVRNA > 400 copies/mL. Linear regression was used to determine the independent predictor variables of log-HIVRNA.

**Results:** There were 475 patients that met inclusion criteria. The median (IQR) age of the patients at the time vitD was obtained was 49 (43–56) years. The median (IQR) vitD level was 26.3 (16.5–34.9). The period prevalence of low vitD (<20 ng/mL) was 34.3%. Variables associated with low vitD are displayed in Table 1. CART was used to identify the breakpoint in vitD associated with HIVRNA > 400 copies/mL. The CART-derived breakpoint was 25 ng/mL and significantly differed by HIVRNA status. There were 192 (45.8%) with vitD levels <25 ng/mL among the 419 patients with HIVRNA <400 copies/mL. Among the 56 patients with HIVRNA ≥400 copies/mL, there were 37 (66.1%) patients with vitD levels <25 ng/mL. In the bivariate analyses, the clinical covariates associated with HIVRNA < 400 copies/mL were age, dyslipidemia, osteoporosis, and antiretroviral therapy. The specific antiretrovirals associated with HIVRNA < 400 copies/mL were abacavir, emtricitabine, lamivudine, tenofovir, efavirenz, lopinavir, enfuvirtide and maraviroc. In the linear regression analyses, the use of antiretroviral therapy and vitD < 25 ng/mL were significant predictors of log-HIVRNA and the resulting linear regression equation was: log-HIVRNA = 3.06 + 0.16\*(vitD < 25 ng/mL) - 1.32\*(antiretrovirals).

**Table 1: Clinical Covariates Associated with Low Vitamin D among HIV-infected Patients**

Covariate	High Vitamin D (n = 312)	Low Vitamin D (n = 163)	P-value
Mean (SD) age	49.9 (9.4)	48.1 (9.9)	0.05
Mean (SD) weight (kg)	78.0 (17.4)	82.3 (19.7)	0.02
Osteopenia	36 (11.5)	9 (5.5)	0.03
Arthritis	45 (14.4)	13 (8.0)	0.04
Obesity	17 (5.4)	18 (11.0)	0.03
Dialysis	1 (0.3)	5 (3.1)	0.02
Any Antiretrovirals	296 (95.2)	148 (90.8)	0.06
• Efavirenz	73 (23.5)	53 (32.5)	0.03
• Lopinavir	37 (11.9)	9 (5.5)	0.03
Multivitamin	159 (51.1)	48 (29.4)	<0.001
Mean (SD) CD4 count	639 (346)	555 (286)	0.005
Mean (SD) log-HIVRNA	1.83 (0.62)	2.04 (1.01)	0.02

All data presented as n (%), unless otherwise noted

**Conclusion:** The prevalence of low vitD is high among HIV patients and levels <25 ng/mL are independently associated with log-HIVRNA.

**P2126 Newly diagnosed cases of HIV infection**

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**Objectives:** The aim of this study is to describe the epidemiological features of patients diagnosed of new HIV infection during 2011 in our hospital. Our hospital gives care to the urban area of Pamplona and is the reference hospital for the region of Navarra (Spain). Since the

beginning of AIDS epidemics until December 2009, 1834 HIV infections have been diagnosed in the population who lives in Navarra. New cases diagnosed in the period 2005–2009 have been related with heterosexual contact in 58%, 21% men who have sex with men, and 10% with injecting drug users (IDUs). The reported HIV diagnoses have increased from 12 748 in 2000 to 24 703 in 2009 in Western Europe. This is an increase from 5.5 cases to 6.7/100 000.

**Methods:** Newly diagnosed cases were found by a search on the Laboratory information system (LIS). The diagnosis were made by means of Chemiluminescence (Abbott Architect HIV Ag-Ab Combo Assay) and the confirmatory test an immunoblot assay (INNO-LIA™), to confirm the presence of antibodies against HIV-1, and HIV-2. HIV 1 viral loads are measured with the COBAS AmpliPrep/COBAS TaqMan HIV-1 Assay (Roche). Study period: January–October 2011.

**Results:** Thirty-four new cases have been diagnosed during the study period. One of them was an HIV two infection. The mean age of the patients was 36 years. Sex: 25 men (73.5%), nine women (26.5%). Mode of acquisition: 33 sexual contact or unknown (97%) and one injecting drug user (IDU) (3%). Geographical precedence: 15 were born in Spain (44%) and the other 19 (56%) were from abroad, mainly from Latin America and Africa. CD4 count/mL was available in 26 patients. In seven CD4 count was <200, in eight CD4 count was between 200 and 500 and in 11 was >500 cell/mL. Viral load was undetectable in four cases and ranged between 1500 and 2 390 751 copies/mL in 22 cases.

**Conclusions:** After a significant drop of incidence rates in the nineties, the rate of HIV infections remains in our region between 4.7 and 5.6 new cases/100 000 habitants. The majority of new cases are diagnosed in young men, and sexual contact is the main transmission route. It is remarkable de high frequency of immigrants (56%) in the new cases as they are about 12% of the population of Navarra, and the frequency of severely immunocompromised patients at the time of diagnosis. Prevention programs must be targeted at these groups, not only to avoid infection but to instruct them on early medical consultation.

**P2127 Evaluation of a new fourth-generation electrochemiluminescence immunoassay for the screening of HIV infection**

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**Objective:** The purpose of this study was to assess whether the Elecsys HIV Combi PT (Roche Diagnostics, Mannheim, Germany), a new fourth-generation electrochemiluminescence immunoassay for the screening of HIV infection, has improved sensitivity and specificity in comparison to the third-generation assays.

**Methods:** A total of 705 routine serum or plasma samples were collected in West China Hospital of Sichuan University, including Anti-HIV-1 Western blot positive adults' plasma, Unselected daily routine adults' plasma, End-stage renal disease patients' plasma, Autoimmune disease patients' plasma and other samples with interrupted virus antibodies. Three assays were compared, the Elecsys HIV Combi and Elecsys HIV Combi PT assays on the Cobas E170 analyzer (Roche Diagnostics, Mannheim, Germany) and enzyme linked immunosorbent assay (ELISA). Besides, commercially available panels (HIV Seroconversion panels, HIV-1 Antigen-lysates and HIV-1 antibody dilution) were tested by the three assays evaluated in this study. Each reactive sample must be re-determined according to manufacturers' instructions and all repeated reactive samples were confirmed with Western blot assay.

**Results:** Among the 705 samples, most results were congruous except for one specimen that was positive tested by Elecsys HIV Combi assay when non-reactive by the other two assays. And according to the results of the HIV Seroconversion panels, we found the fourth-generation Elecsys HIV Combi PT assay could detect the HIV infection earlier.

**Conclusions:** The concordance of the Elecsys HIV Combi PT assay and the Elecsys HIV Combi assay was found to be 99.86% (704/705), and to be 1 between Elecsys HIV Combi PT and ELISA. The Elecsys

HIV Combi PT assay has a shorter window period than the third-generation ELISA and ECLIA assays and this test showed satisfactory reproducibility and clinical sensitivity.

**P2128 Correlation between inflammatory markers and bone mineral density in treatment experienced HIV-seropositive patients**

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**Objectives:** HIV infection induces a chronic inflammatory syndrome, even when the viral replication is suppressed under combined antiretroviral therapy (cART). The immune activation leads to a variety of comorbidities, including the loss of bone mineral density and increased risk of fractures. Our aim was to evaluate the correlation between inflammatory markers and bone mineral density (BMD) in young HIV-positive patients under cART.

**Methods:** We conducted a cross-sectional study on HIV-infected patients undergoing stable cART for at least 6 months, in a tertiary care hospital – INBIMB, during a period of 6 months. Patients aged more than 50 years were excluded. Patients were evaluated by CD4, HIV viral load, TNF- $\alpha$ , IL-6, MCP-1, hs-PCR and DEXA scan. We used BioSource EASIA for inflammatory markers and Lunar DEXA scanner for bone mineral density. Spearman correlation was performed for statistical analysis. This study was part of an ongoing prospective Romanian research grant (SLD ART – PNCD12 no.62077/2008) on experienced HIV positive patients.

**Results:** We included 56 patients, with median age of 29.5 years and mode age of 20 years, sex ratio M/F 1.43, median CD4 cell count 536/mm<sup>3</sup>, undetectable viremia in 76% of cases. A quarter (23.6%) of patients had a T-score < -1, the majority of them being osteopenic. There were no sex statistical significant differences of the T-score. The T-score and total BMD were correlated with plasma levels of MCP-1 ( $p = 0.022$ ,  $r = 0.4$  respectively  $p = 0.038$ ,  $r = 0.36$ ) and with hs-PCR ( $p = 0.006$ ,  $r = 0.525$  respectively  $p = 0.002$ ,  $r = 0.6$ ). There were no correlation found between TNF- $\alpha$ , IL-6 and T-score or total BMD.

**Conclusions:** In our study the plasma levels of MCP-1 and hs-PCR were correlated with total bone mineral density and T-score and could predict disturbances in bone metabolism in HIV seropositive patients undergoing cART, being a useful tool in the patients' follow-up.

**P2129 Bioinformatic application to facilitate the genotypic determination of HIV-1 tropism**

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**Objectives:** We have developed a bioinformatic tool in order to simplify the computer analysis of the genotypic study of HIV tropism by means of the V3 loop of the gp120 protein. To get this, we have to analyze the FASTA sequence obtained after sequencing the V3 region by Geno2pheno (G2p) and/or WebPSSM (WP) algorithms individually. With this tool, we can obtain simultaneously the interpretation of both algorithms. Furthermore, with G2p analysis you can obtain results with two false positive ratios (% FPR). The first one is the analysis from MOTIVATE clinical data: (2% and 5.75% FPR). The second one is the Recommendations from the European Consensus Group (10%FPR).

**Methods:** We have analyzed with G2p and WP a total of 653 protein sequences of V3 regions of HIV-1 obtained from Los Alamos database and sequences analyzed in the laboratory of Molecular Microbiology of the Valme University Hospital. These sequences correspond to 443 sequences with CCR5 (R5) genotype and 123 sequences with CXCR4 (X4) genotype (X4 or R5X4). Eighty-seven sequences with discordant results (nine sequences R5 by G2p but X4 by WP, and 78 sequences X4 by G2p but R5 by WP) were also included. These sequences belong to subtypes A (80 sequences, including A1 and A2 subtypes), B (501 sequences) and C (72 sequences) of HIV-1. (The next update will

include approximately 1600 sequences with different subtypes of HIV-1 and HIV-2 and several CRFs).

**Results:** At running a new sequence, the result will show a % of similarity to any of the sequences of our database that will be used as reference sequence, and three tropism predictions of the model sequence: (i) the result obtained by analyzing the sequence of G2p with a FPR = 10%, (ii) the result obtained by analyzing the sequence by G2p with a FPR = 2.5% and 5.75% and (iii) the result obtained by analyzing the sequence with PSSM matrix using the "subtype B: X4/R5" (for C subtype we used the subtype C SI/NSI matrix). Besides, this application gets an automatically full expansion of your sequence. To test this application ten sequences randomly selected were used obtaining the same tropism interpretation in nine cases.

**Conclusion:** (i) We oversimplified the methods for tropism analysis unifying the bioinformatics tools used for determining it. (ii) We had obtained excellent results using this application, but it is necessary to increase the number of sequences in our database to optimize results and minimize discordant results that are generated after entering the sequence of study.

**P2130 Outcome of HAART-treated HIV infected patients in a resource-limited setting: the Belgrade cohort study**

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**Objectives:** To evaluate HAART we analyzed the clinical outcome of HIV-infected patients treated at the Infectious and Tropical Diseases Hospital in Belgrade, Serbia.

**Methods:** The study involved a total of 840 unselected HIV-infected patients, which were evaluated after one and after a mean  $6.6 \pm 3.4$  years of treatment. HAART was considered favorable if undetectable viremia and a rise in the CD4 count to above 350/ $\mu$ L were achieved.

**Results:** Of the 544 patients available for follow-up to the end of the study, 34 (6.2%) had treatment failure, while of the 510 (93.7%) with full virological suppression 70.1% had optimal immune reconstitution. A favorable response after the first year of treatment was the single independent predictor of a favorable response at the end of follow-up, while pre-treatment with mono or dual ART, HCV co-infection, AIDS, and baseline CD4 cell counts below 200/ $\mu$ L, were all factors preventing a favorable response ( $p < 0.01$ ). A favorable treatment response after a mean 6.6 years of HAART was the independent predictor of survival ( $p < 0.01$ ).

**Conclusion:** If patients with advanced HIV disease initiate HAART, remain compliant; reach undetectable viremia and immune recovery after 12 months, which they maintain at 6.6 years, there is a 90% probability of surviving over 14 years.

**P2131 Prevalence of transmitted antiretroviral resistance and distribution of HIV-1 subtypes among recently infected patients in Gran Canaria, Spain between 2003 and 2011**

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**Objectives:** The aims of this study were to assess the frequency of HIV-1 transmitted drug resistance (TDR) and subtypes in recently infected patients in Gran Canaria (Spain) and to describe their epidemiological characteristics.

**Methods:** The study was performed between 2003 and 2011 and included all HIV-1 recently infected patients diagnosed by antibody seroconversion observed in two samples in the last 12 months or the presence of an acute retroviral syndrome in a patient with a risk contact and a previous seronegative sample. HIV-1 reverse transcriptase and protease genes were genotyped using HIV-1 ViroSeq Genotyping System (Abbott Molecular). FASTA sequences were analyzed using the HIVDB program for the detection of resistance and the REGA HIV-1 system for subtyping. The medical records of patients were reviewed to

collect clinical and epidemiological data. Differences were examined using chi test. A p value of <0.05 was considered significant.

**Results:** A total of 80 recently infected patients were included. Seven (8.8%) patients had TDR, five (6.2%) to non nucleoside reverse transcriptase inhibitors (RTIs) with K103NS mutation and two (2.5%) to nucleosides RTIs (one with M184I and one with D67N and K219Q mutations). The prevalence of TDR was 30% between 2003 and 2005, 7.9% between 2006 and 2008 and 3.1% between 2009 and 2011 (p < 0.05). The subtype was obtained in 79 patients, 10 (12.6%) of them infected with non-B subtypes (eight CRF02\_AG, one D and one G subtypes). Recent transmission of non-B subtypes was first detected in 2006. The patient characteristics are presented in the Table 1.

**Table 1.** Clinical-epidemiological characteristics of patients with TDR and non-B subtypes

	N (%)	Patients with TDR (n:7) N <sup>o</sup> (%)	Non B subtypes (n:10) N <sup>o</sup> (%)
<b>Sex</b>			
Male	73 (91.2)	7 (100.0)	8 (80.0)
Female	7 (8.8)	0 (0.0)	2 (20.0)
<b>Age (years)</b>			
<30	32 (40.0)	2 (28.6)	4 (40.0)
30-40	30 (37.5)	3 (42.8)	3 (30.0)
40-50	15 (18.8)	2 (28.6)	1 (10.0)
>50	3(3.7)	0 (0.0)	2 (20.0)
<b>Origin</b>			
Immigrant	11 (13.7)	1 (14.3)	1 (10.0)
Natives	69 (86.3)	6 (85.7)	9 (90.0)
<b>Route of transmission</b>			
Men who have sex with men	59 (73.7)	6 (85.7)	5 (50.0)
Heterosexual	15 (18.8)	1 (14.3)	5 (50.0)
Intravenous drug use	1 (1.2)	0 (0.0)	0 (0.0)
Unknown	5 (6.3)	0 (0.0)	0 (0.0)
<b>Plasma RNA HIV-1 (log<sub>10</sub>)</b>			
<5.0	54 (67.5)	4 (57.1)	8 (80.0)
>5.0	26 (32.5)	3 (42.8)	2 (20.0)
<b>CD4 cell count/mm<sup>3</sup></b>			
≤350	3 (3.7)	0 (0.0)	0 (0.0)
350-500	4 (5.0)	0 (0.0)	0 (0.0)
>500	73 (91.3)	7 (100.0)	10 (100.0)
<b>HIV Subtype (n=79)</b>			
Non B	10 (12.6)	1 (14.3)	-
B	69 (87.3)	6 (85.7)	-
<b>TDR</b>			
Yes	7 (8.7)	-	1 (10.0)
No	73 (91.2)	-	9 (90.0)

N<sup>o</sup>(%): Number (percentage)

TDR: Transmitted drug resistance

**Conclusions:** The rate of TDR decreased in the last years, from 30% between 2003 and 2005 to 3.1% between 2009 and 2011. The TDR was detected in both B and non-B subtypes. Non-B subtypes have been detected since 2006 and they have represented 12.6% of the cases; 90% in native population and 50% in the patients infected by heterosexual route.

#### P2132 Trends in HIV incidence in Siberia, Russia (2000–2010)

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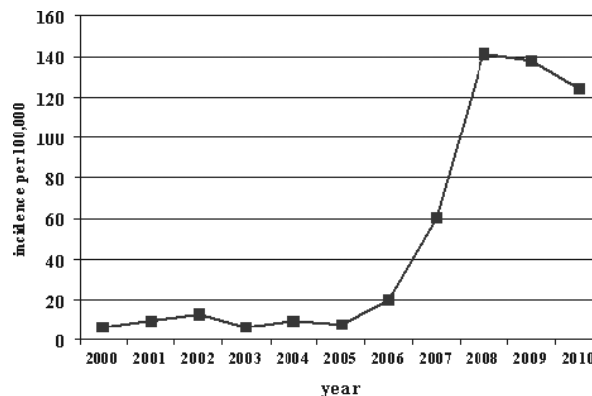
**Objective:** Currently the spread of HIV infection is acquiring an epidemic pattern in Russia. HIV incidence rate in Siberia accounts for 17.0% of all HIV infected persons in Russian Federation. Additionally, the HIV incidence rate is higher than average in Russia by 13.1%. The aim of the present study was to analyze the epidemiology of HIV infection in Novosibirsk (Western Siberia) over period from 2000 through 2010.

**Methods:** Novosibirsk is a main city of Siberia, and third in Russia according to population amount and geopolitical importance. Annual reports from district STD clinics were obtained and registered in the departments of social statistics of the regional committees of statistics.

**Results:** Trends in the incidence of HIV infection over period studied are shown in figure. These parameters raised enormously during last 4 years. The majority (65.0%) of HIV cases were unemployed, homeless, and/or prisoners. Migrants accounted for only 1.5% of those tested. Male: female ratio changed from 3:1 to 2:1 over last 2 years, whereas incidence among adolescence raised by 22%.

The analysis showed that usual routes of transmission were parenteral administration of drugs (65.6%), and sexual transmission with heterosexual contacts (20.9%). Perinatal transmission (from mother to child) accounted to 0.9%. None nosocomial infection through transfusion of

blood products was found (0%). Unknown route cases were registered in 12.6%.



**Conclusion:** At present, onset of a concentrated HIV infection phase turns to a generalized epidemic in Siberia. This tendency lags from european part of Russia for a half decade. Thus, governmental and local intervention programmes are urgently needed to prevent the forthcoming HIV epidemic in Siberia, especially among target groups.

#### P2133 Quantifying the incidence and magnitude of liver injury among HIV-infected patients receiving raltegravir-containing antiretroviral therapy

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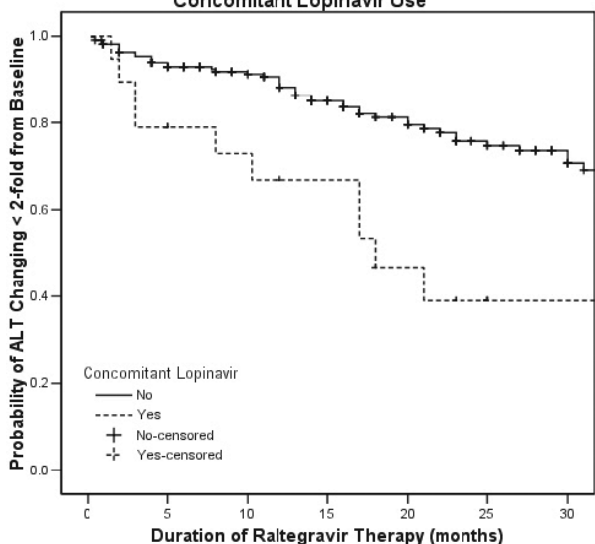
**Objectives:** (i) Quantify the incidence of liver injury among patients receiving raltegravir (RAL) and (ii) characterize the magnitude of liver injury associated with raltegravir (RAL) use.

**Methods:** A retrospective cohort study was performed at the Albany Medical Center between January 2007 and July 2011. Inclusion criteria were: (i) age ≥18 years, (ii) HIV-infection, (iii) availability of alanine aminotransferase (ALT) values in the medical chart and (iv) RAL use ≥1 month. The following were extracted from the patients' medical records: demographics, co-morbid conditions, medication histories and laboratory values. Liver injury was defined in two ways: (i) ALT increase 3× the upper limit of normal (ULN) from baseline ALT value and (ii) a relative change in ALT from baseline, calculated as the most extreme ALT value divided by the baseline ALT. For this second outcome, liver injury was defined as a twofold increase in ALT from baseline. Descriptive statistics were used to quantify the incidence and magnitude of liver injury. Kaplan Meier plot was generated and survival distributions were compared using log-rank test.

**Results:** There were 238 patients included. The mean (SD) age of the patients was 48.6 (9.0) years. The median (IQR) baseline ALT was 27.5 (20.0–41.0) IU/L. The median (IQR) value of the most extreme ALT value on RAL therapy was 43 (28–72.3) IU/L, occurring after a median (IQR) of 7 (2–15.3) months. Concomitant hepatotoxic drugs were used by 214 (89.9%) patients. Among these patients, the mean (SD) number of concomitant hepatotoxic drugs was 2.5 (1.4). Absolute ALT increases 3× the ULN were observed in eight (3.9%) patients. Concomitant lopinavir use was the only covariate associated with this outcome and was more frequent among patients that experienced an ALT increase 3× ULN than those that did not, 37.5% vs. 6.6%, respectively. There were 60 (25.2%) patients that experienced a doubling (2-fold increase) in ALT from baseline. The covariates associated with a doubling ALT were alcoholism, baseline ALT, concomitant lopinavir use and duration of RAL therapy. Time-to-event distribution of the probability of ALT changing <2-fold from baseline, stratified by concomitant lopinavir is displayed in the figure. Survival distributions differed significantly

( $p = 0.001$ ) and was most pronounced for patients using concomitant lopinavir.

#### Probability of ALT Changing < 2-fold from Baseline, Stratified by Concomitant Lopinavir Use



**Conclusion:** The incidence of ALT increases  $3 \times$  ULN was low among patients receiving RAL. Doubling ALT was more common and modified by concomitant lopinavir use.

#### P2134 High prevalence of cognitive impairment in HIV – interim results of a cross-sectional study of 330 patients

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**Objectives:** Cognitive impairment occurs in 20–50% of patients with HIV. The incidence of HIV dementia has decreased but mild to moderate cognitive impairment continues to be an on-going clinical issue even for those patients who are virally suppressed and with stable CD4 counts. We undertook this study to assess the prevalence of cognitive impairment in the HIV positive population attending St. James's Hospital, Dublin and to identify risk factors for the development of cognitive impairment.

**Methods:** Patients attending the HIV clinics at St. James's Hospital, Dublin were offered screening for cognitive impairment. Inclusion criteria were as follows: HIV positive; over the age of 18; capable of giving consent; have sufficient English to partake; and be willing to participate in the study. Screening was carried out using the Brief NeuroCognitive Screen which consists of Trails A, Trails B and Digit Symbol Test.

**Results:** Three hundred and thirty patients have been screened: 77% were men; 23% were women; 62% were Irish. Median age was 39 (range 18–77) and median length of diagnosis was 6 years (range 1 month to 27 years). The most common mode of transmission in 48% was men who have sex with men; 76% were on highly active antiretroviral therapy; 87% were virally suppressed; and median nadir CD4 count was 228 (range 1–907). Median number of years of education was 15 (range 6–26). We found cognitive impairment in 52% of patients. A positive screen for cognitive impairment was associated with female gender ( $p \leq 0.001$ ), younger age ( $p = 0.029$ ), a country of birth outside of Ireland ( $p \leq 0.001$ ), heterosexual mode of transmission ( $p = 0.013$ ) and a positive screen for depression on the hospital and anxiety depression scale ( $p = 0.008$ ). There were no significant relationships to recreational drug use.

**Conclusion:** We observed cognitive impairment in over 50% of our sample of HIV positive patients. The screening tests focussed primarily on executive function and were chosen because they are validated in

HIV and HIV commonly affects frontal lobe function. The positive screening tests overly represent executive dysfunction. One of the limitations of the study is the lack of available normative data for extensive populations particularly for Africans. We plan to continue screening patients for cognitive impairment and to perform detailed cognitive assessment on these patients in order to characterise the profile of cognitive impairment that occurs in HIV positive patients.

#### P2135 Screening for strongyloidiasis in HIV-positive immigrants from endemic areas

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**Background:** *Strongyloides stercoralis* is a nematode parasite, which is endemic in tropical and subtropical regions. Infection usually remains asymptomatic, but in immunocompromised hosts severe and life-threatening manifestations such as hyperinfection syndrome and disseminated disease might occur. We describe the results of a screening programme conducted in the HIV-positive immigrant population arriving from endemic areas who attended the Tropical Medicine Unit of Hospital Universitario Central of Asturias during 2008–2011.

**Methods:** A prospectively observational study was conducted. We determined the presence of strongyloidiasis with a combination of repeated examination of three concentrated stool samples, culture in blood agar and enzyme-linked immunosorbent assay for serum anti-*S. stercoralis* antibodies. Infection exits if the microscopic visualization of larvae in stool sample and/or the Elisa was positive. The presence of other nematodes or filarias were discarded.

**Results:** We analysed 46 HIV-positive immigrants, of which 15 (32.6%) had a positive antibody test for *Strongyloides*. No patients had stool test positive. The most frequent countries of origin were: Equatorial Guinea (43.4%), Colombia (15%), Ecuador (10.6%), Brazil and Paraguay (9.5% each), Nigeria and Senegal (4% each), Bolivia and Argelia (2% each). The countries of origin in patients with strongyloides were: Equatorial Guinea (53.5%), Paraguay (20%), Colombia (13.3%) and Brasil y Bolivia (6.6% respectively) without statistically differences. In the positive cases the mean time in Spain was 791 days (limits 34–1576).

Ten patients has blood eosinophilia (mean: 1320 cells/mL; limits 618–2367) and the rest were asymptomatic. The mean of CD4+ cells was 352 cells/mm<sup>3</sup> and the viral were 191 303 RNA copies/mL. No patients had a hyperinfestation syndrome.

**Conclusions:** Strongyloidiasis is frequent in immigrant HIV positive patients, specially proceeding from Equatorial Guinea. These cases have a high risk of not being correctly diagnosed, therefore adding to the seriousness and lethality of the disease. Screening for Strongyloidiasis, even in asymptomatic patients should be taken fully into account and the relevant parasitological tests must be performed.

#### P2136 Incidence of recent HIV infection among persons seeking voluntary, anonymous counselling and testing services in Taiwan, 2006–2010

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**Background:** The annual case number of persons who are newly diagnosed with human immunodeficiency virus (HIV) infection continues to increase in Taiwan after successful control of HIV outbreak among injecting drug users. Whether the increasing case number is related to increased awareness and HIV testing activities or increasing incidence in high-risk populations, such as men who have sex with men (MSM), remains to be investigated.

**Objectives:** In this study, we aimed to estimate the incidence rate of recent HIV infection among persons seeking voluntary, anonymous counseling and testing services (VCT) at a university hospital and try to find the factors associated with the incidence rate.

**Methods:** Between 1 April 2006 and 31 December 2010, 10 198 persons sought VCT services for HIV testing at the National Taiwan University Hospital, which was sponsored by the Taiwan Centers for Disease Control (CDC). Anti-HIV antibody was tested using particle agglutination (SFD HIV 1/2 PA, Bio-Rad FUJIREBIO, Japan) and HIV infection was confirmed using Western blot. Demographics and behavioral data were obtained at the time of counseling. The BED IgG-Capture Enzyme Immunoassay (BED assay) was used to estimate HIV incidence for recent infection (within 153 days). Trends in HIV incidence were analyzed by weighted linear regression. The odds ratios between risk populations were analyzed by conditional logistic regression in the case-control study.

**Results:** During the study period, 360 individuals (3.5%) were test positive for HIV infection (3.5%). The overall incidence rate of recent HIV infections was found 3.83 per 100 person-years (PY). Although the trend in the incidence rate in MSM was not significant during the study period by the BED assay ( $p = 0.6388$ , weighted linear regression), the incidence rate in MSM was significantly higher than that in heterosexuals in the case-control study (odds ratio, 9.31; 95% CI, 4.77–18.20). Five behaviors/characteristics: “ever use illicit drug,” “the baseline RPR  $\geq 4$ ,” “condom use in anal sex  $< 100\%$ ,” “confirmed sexual partner to be HIV-positive,” or “having anal sex” were risk factors for recent HIV infection among either all clients or MSM ( $p$  values  $< 0.05$ ).

Adjusted Odds Ratio Estimates			
Effect	Point Estimate	95% Wald Confidence Limits	
Confirmed sexual partner to be HIV (+)	2.755	1.261	6.022
Condom-use $< 100\%$	1.887	1.024	3.477
Illicit drug use	2.616	1.328	5.151
Baseline RPR $\geq 4$	4.899	1.716	13.991
Having anal sex	4.121	2.260	7.515

**Conclusions:** From 2006 to 2010, the incidence rates of HIV infections among clients of this VCT program did not decrease. MSM had a significantly higher incidence rate of recent HIV infections than heterosexuals, especially those with illicit drug use or other high risk behaviors.

#### **P2137** Abnormal tubular function and chronic kidney disease in HIV-infected patients, ANRS CO3 Aquitaine Cohort

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**Objectives:** Proximal renal tubular dysfunction (PRTD) is frequent in the course of HIV infection. We investigated the consequences of PRTD on chronic kidney disease (CKD) occurrence in a cohort of patients living with HIV and under routine follow-up.

**Methods:** Consecutively enrolled HIV-infected subjects participating to the Aquitaine Cohort were prospectively evaluated for kidney function using concomitant blood and urine samples. Patients with baseline estimated glomerular filtration rate (eGFR)  $> 60$  mL/minute/1.73 m<sup>2</sup>, calculated with simplified MDRD Study Group equation, were followed prospectively. PRTD diagnosis (Fanconi's syndrome) was assessed at baseline. CKD was defined during follow-up as a confirmed eGFR  $\leq 60$ . The occurrence of CKD was estimated with Kaplan-Meier survival techniques during the first 18 months of follow-up by an on-treatment approach. We investigated baseline patients' characteristics associated with the outcome.

**Results:** Three hundred and sixty-seven patients (284 males) were included in this cohort. Median age at baseline was 48 years (Inter-

quartile Range [IQR]: 42–54), median CD4 count 480/mm<sup>3</sup> (IQR: 353–640), 322 (87.7%) were treated with  $\geq 3$  antiretroviral drugs and 297 (80.9%) had HIV-RNA  $< 40$  copies/mL. Fifty-six percent and 55% of patients were treated with tenofovir- and boosted protease inhibitors-containing regimens, respectively.

PRTD was present in 20 patients at baseline. At the closing date, CKD was diagnosed in 11 more patients. Cumulative probability of CKD was 1.7% at 6 months (95% confidence interval [CI]: 0.8–3.7%), 2.9% at 12 months (CI: 1.6–5.3%) and 3.4% at 18 months (CI: 1.8–5.8%).

Baseline factors associated independently with CKD diagnosis were: age per additional 5 years ( $p = 0.031$ ), high eGFR ( $p < 10^{-4}$ ), urine protein/creatinin ratio  $\geq 30$  mg/mmol ( $p = 10^{-3}$ ), urine albumin/creatinin ratio  $\geq 6$  mg/mmol ( $p = 10^{-3}$ ). PRTD was borderline significance ( $p = 0.081$ ).

**Conclusion:** Periodic monitoring of kidney function with serum and urinary markers might allow the early identification of patients predisposed to progression to CKD.

#### **P2138** Antiretroviral treatment and viral load responses in HIV-infected patients accessing specialist care in Ireland

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**Objectives:** The six specialist HIV centres in the Republic of Ireland (ROI) collaborated to describe the adult HIV population engaged in care.

**Methods:** Patients attending at least one medical outpatient appointment in the 12-month period 1 July 2009 to 30 June 2010 were included. Gender, age, probable route of acquisition, country of birth, number of ambulatory attendances, recent CD4 count, viral load and anti-retroviral treatment regimens were recorded.

**Results:** Three thousand two hundred and fifty-four HIV infected adults attended specialist care in the 12-month period. Two thousand and twenty-three (62%) were male, 1761 (54%) Irish and 1048 (32%) African. One thousand nine hundred and twenty-one (59%) resided in the Dublin area. The mean age was 40 (range 17–78); probable route of acquisition was available for 2898 (89%); heterosexual acquisition accounted for 1442 (50%), men who have sex with men (MSM) 777 (27%) and injecting drug users (IDU) 598 (21%). A recent CD4 count was available on 3193; the mean was 521. Two thousand five hundred and seventy-four (79%) were on anti-retroviral therapy (ARV). On ARV HIV RNA values were available on 2528 (98%); 2208 (87%) were  $< 50$  copies/mL (cpm) and 2384 (94%) were  $< 500$  cpm. Those who were virally suppressed (VL  $< 50$  cpm) were older (41 vs. 38 years  $p < 0.05$ ) and had a higher CD4 count. (542 vs. 422  $p < 0.01$ ) 1401 (89%) of men on treatment had VL  $< 50$  cpm vs. 803 (85%) of women ( $p < 0.05$ ). Nine hundred and ninety-four (90%), 545 (92%) and 385 (79%) of the heterosexual, MSM and IDU populations had VL  $< 50$  cpm respectively. Virological failure was lower in the MSM population ( $p < 0.05$ ) and significantly higher in the injecting drug users ( $p < 0.005$ ). ARV regimen was recorded for 2502. One thousand and sixty-five (43%) and 1073 (43%) were on a first line nucleoside/nucleotide reverse transcriptase inhibitor backbone (NRTI) with a non-nucleoside reverse transcriptase inhibitor (NNRTI) or protease inhibitor (PI) based regimen respectively.

**Conclusion:** We present the first national descriptive profile of HIV infected patients in care in ROI. Seventy-nine percent of patients are on ARV and 94% had HIV RNA values  $< 500$  cpm.

#### **P2139** Impact of some demographic and clinical factors in recommended lopinavir minimal plasma concentration cut-offs in antiretroviral naïve and experienced patients

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**Objectives:** Lopinavir/ritonavir (LPV/r) co-formulation is a first line therapeutic option for human immunodeficiency virus (HIV) infection in both American and European guidelines. LPV/r was first introduced

in 2001 as soft gelatin capsules and in 2005 the formulation was changed to solid tablets reducing the pill daily intake and pharmacokinetic variability. Yet, inter-individual variability of LPV plasma concentrations (C<sub>plasm</sub>) has been verified with factors like age, weight, pregnancy, co-morbidities, etc. In this study we assess the impact of some demographic and clinical variables in LPV C<sub>plasm</sub> subtherapeutic recommended cut-offs.

**Methods:** From January 2006 to August 2011, LPV C<sub>plasm</sub> were assessed using solid phase extraction and a validated high performance liquid chromatography method. The two recommended subtherapeutic trough cut-offs were applied (1000 ng/mL for antiretroviral naïve patients and 4000 ng/mL for experienced patients). Analytical statistics was performed with Mann–Whitney (non-normal continuous variables) and Qui-square (discrete variables) tests.

**Results:** A total of 214 LPV C<sub>plasm</sub> results were selected for this study (age >18 years and time after dose intake from 9 to 15 hours). The mean (standard deviation) time after LPV intake was 11.9 (1.19) hours and the median (interquartile range, IQR) LPV C<sub>plasm</sub> was 4230 (2097–8467) ng/mL.

The statistical significance of age, body mass index (BMI), gender, undetectable viral load (VL) (<50 HIV-RNA copies/mL) and reference to hepatic disease were analyzed for both LPV subtherapeutic cut-offs. The annexed table summarizes the results.

Factor	Total (n=214)	Cut-off 1000 ng/mL (n=27)	P	Cut-off 4000 ng/mL (n=104)	P
Age (median, IQR) (years)	40.7 (34.7-45.8)	39.6 (32.9-44.8)	0.108	39.8 (32.9-45.3)	0.041
BMI (median, IQR)	23.17 (21.26-25.18)	23.14 (21.29-27.58)	0.751	23.59 (21.28-26.53)	0.088
Gender (%)					
M	142 (66.4)	16 (59.3)		67 (64.4)	0.561
F	72 (33.6)	11 (40.7)	0.404	37 (35.6)	
VL <50 copies/mL (%)	137 (64.0)	9 (33.3)	<0.001	51 (49.0)	<0.001
Hepatic disease (%)	23 (10.7)	2 (7.40)	0.549	8 (7.70)	0.161

**Conclusion:** The wide inter-individual pharmacokinetic variability and correlation with demographic and clinical individual data reinforce the importance of TDM in clinical management of patients treated with LPV, particularly in antiretroviral experienced individuals, as a higher impact of factors like age (p = 0.041) and body mass index (p = 0.088) was verified with the cut-off 4000 ng/mL. The significance of the viral load clearly show that the two recommended LPV C<sub>plasm</sub> cut-offs are good virologic failure predictive factors.

#### **P2140** Risk factors of adverse outcome in HIV positive patients with bloodstream infections: an observational retrospective case control study in Milan, 1987–2010

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**Objectives:** Bloodstream infections (BSI) may be a critical event in HIV infection and are associated with increased mortality. Epidemiological, clinical, microbiological and therapeutic patterns may influence the outcome. The aim of this study was to identify factors associated with mortality. Moreover, we evaluated how BSI changed during the last decades, mainly after the introduction of highly active antiretroviral therapy (HAART).

**Methods:** We performed an observational retrospective case control study in Milan during the period 1987–2010. Three hundred and thirty-three consecutive HIV adult patients with bacterial BSI were included. Cases were patients whose death was due to BSI; the remaining subjects formed the control group. Demographical (sex, age), clinical (HAART, previous diagnosis of AIDS, apache II score, nosocomial or community acquired infection), laboratory (neutrophils, CD4), microbiological (source of infection, pathogen, susceptibility test) and treatment data were collected for each patient. A multiple logistic regression analysis was carried out to evaluate the impact of each variable on attributable mortality. Moreover, for each variable we evaluated the difference between pre HAART (1987–1997) and HAART era (1998–2010).

**Results:** Of the 333 patients included in the study, 64 died because of BSI whereas 242 formed the control group (data about attributable

	PERIOD		P VALUE
	PREHAART (n=177)	HAART (n=156)	
SEX (n (%))			
- F	32 (18)	48 (31)	
- M	145 (82)	108 (69)	0.810
AGE (YEARS)	32 (23–64)	42 (25–73)	<0.0001
APACHE II SCORE	11 (6-26)	12 (2-36)	0.007
NEUTROPHILS (n (%))			
- >10000	144 (81)	131 (87)	0.381
- <10000	29 (17)	19 (12)	
CD4 (n%)	20 (11%)	146 (93%)	<0.0001
TYPE OF INFECTION (n (%))			
- COMMUNITY	71 (41)	90 (58)	0.004
- NOSOCOMIAL	101 (58)	66 (42)	
SOURCE OF INFECTION (n (%))			
- NO	130 (73)	125 (80)	0.191
- YES	47 (27)	31 (20)	
GRAM (n (%))			
- POS	131 (74)	103 (66)	0.236
- NEG	36 (20)	44 (28)	
- POS & NEG	10 (6)	9 (6)	
MULTIDRUG RESISTANCE (n (%))			
- NO	83 (46)	99 (63)	0.002
- YES	64 (36)	47 (30)	
ANTIBIOTIC REGIMEN (n (%))			
- COMBINATION THERAPY	51 (29)	54 (35)	0.146
- MONOTHERAPY	122 (69)	95 (61)	
- NONE	3 (2)	7 (4)	
SUITABLE THERAPY (n (%))			
- YES	47 (28)	46 (29)	0.993
- NO	113 (64)	110 (71)	

mortality were not available for 27 subjects). Neutrophils <1000/μL (adjusted odds ratio [AOR] 3.75 [95% CI 1.41–9.95]), CD4 < 100/μL (AOR 3.08 [1.22–7.74]) and inappropriate therapy (AOR 2.66 [1.14–6.23]) were putative risk factors of death in HIV patients with BSI. Male gender (AOR 1.75 [0.77–3.96]), age >40 years (AOR 1.00 [0.41–2.45]), absence of HAART (AOR 2.47 [0.76–7.99]), APACHE II score >16 (AOR 1.40 [0.53–3.72]), nosocomial infection (AOR 1.52 [0.66–3.49]), presence of multidrug resistant pathogens (MDR) (AOR 1.36 [0.60–3.10]) and monotherapy (AOR 0.87 [0.39–1.91]) were not associated with a statistically significant increased risk of mortality. Comparing BSI in pre HAART and HAART era, in the second period patients were older (p < 0.0001) and with an improved immunological status (p < 0.0001), more frequently BSI were acquired in the community (p = 0.004) and a decreased number of pathogens were MDR (p = 0.022).

**Conclusion:** Alterations in immunity (low neutrophils and CD4 counts) and inappropriate antibiotherapy are life threatening conditions in HIV patients with BSI. An evolution of BSI in HAART era is detectable.

#### **P2141** Comparative evaluation of electrochemiluminescence immunoassay and ELISA for HIV screening in a multi-ethnic region of China

X. Bi\*, C. Tao, H. Ning, D. Li, T. Wang (Chengdu, CN)

**Objective:** Although automated chemiluminescence immunoassay (CLIAs) are gradually replacing the enzyme immunoassays (EIA), there are limited published studies on the comparative evaluation of these two different assays. We compare the performance of fourth-generation electrochemiluminescence immunoassay (ECLIA) and third-generation enzyme linked immunosorbent assay (ELISA) for human immunodeficiency virus (HIV) screening and gauge whether the shift from ELISA to ECLIA or alternative algorithms could be better in a multiethnic region of China.

**Methods:** We identified a large number of specimens (345 492) tested under routine conditions of a hospital laboratory using two different assays from January 2008 to August 2011 in urban centers with high sample throughput. Until October 2010, screening for HIV infection was carried out with a third generation ELISA. Since November 2010, a fourth-generation immunoassay ECLIA has been used at our laboratory for both diagnosis and screening purposes. Specimens with initially repeatedly reactive and western blot negative or indeterminate results were considered false-positive. Among all initially repeatedly reactive specimens, we evaluated the proportion of false-positive, positive predictive value (PPV), and western blots results in relation to ratios.

**Results:** The reproducibility of assays was determined by intra-class correlation coefficient (ICC). Precision results for assays are 0.994 (ELISA) and 0.998 (ECLIA). HIV prevalence was 0.23% using ELISA and 0.26% using ECLIA. The false-positive rate was lower for ELISA than ECLIA (0.03% vs. 0.11%, odds ratio 0.28 [95% confidence

interval 0.21, 0.37]). The PPV for ELISA (87.9%) was significantly higher than that for ECLIA (69.4%).

**Conclusions:** The performance of both assays was satisfactory in this setting. Increased specificity without compromising sensitivity can be achieved by slightly increasing COI ratio in ECLIA. Caution is needed in changing a test or combination of tests. Performance is important, but not sufficient. Since none of these tests are perfect, different testing algorithms should be developed for different clinical settings, taking into account the local conditions.

#### **P2143** Late HIV diagnosis in Georgia: reasons and consequences

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**Objectives:** Since 2004 Georgia achieved universal access to antiretroviral therapy (ART). However, the substantial proportion of HIV patients in Georgia are diagnosed late, compromising benefits of ART. The objective of this study was to identify risk factors for late HIV diagnosis and to its impact on short-term mortality.

**Methods:** Study enrolled consecutive 300 adult (age > 18) patients newly diagnosed with HIV between 1 June 2010–3 January 2011. Patients were interviewed between 3 November 2011–1 March 2011, and were followed until 1 July 2011. Clinical data were abstracted from medical records. Late diagnosis was defined as patient presenting with AIDS or CD4 count <200 cells/mm<sup>3</sup> at the time of HIV diagnosis. Comparisons were tested using Pearson's chi-square or Fisher's exact tests. Risk factors for late diagnosis were evaluated in multivariate logistic regression.

**Results:** Of 300 patients enrolled 64.0% were males and the median age was 36 years. 69.7% were unemployed and 50.3% reported history of living abroad primarily as temporary labour migrants. 43.0% had history of injection drug use (IDU). 18.0% patients had ever been tested for HIV prior to diagnosis. 47.7% reported referral to healthcare facility for conditions possibly indicative of HIV, including prolonged fever, pneumonia, chronic diarrhoea. The median time from first medical encounter to HIV diagnosis was 26 months. 48.7% had CD4 count <200 cells/mm<sup>3</sup> and 41.3% had AIDS-defining illness. Overall 158 (52.7%) patients met the criteria of late diagnosis. In multivariate regression factors significantly associated with late diagnosis were: increasing age (OR: 3.6, 95% CI: 1.7–7.6), unemployment (OR: 2.7, 95% CI: 1.5–4.9), history of living abroad (OR: 2.5, 95% CI: 1.29–4.7), IDU (OR: 2.1, 95% CI: 1.0–4.7), history of referral to healthcare facility (OR: 5.4, 95% CI: 3.0–9.5). Patients were followed for median 5.5 months, with total 141 person-years (py). Fifteen cases of death were reported during the follow-up (13 among late presenters). Mortality was significantly higher among late presenters: 18.3 per 100py (95% CI: 10.6–31.5) vs. 2.9 per 100py (95% CI: 0.7–11.5).

**Conclusion:** The findings suggest that current HIV testing practices in the country fail to identify a substantial proportion of HIV patients earlier in the course of their disease leading to excess early mortality. Our findings support implementation of proactive approach to HIV testing especially among IDUs.

#### **P2144** Persistent very low level viraemia is associated with non-subtype B in HIV-1 infected patients with virological success following HAART introduction

J. Saison, T. Ferry\*, C. Scholtes, V. Icard, D. Peyramond, C. Chidiac, P. Andre, R. Ecochard, J.-C. Tardy (Lyon, FR)

**Objectives:** After at least 6 months under HAART around 80% of patients obtain virological success defined as an HIV-1-RNA or viral load (VL) below the quantification limit (QL) 40 copies/mL (or 20 copies/mL, depending on the technique used). However a persistent very low level viraemia (VLLV) can still be detected, and may reflect a residual viral replication. Among patients in virological success some achieve undetectability, while others retain a persistent VLLV between 1 copie/mL and the QL. We wondered what were the predictive factors

associated with undetectability following HAART introduction, in patients achieving virological success.

**Methods:** We conducted a prospective observational study in the cohort of HIV infected patients followed at Hopital de La Croix-Rousse, Lyon, France. Inclusion criteria were to be HIV-1 positive patient beginning HAART between January 2009 and December 2009 and achieving virological success. The end point of the study was March 2011. Amplification levels were recovered from Abbott m2000 RealTime HIV-1 software. Each VL was classified as "detectable VL" (VLLV) or "undetectable VL". The primary end point was the probability of obtaining undetectability. A Cox model adapted for interval censored data was used to identify independent predictors of undetectability among epidemiological, clinical, virological and immunological factors.

**Results:** Seventy patients met inclusion criteria. In univariate model subtype B was associated with a higher probability of obtaining undetectability (HR = 1.56, p = 0.11) compared with non subtype B (Fig 1). Undetectability was not significantly associated with age at baseline, sex, contamination modes, metabolics data, coinfections with hepatitis viruses, CDC stage, CD4 nadir, viral load at baseline nor treatment regimen (5/70 patients had an NNRTI-base regimen). In multivariate model only subtype B was associated as an independent predictor of obtaining undetectability (HR 2.17 95% CI: 1.11–4.20; p = 0.025) vs. non-subtype B.

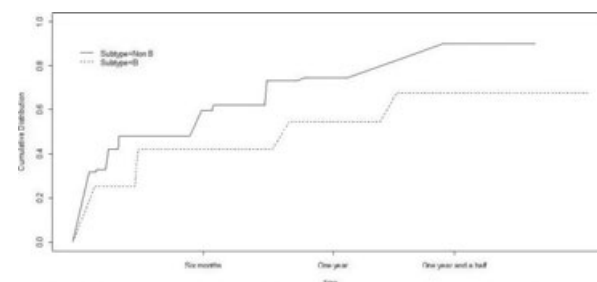


Fig. 1. Subtype B is associated with an increased probability of achieving undetectability, compared with non-subtype B (p log-rank = 0.11)

**Conclusions:** In this prospective observational study, non-subtype B patients remained more often with a very low level viraemia. The causes and consequences of the persistence of VLLV in HIV-1 non-subtype B infected patients have to be further investigated.

#### **P2145** HIV-2 transmission and molecular epidemiology in a community in rural Guinea-Bissau

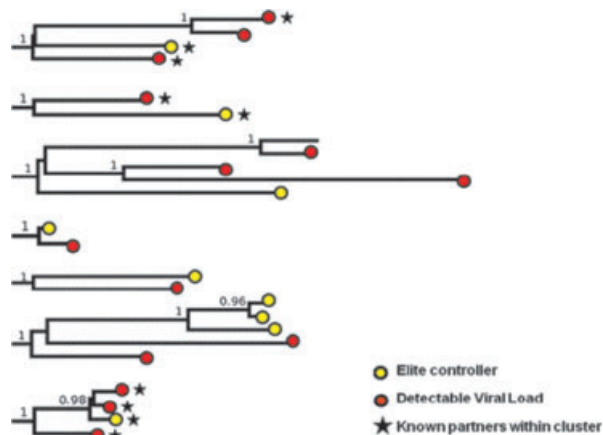
T.I. de Silva, C. van Tienen\*, C. Onyango, A. Jabang, T. Vincent, M.F. Schim van der Loeff, R.A. Coutinho, S. Rowland-Jones, H. Whittle, M. Cotten, S. Hué (Fajara, GM; Rotterdam, NL; Kilifi, KE; Amsterdam, NL; Oxford, UK; Cambridge, London, UK)

**Background:** HIV-2 is less virulent than HIV-1. It can lead to AIDS indistinguishable from AIDS caused by HIV-1, but disease progression occurs only in a minority of HIV-2 infected individuals. HIV-2 prevalence and incidence in the most affected region of the world, West Africa, are declining. However, new infections do still occur. We combined phylogenetic analyses with clinical and epidemiological data from a rural community in Guinea-Bissau to gain insights into the transmission of HIV-2.

**Methods:** Samples were obtained from population surveys held in 1989, 1997 and 2007. Gag p26 (690 basepairs) and/or env (1350 basepairs) sequences were isolated from 103 adults from the Caio community. Infections were designated "incident" (when a person had a previous HIV-2 negative sample) or "pre-1989" (when a person was diagnosed with HIV-2 in the first survey in 1989). Dated phylogenies were reconstructed using the program BEAST with gamma-distributed rate heterogeneity and a relaxed molecular clock.

**Results:** Sequences from incident infections were found more often in transmission clusters. Consistent with the phylogenetic linkage, viruses derived from known (sexual) partners were observed in the clusters.

The median CD4% was lower and the viral load higher in people whose viruses appeared in clusters than in people with non-clustering viruses. Some sexual partners within clusters had discordant viral loads (undetectable vs. detectable viraemia). Timed phylogenies estimated a median inter-transmission interval of 0.75 years in gag and 1.32 years in env phylogenies.



**Conclusions:** The HIV-2 phylogenies from this community-based study suggest incident infections contribute to onward transmission. There is no evidence to suggest specific viral lineages that are more successfully transmitted. Discordant viral loads of sexual partners suggest host factors play an important role in the outcome of the infection. Estimated median maximum inter-node intervals were relatively short. Along with the finding that incident cases cluster more often with other incident cases, this may indicate that when HIV-2 is transmitted, it occurs relatively early during infection.

**P2146** **Treatment of HIV infection in subjects with tuberculosis: prospective, randomised, multicentre study comparing a PI-containing regimen (lopinavir/TDF/3TC) with an NNRTI-containing regimen (efavirenz/TDF/3TC)**

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**Trial Design:** Prospective, multicenter, open-label, randomised.

**Methods:** *Participants:* Consecutive patients with HIV infection and diagnosis of active tuberculosis observed at 18 Italian study sites. Eligible HIV patients had peripheral CD4+ T cell counts  $\leq 300/\text{mm}^3$ . *Intervention:* Eligible subjects were randomly assigned to one of the following regimens: arm A standard 4-drug TB regimen for 2 months followed by a 2-drug continuation phase in association with efavirenz 800 mg/QD and a standard backbone of emtricitabine 200 mg + tenofovir 300 mg QD. Arm B: TB regimen with rifabutin as substitute for rifampicin 150 mg every other day in association with lopinavir/ritonavir 400/100 mg BID and the standard backbone.

**Objective:** To compare the completion rate of combined TB and HIV treatment regimens.

**Outcome:** The primary outcome was the rate of completion of dual TB and HIV treatment, measured at the end of standard TB therapy.

**Randomisation:** Patients were assigned to one of the study arm according to a central randomisation list at TB treatment initiation. Eligibility criteria were evaluated before randomisation and re-checked at the time of final diagnosis.

**Results:** Patients were enrolled between July 2005 and December 2010. Recruitment was closed before reaching the target sample of 200 patients: 121 patients were randomised, 61 to arm A and 60 to arm B.

Ninety-six patients (79.3%) were eligible for the study, 49 in arm A and 47 in arm B. Most of eligible patients were male (74%), Italian (71%), had acquired HIV infection heterosexually route (50%), had a mean age of 42 years (SD + 11 years) and a mean baseline CD4+ T cell count of 147 (SD + 144). Demographic clinical and viro-immunological characteristics at baseline were similar in the two groups.

The completion rate was 57.1% (28/49) in arm A compared to 48.8% (22/47) in arm B ( $p = 0.41$ ). The initial treatment was not completed due to major adverse events in 10.2% (5/49) of patients in arm A vs. 17.0% (8/47) in arm B ( $p = 0.38$ ). Eighteen percent of patients (9/49) in arm A vs. 25.2% (25/47) in arm B were lost to follow up. Two persons died in each arm.

**Conclusions:** The treatment completion rate was similar in the two arms. There is a trend towards a higher rate of major adverse events and default among patients in the arm B.

Trial registration: EUDRACT number 2005-245097-05.

**Funding:** The study was partially funded by the V and VI Italian AIDS Research Programme.

## Bacterial pathogenesis: from intracellular bacteria to biofilm

**P2147** **Regulation and function of CEACAM8 secreted by human granulocytes on bronchial epithelium**

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**Objectives:** Chronic obstructive lung disease is characterized by neutrophilic inflammation in the human airways. However, pathological bacterial colonization is common in the lower airways of affected patients that is associated with frequent exacerbations. In recent work we demonstrated that the soluble form of CEACAM8 that is solely expressed on human granulocytes binds to CEACAM1 expressed on human bronchial epithelium. We hypothesized that binding of soluble CEACAM8 to CEACAM1 is able to inhibit TLR2 receptor signaling on bronchial epithelium as it has been recently demonstrated for the specifically CEACAM1 binding pathogens *Moraxella catarrhalis* or *Neisseria meningitides*.

**Methods:** Investigation of the CEACAM8 secretion (soluble and cell bound) by human granulocytes in relation to different stimuli by FACS analysis and ELISA. Analysis of the signaling pathways that are activated in response to the CEACAM8-CEACAM1 interaction in primary human bronchial epithelial cells and in relation to stimulation of bronchial epithelial cells with the TLR2 agonist Pam3Cys.

**Results:** We found that binding of soluble CEACAM8 secreted by human granulocytes to CEACAM1 resulted in reduced Toll-like receptor 2-initiated transcription factor NF-kappaB-dependent inflammatory responses of primary pulmonary epithelial cells. These inhibitory effects were mediated by tyrosine phosphorylation of the immunoreceptor tyrosine-based inhibitory motif of CEACAM1 and by recruitment of the phosphatase SHP-1, which negatively regulated Toll-like receptor 2-dependent activation of the phosphatidylinositol 3-OH kinase-Akt kinase pathway. Our results identify a neutrophil-specific CEACAM1-dependent strategy for the reduction of airway inflammation.

**Conclusion:** Our results suggest a new mechanism how neutrophils reduce proinflammatory immune responses by the secretion of soluble CEACAM8 in neutrophil-driven infections in the human airways, thus acting as resolution-associated molecular pattern on human bronchial epithelial cells. These interactions might play a role for the reduced capacity to clear the lower respiratory tract from bacterial colonization in COPD-patients.

**P2148** **Fas-activated serine threonine phosphoprotein is a negative regulator of phagocytosis in macrophages**

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**Objectives:** We have recently described the importance of FASTK protein (Fas-activated serine threonine phosphoprotein) in the innate



immune response. A model of acute lung injury induced by lipopolysaccharide in mice lacking FASTK (FAST KO) has shown reduction in neutrophil infiltration and the cytokine and chemokine concentrations in bronchoalveolar lavage (Simarro et al. *J Immunol* 2010; 184(9): 5325–5332). The analysis of bone marrow chimeras and in vitro assays demonstrated that alveolar macrophages are the cells responsible for the phenotype.

The purpose of this study was to explore the effect of FAST deficiency in other macrophage functions such as phagocytosis and intracellular killing of gram positive and gram negative bacteria.

**Methods:** In vitro experiments were done using thioglycolate-elicited macrophages from wild-type and FASTK KO mice, and human THP1 macrophages with silenced FASTK expression (with siRNA). Phagocytosis of FITC-labeled non opsonized bacteria (*Escherichia coli* DH5-alpha and *Staphylococcus aureus* Cowan), was evaluated using flow cytometry and microscopy. Bactericidal activity was measured using a gentamicin-protection assay. Respiratory burst activity, binding assays and expression of toll-receptors and maturation markers were analyzed by flow cytometry.

In vivo phagocytosis assays were performed on wild type and FAST KO mice by injecting FITC-labeled bacteria into the peritoneal cavity.

**Results:** There was an increase in the phagocytic index for *Escherichia coli* and *Staphylococcus aureus* (>2 and 1.3 fold increase, respectively) in FASTK KO macrophages compared to wild type mice. Evaluation of the expression of the receptors TLR2 and TL4 and maturation markers revealed no significant differences between wild-type and FASTK KO macrophages. Both cell types also showed a similar ability to kill bacteria and to produce reactive oxygen species. Bacterial counts at the early time points in the gentamicin protection assay correlated well with the phagocytic indexes. Increased *Escherichia coli* phagocytosis by FAST KO macrophages was confirmed in vivo. Consistent with the findings in FAST KO macrophages, human THP1 macrophages with silenced FASTK expression showed increased phagocytic index for *Escherichia coli* compared with control cells.

**Conclusions:** FASTK is a negative regulator of phagocytosis in human and mouse macrophages.

**Acknowledgement:** Dolores Rico and Julia Panero for their technical support.

#### **P2149** Interaction of *Treponema pallidum* with microglial cells

A. Marangoni\*, C. Foschi, P. Nardini, I. Russo, R. Cevenini (Bologna, IT)

**Objectives:** *Treponema pallidum*, the agent of syphilis, exerts tropism for the central nervous system, in the course of natural infection. In the present study we investigated *T. pallidum* susceptibility to phagocytosis by primary microglia rabbit cells in opsonic and non-opsonic conditions.

**Methods:** Bacterial strains and culture conditions: *T. pallidum*, Nichols strain, was maintained by testicular passage in adult male New Zealand white rabbits. To be used as a working stock of antigen, treponemes were resuspended in PBS supplemented with 2% (v/v) heat inactivated non-infected rabbit serum to  $5 \times 10^8$  organisms/mL. As control, *Leptospira interrogans* serovar icterohaemorrhagiae was used at the same concentration.

**BV-2 cells:** The cell line was maintained in vitro in RPMI 1640 medium supplemented with 10% heat-inactivated FBS, gentamycin (50  $\mu$ L/mL) and L-glutamine (2 mM). Cells were detached by vigorous shaking and fresh cultures were started at a cell concentration of  $5 \times 10^4$ /mL.

**Measurement of phagocytosis:** Phagocytosis was evaluated on adherent BV/2 cells by immunofluorescent assay. Opsonisation of treponemes. When indicated, treponemes were incubated for 30 minutes with normal or immune human serum at a concentration of 10%.

**Results:** The phagocytosis of viable *T. pallidum* by BV/2 cells, studied by immunofluorescence staining of cells-associated bacteria, showed that ingestion of live, unopsonized treponemes was slow. Microglial cells started to be positive 30 minutes after infection, when only 3% of

the cells presented small round fluorescent inclusion-like bodies. Thereafter, the number of positive cells progressively increased with time: 10% and 21% of BV/2 cells were positive, respectively, 1 and 2 hours after infection. Opsonisation of *T. pallidum* with human immune serum did not substantially modify the percentage (5%) of microglial cells ingesting *T. pallidum* 30 minutes after infection, whereas opsonisation increased phagocytosis after 1 and 2 hours of incubation, when 15% and 48% cells were positive, respectively. When *L. interrogans* was used, numbers of positive cells at 30 minutes, 1 hour and 2 hour post infection were 68.7%, 76.3% and 88.1%, respectively.

**Conclusions:** Microglial cells were much more effective in binding and ingesting unopsonized leptospire than treponemes. Opsonization of treponemes did not affect ingestion at 30 minutes and 1 hour of incubation, whereas it significantly ( $p < 0.01$ ) increased phagocytosis at 2 hours post-infection.

#### **P2150** The role of matrix metalloproteinase 14 in the pathogenesis of tuberculosis

T. Sathyamoorthy\*, P. Elkington, J. Friedland (London, UK)

**Introduction:** Morbidity in *Mycobacterium tuberculosis* (Mtb) infection results from immunopathology, which causes tissue destruction and pulmonary cavity formation. Matrix metalloproteinases (MMPs) have a unique ability to degrade all extracellular matrix components, and are implicated in TB pathology. MMP-14 is a membrane bound collagenase which is key for monocyte migration and tissue destruction. Therefore we hypothesise that MMP-14 has a critical role in immunopathology in human tuberculosis (TB).

**Objectives:** To investigate: 1 Regulation of MMP-14 expression by Mtb infection.

2 MMP-14 expression in patients with TB.

3 Functional effects of MMP-14 upregulation in Mtb infection

**Methods:** Human monocytes were infected with Mtb H37Rv or stimulated with Conditioned Media from Tb infected human monocytes (CoMTb). Gene expression was measured by real time PCR, total protein expression by western blot analysis and fluorescent microscopy, and surface expression by flow cytometry. Immunohistochemistry was performed on lung sections from controls and patients with TB.

**Results:** Mtb infection upregulated MMP-14 gene expression in human monocytes fivefold ( $p < 0.001$ ) and CoMTb 10 fold ( $p < 0.001$ ), relative to control uninfected/unstimulated monocytes at 6 hours. At 24 hours, MMP-14 was upregulated 36 fold by Mtb ( $p < 0.001$ ) and 23 fold by CoMTb ( $p < 0.001$ ). MMP-14 total protein expression in monocytes, measured by western blotting, was upregulated 3.2 fold by Mtb and 2.5 fold by CoMTb relative to control at 48 hours. This was qualitatively confirmed by fluorescent microscopy images of Mtb infected and CoMTb stimulated monocytes at 24 hours. MMP-14 cell surface expression, measured by flow cytometry, showed a 1.8 fold increase in median fluorescence intensity in CoMTb stimulated monocytes at 24 hours, compared to control unstimulated monocytes. 50.8% of CoMTb stimulated monocytes had positive fluorescence for MMP-14, compared to 19.2% of control. To demonstrate the significance of these findings in patients with TB, we performed immunohistochemical analysis of lung biopsies from cases of pulmonary TB and found MMP-14 expression in macrophages surrounding the TB granuloma.

**Conclusion:** Mtb infection upregulates MMP-14 expression and MMP-14 is expressed in patients with TB, supporting the hypothesis that MMP-14 activity contributes to immunopathology in human TB. To our knowledge these are the first findings that implicate MMP-14 in the pathogenesis of TB.

#### **P2151** Matrix metalloproteinase-1 expression in tuberculosis is regulated by histone acetylation

R.C. Moores\*, L. Rand, P.T.G. Elkington, J.S. Friedland (London, UK)

Pulmonary cavitation is fundamental to the pathogenesis and transmission of *Mycobacterium tuberculosis* (Mtb) infection. Matrix metallo-

proteinases (MMPs) are host enzymes uniquely able to degrade the pulmonary extracellular matrix and we have previously demonstrated a key role for the collagenase MMP-1 in tuberculosis. The role of chromatin modifications, including histone acetylation, in regulating pro-inflammatory gene expression is increasingly recognised. We hypothesised that epigenetic mechanisms contribute to pathological over-expression of MMP-1 in tuberculosis.

**Objectives:** To define the role of chromatin modification by histone acetylation in the regulation of MMP-1 expression in an in vitro model of tuberculosis.

**Methods:** Human monocyte-derived macrophages (MDMs) from healthy donors were infected with Mtb strain H37Rv. MMP-1 mRNA was measured by quantitative PCR and protein secretion by Luminex bead array. Acetyl-histone H3 and H4 were quantified by Western blot. Histone acetyltransferase (HAT) and histone deacetylase (HDAC) activity was measured using indirect ELISAs. HDAC expression was measured by quantitative PCR and Western blot. Chromatin immunoprecipitation assays were used to study acetyl-histone H3 and H4 binding to the MMP-1 promoter region.

**Results:** MDM MMP-1 secretion was up-regulated >100-fold by Mtb infection. The non-selective HDAC inhibitor (HDACi) Trichostatin A 100 ng/mL reduced Mtb-induced MMP-1 mRNA accumulation at 24 hours by 89% and secretion at 72 hours from 8886 to 830 pg/mL ( $p < 0.01$ ). The selective Class I HDACi MS-275 1  $\mu$ M similarly inhibited MMP-1 expression by >90% ( $p < 0.01$ ). HAT inhibition with anacardic acid 1  $\mu$ M reduced MMP-1 secretion by Mtb-infected cells. No change in total histone acetylation was observed up to 4 hours after Mtb infection. Total HAT and HDAC activity in nuclear extracts was unaltered in Mtb-infected vs. uninfected MDMs. Mtb infection reduced HDAC-1, -2, -3 and -8 mRNA at 24 hours by 32%, 32%, 24% and 29% respectively compared to control (all  $p < 0.01$ ).

**Conclusion:** These findings demonstrate regulation of MMP-1 expression in tuberculosis at the level of histone acetylation and deacetylation. Epigenetic mechanisms regulating macrophage expression of pro-inflammatory and matrix-degrading molecules in response to Mtb infection may contribute to the excessive tissue destruction observed in patients.

**P2152** **Unfolding a secret: *Francisella tularensis* LVS protein Pile4 interacts with brain microvascular endothelial cells through ICAM-1 molecule**

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**Objectives:** *Francisella tularensis* (F.t.) is an emerging candidate in blood-brain barrier (BBB) invasive group of bacteria. F.t. is a highly infectious bacterium that invades various organs, including CNS. Molecular principles of BBB crossing by F.t. are still unknown. Present study is aimed at investigation of the molecules responsible for adhesion of F.t. to the brain microvascular endothelial cells (BMEC), which may be the crucial step in BBB translocation.

**Methods:** In our previous study we found that F.t. LVS interacts with ICAM-1 receptor of rat BMEC. To determine the protein ligand of F.t. interacting with ICAM-1, we performed magnetic bead based immobilized metal ion affinity chromatography (MB IMAC, Bruker), wherein His-tagged ICAM-1 was fused on affinity beads and hybridized with whole cell lysate of LVS. After stringent washings protein complexes were eluted and fractionated on SDS-PAGE. Interacting protein partner of ICAM-1 observed on PAGE (~34 kDa) was excised and subjected for peptide mass fingerprinting which identified this protein candidate as Pile4. To confirm the interaction between Pile4 and ICAM-1, and to assess binding abilities of Pile4 associated molecules of Pil complex we constructed His-tagged forms of Pile4, Pile5 and PilW and were used in far western blotting. BMEC proteins were fractionated by non-reducing SDS-PAGE and transferred onto nitrocellulose membrane, blocked with 2% BSA in TBST and hybridized with recombinant His-tagged proteins. Bound His-tagged candidates were detected with Ni-HRP conjugate and ECL.

**Results:** Only Pile4 protein, but not Pile5 and PilW, interacted with BMEC. This Pile4:ICAM-1 interaction was also confirmed by pull down assay where His-tagged Pile4 was bound on affinity beads and hybridized with whole cell lysate of BMEC. Complex was eluted and subjected directly for MALDI mass spectrometry. Mass spectrometry clearly showed presence of His-tagged Pile4 (~41.9 kDa) and its interacting partner ICAM-1 (peak at ~57.4 kDa corresponding to the molecular weight of ICAM-1).

**Conclusion:** Results from this study confirm that Pile4, but not Pile5 and PilW, is a potential adhesive molecule of F.t. LVS crucial for pilus-mediated adhesion to BMEC.

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**P2153** **Usefulness of an imaging technique for genital *Chlamydia* infection assessment in the murine model**

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**Objectives:** Untreated *Chlamydia trachomatis* infection can wreak havoc on the reproductive organs profoundly affecting fertility in women. Taken together, the high rate of asymptomatic infections and the severity of the infection related pathology indicate that control of chlamydial infections would require the development of new diagnostic non-invasive techniques for genital infection. Here we report about a comparison between Positron Emitting Tomography and traditional histology, in a mouse model of genital *C. muridarum* infection.

**Methods:** Animals used were 22 female Balb/c mice, 6–8 weeks old. All animals received 2.5 mg of medroxyprogesterone acetate i. m. 9 and 2 days prior the infection. Eighteen mice were challenged intravaginally with  $10^6$  IFUs of *C. muridarum* under Ketamine anaesthesia. As control, four animals were challenged with sucrose phosphate buffer. At 5, 12 and 18 days after challenge nine infected mice and one control underwent a 11C-Choline PET. Each animal was anaesthetised and injected with approximately 20 MBq of 11C-Choline. Images were acquired with a small animal PET tomograph for 20 minutes. Standard uptake value (SUV) was calculated measuring concentration of labelled tracer in the region of interest and correcting it for body weight and injected dose. Twelve infected mice were sacrificed to study histology of genital tract at 5, 12, 18 days after infection (three infected animals, plus one control at each point). Sections of hysterectomy specimen were preserved in 10% neutral buffered formalin for 48 hours and put in embedding cassettes, then processed in automatic tissue processor. After dehydration they were infiltrated with molten paraffin wax. Four-micrometres thick sections were cut with microtome, stained with haematoxylin-eosin and mounted on glass microscope slides.

**Results:** At 5 days, mean vaginal SUV ( $\pm$ SD) of cases was  $0.83 \pm 0.39$ , whereas the control showed a value of 0.278. At 12 and 18 days, SUV of cases increased to  $0.94 \pm 0.25$  and  $1.17 \pm 0.21$ , respectively, whereas SUV of the control was 0.299 and 0.302, respectively. All sections of hysterectomy of infected mice showed an intense grade of inflammation, characterized by an acute infiltrate in the initial phase, and by a prevalent chronic infiltrate in the successive period. Tissues collected from the three control mice showed only mild proliferation of endometrium.

**Conclusion:** 11C-Choline PET seems to be a promising diagnostic technique to assess inflammation due to *Chlamydia* genital infection.

**P2154** **Meningococcal invasion of the perivascular space in chronic meningococcaemia cutaneous lesions involves meningococcus-triggered remodelling of peripheral endothelial barriers**

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**Objectives:** Chronic meningococemia is a form of invasive meningococcal infection that involves recurrent fever for at least

1 week, frequent arthralgia and polymorphous skin eruption in the absence of meningitis. The presence of meningococci in skin lesions in the course of invasive meningococemia is well described. However, unlike acute forms of invasive meningococcal diseases such as meningitis for which in vitro models of infection have been developed, little was known on the route by which meningococci might gain access to the peripheral extravascular compartment in chronic meningococemia.

**Methods:** Purpuric and non-purpuric skin biopsies were obtained in the course of a clinical case of chronic meningococemia, and analysed with histologic, 3D-immunofluorescence and transmission electron microscopy. Pathophysiological hypothesis drawn from in vivo data were further tested in vitro, using a human dermis endothelial (HDMEC) cells model of meningococcal infection.

**Results:** We show that, in vivo, purpuric and non-purpuric cutaneous lesions of chronic meningococemia are both associated with the colonization of dermis capillaries by piliated meningococci, and that the bacterial translocation towards the perivascular compartment does not require vascular dislocation. Beside internalization of bacteria into endothelial cells, the remodelling of VE-cadherin junctions observed in vivo at paracellular sites, where meningococci were present, could be reproduced in vitro using an HDMEC cells infection model. Moreover, the meningococcus-triggered remodelling of VE-cadherin junctions was not restricted to a particular serogroup.

**Conclusions:** Taken together, the combination of in vivo and in vitro data suggests that, in the course of chronic meningococemia, a meningococcus-triggered remodelling of endothelial junctions opens a paracellular route by which meningococci gain access, from the lumen of peripheral capillaries, to the extravascular compartment.

#### **P2155** Inducible nitric oxide synthase expression in *Helicobacter pylori* CagA+ strains infections

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Increased expression of inducible nitric oxide synthase (iNOS) has been observed in patients with chronic inflammatory diseases of the gastrointestinal tract leading to sustained production of nitric oxide (NO) which may induce DNA damage.

**Objectives:** *Helicobacter pylori* (*H. pylori*) infection produces a state of chronic immune-stimulation in the gastric epithelium and a causal relationship between *H. pylori* CagA+ strains infection and gastric cancer has been suggested, therefore, our aim was to evaluate the significance of iNOS expression in gastric lesions induced by *H. pylori* CagA+ strains with correlation to the encountered endoscopic and pathological diagnoses.

**Methods:** Eighty-four dyspeptic patients underwent endoscopic examination. Four antral gastric biopsies were obtained for detection of *H. pylori* by histopathological assessment (Giemsa staining), urease test and gene expression of *H. pylori* using PCR assay. Immunohistochemical staining for iNOS expression and quantitative detection of anti-CagA antibodies were performed.

**Results:** *H. pylori* infection was detected in 64.3%, anti-CagA seropositivity in 54.8% and iNOS expression in 61.9%. Anti-CagA antibodies seropositivity and iNOS immunorexpression were significantly related to *H. pylori* infection. The positive rates of iNOS immunostaining increased with the lesion progression from chronic superficial gastritis to chronic atrophic gastritis to intestinal metaplasia (45.2%, 87.5% and 92.8% respectively). Positive immunostaining rates of iNOS correlated significantly with *H. pylori* CagA seropositivity with respect to both endoscopic and pathologic diagnoses.

**Conclusion:** CagA+ *H. pylori* strains are associated with enhanced immunorexpression of iNOS in *H. pylori* -related gastric diseases, therefore they might contribute as risk cofactors that conduces to gastric carcinogenesis. Give the high prevalence of *H. pylori* gastric diseases and frequent performance level of endoscopic gastric examinations among Egyptian patients, prompt identification of gastric infections

caused by *H. pylori* harboring CagA virulence factor is necessary for the early eradication of infection before the development of pre-neoplastic lesions.

#### **P2156** *Helicobacter pylori* with higher motility enhances bacterial density and inflammatory response in dyspeptic patients

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**Objectives:** Motility mediated by the flagella of *Helicobacter pylori* is important to move toward the gastric mucus in niches adjacent to the epithelium, and then *H. pylori* uses adhesin SabA to interact with sialyl-Lex on inflammatory host cells for persistent infection. Here, we revealed the clinical association of bacterial motility, SabA expression, and pathological outcomes.

**Methods:** Ninety-six clinical isolates were used to determine the bacterial motility, and the expression of SabA of each isolate was confirmed by western blotting. *H. pylori* infected patients were assessed for their bacterial density, sialyl-Lex expression, inflammatory scores and clinical diseases.

**Results:** The mean diameter of motility assay was 17 mm, and 8 (8.2%) strains have lower motility with a diameter <5 mm. The *H. pylori* density in cardia, the acute inflammatory score in corpus locus and the prevalence rate of gastric atrophy were increased in patients infected with higher motility strains ( $p = 0.023$ ,  $<0.001$ , or  $<0.001$ , respectively). The total inflammatory scores (both acute and chronic) and bacterial density dramatically increased in patients expressing sialyl-Lex antigen and infected with higher motility, SabA-positive *H. pylori* ( $p = 0.016$ ,  $0.01$ , or  $0.005$ , respectively).

**Conclusion:** The higher motility *H. pylori* enhances pathological outcomes, and the SabA-sialyl-Lex interaction has synergy effect when patients infected with the higher motility strains.

#### **P2158** Novel long-incubation organ explant system for dynamic high-resolution confocal imaging, and simultaneous cytokine measurements of infectious processes on mucosal interfaces

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**Objectives:** The mucosal interface of the human body is the most important entry route for pathogenic microbes, as well as the site of multiple colonisations and complex interactions. The processes taking place during the critical initial hours of infection or colonization, adhesion, invasion etc. are still poorly understood. Today, there are only few models available and especially the internal mucous membranes such as stomach, gut or urinary bladder are very difficult to study under in vivo conditions.

**Methods:** We developed a novel organ-explantation-based system. The setup is based on a custom-built reusable organ chamber compatible with standard microscopes. Luminal and basal side of the explanted mucosal organs are connected to separate channels for optimized incubation conditions and almost real time cytokine measurements. Oxygen is provided via a specially constructed membrane oxygenation device. Dynamic imaging with confocal microscopy permits a detailed analysis of the dynamics of pathogen-host cell interactions at the mucosal interface and the neighbouring tissue at highest spacial and temporal resolutions in multiple colours. Data are complemented by parallel cytokine measurements. We studied the infection of the mouse bladder with uropathogenic *E. coli* (UPEC), the small intestine with *Salmonella* sp. as well as the large intestine infected with *Entamoeba histolyticum* in the described setup. We analysed the intracellular bacterial communities (IBC) of UPEC, the invasion of *E. histolytica* and looked at inflammatory responses evoked during the infection.

**Results:** In our model system pre-infected organs as well as native organs which were infected ex vivo were analysed. We could show for

the first time a high degree of motile bacteria within IBCs which were considered to be in a biofilm like resting state. We further analysed the host's cytokine reaction triggered by UPEC infection. By that, we could for the first time demonstrate the immuno-suppressive effect evoked by live UPEC at an organ level and the growth phase dependency of this effect. Further, invasion of *E. histolytica* could for the first time be imaged in 3D in high resolution in a model of flushed explanted mouse gut.

**Conclusion:** We present an easy to use and cost effective new method to study infectious processes on mucosal membranes. New insights into the pathogenesis of urinary tract infections could be gained, further a new method to study Entamoeba infection could be established.

#### P2159 Mouse gut enterotypes identified by metaproteomics: a model for neonatal gut microbiota

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**Objectives:** An exhaustive description of human gut phylotypes is essential to highlight gut microbiota homeostasis and perturbation, in fact, classical microbiology is unable to provide unbiased representation of the gut microbial community. In order to unravel microbial community multiplicity, the mouse gut, which is characterized by a lower complexity than the human one, was herein selected as a model to design an original bioinformatic algorithms able to interpret metaproteomics massive data.

**Methods:** Mucosal biopsies from 24 Balb/c and Rag2KO mouse babies, after homogenization, were inoculated in Brain Heart Infusion (BHI) for ON growth. Culture pellets were trypsin digested. LC-MS2 was performed with a Proxeon EASY-nLC™ and an amaZone Ion Trap mass spectrometer equipped with a nanoFlow ESI Sprayer. Mascot Distiller software processed data under AutoMSn mode. Database searching (Swiss-Prot, bacteria taxonomy) and home-made designed microbiology algorithm was applied.

**Results:** For all samples, LC-MS2 runs of tryptic digests, yielded a number of molecular spectral features ranging from 3200 to 4000. However, a selected number of protein hits in the Mascot output varied between a minimum of 126 and a maximum of 314. Each protein hits, associated with consistent taxon unit (TU), allowed us to select 310 bacterial strains associated with the entire set of mice. With respect to microbe features, ecology, growth requirements, host and organ specificity, and pathogenicity traits, we have introduced four groups of microorganisms: (i) highly probable; (ii) not assignable; (iii) not compatible with the healthy status of the animal; (iv) highly improbable. The highly probable inferred TUs corresponded to 39 strains, associated with 23 species for the entire set of animals.

**Conclusion:** The above mentioned approach appears suitable to manage a wide metaproteomics data set and to interpret data from human gut samples, especially in newborns. Moreover, further analyses, based on metagenomic approach, are necessary to corroborate bacterial TUs and compare metaproteomic data with already known metagenomic indications.

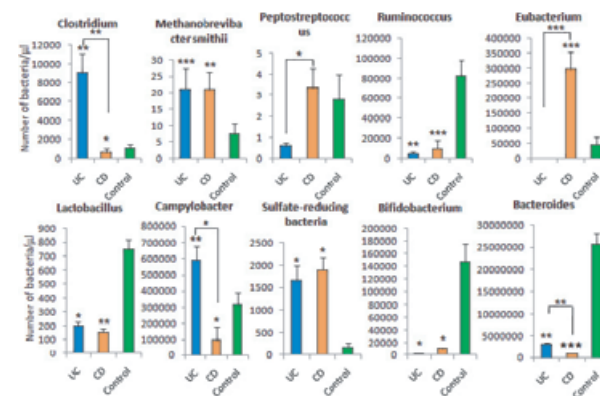
#### P2160 Real-time quantification of mucosal-associated bacterial flora of human colon in IBD patients of India: a case study

R. Verma\*, V. Ahuja, J. Paul (New Delhi, IN)

**Objectives:** The dynamic balance between microbes, particularly commensal flora, and host defensive responses at the mucosal frontier has a pivotal role in the initiation and pathogenesis of IBD. So the bacterial flora associated with the intestinal mucosa of IBD patients has been examined and compared with the mucosal flora of a control group.

**Methods:** Mucosa associated bacterial flora were evaluated between control and IBD patients by Real Time analysis using 16S rRNA based genus-specific primers. Members of *Bacteroides*, *Bifidobacteria*, *Lactobacillus*, *Peptococcus*, *Clostridium*, *Campylobacter*, *Methanobrevibacter smithii*, *Eubacterium*, *Ruminococcus* and Sulphate reducing bacteria were considered for this study.

**Results:** Among the predominating commensal flora incidence rate of *Bacteroides*, *Clostridium*, and *Ruminococcus* did not change significantly both in UC and CD patients except *Bifidobacterium* that decreased significantly ( $p = 0.0201$ ) in CD patients. Incidence rate of *Lactobacillus* decreased sharply ( $p = 0.01$ ) in UC patients. Among sub dominant flora incidence rate of *Methanobrevibacter smithii* ( $p = 0.0001$ ) and Sulfate reducing bacteria (SRB) ( $p = 0.0103$ ) were high in UC and CD patients. Real time analysis showed significant increase in concentration of *Eubacterium* ( $p = 0.0054$ ), *Methanobrevibacter smithii* ( $p = 0.017$ ), SRB ( $p = 0.0287$ ) in CD patients and increase in *Clostridium leptum* sub group ( $p = 0.0153$ ), *Methanobrevibacter smithii* ( $p = 0.0069$ ), *Campylobacter* ( $p = 0.017$ ), SRB ( $p = 0.0491$ ) in UC patients. Decrease in concentration of *Ruminococcus* ( $p = 0.0094$ ; 0.0036), *Bacteroides* ( $p = 0.0108$ ; 0.0088), *Lactobacillus* ( $p = 0.0206$ ; 0.016) and *Bifidobacteria* ( $p = 0.0311$ ; 0.0353) in UC and CD patients respectively.



**Conclusion:** When the clinical status of the UC patients were compared, population of *Campylobacter*, *Eubacterium* and *Ruminococcus* showed a tendency of restoration to normal values during remission. It is evident from our study that the subset of bacteria participating in the pathogenesis of UC and CD are different. High concentration of H<sub>2</sub>-consuming microorganisms – methanogens and SRB during disease conditions indicate that end products of their metabolism have an important impact on the pathogenesis of IBD.

#### P2161 A specific intestinal microbiota profile predisposes to severe chemotherapy-induced diarrhoea

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**Objectives:** The role of the intestinal microbiota in the pathophysiology of chemotherapy-induced diarrhea (CID) remains poorly understood. The objectives of our study were to describe the intestinal microbiota during chemotherapy and to investigate pre-chemotherapy patterns that could predispose to CID.

**Methods:** Patients undergoing BEAM conditioning chemotherapy for bone marrow transplantation were eligible. Exclusion criteria were inflammatory bowel disease, intake of probiotics, steroids, immunosuppressants, antibiotics during 1 month prior to study or during the chemotherapy. Fecal samples were collected before (S1) and after (S2) the onset of the chemotherapy. For culture of samples, fecal samples in BHI were thawed and serial dilutions ranging from 10<sup>-2</sup> to 10<sup>-6</sup> were spread on various media using an automatic spiral system. We looked for *Escherichia coli*, *Enterococcus*, *Streptococcus*, *Lactobacillus*, *Bifidobacterium*, total aerobic and anaerobic bacteria. For culture-independent molecular analyses, total DNA was extracted using the bead-beating method coupled with QIAmp DNA stool mini kit. The V6 to V8 region of the 16S rRNA gene was amplified. Purified PCR products were separated by dHPLC on a DNASep® HT cartridge (Transgenomic).

**Results:** Eight patients (five men/three women, mean age 50.5 years, SD 10.8) were included. Significant increase in bacterial counts between pre-chemotherapy and post-chemotherapy were observed for *Escherichia coli* ( $p = 0.002$ ), *Streptococcus* spp. ( $p = 0.02$ ) and anaerobic bacteria ( $p = 0.009$ ). Using dHPLC, hierarchic cluster analysis showed that fecal samples collected before chemotherapy clustered separately from those collected after chemotherapy. A Principal Component Analysis was performed on S1 samples to investigate differences in pre-chemotherapy fecal samples between patients who developed CID and patients who didn't. The score plot showed that two patients who developed CID with the most severe symptoms were separated from the six others.

**Conclusion:** Our data indicate that intestinal microbiota rapidly alters in patients during BEAM conditioning chemotherapy for bone marrow transplantation. Our results suggest that a specific initial distribution of dominant microbiota may predispose to severe CID.

This work was supported by Mérieux Research Grant and by Hospital Grant.

#### P2162 Screening and characterisation of antibiofilm compounds from Palk Bay metagenome

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**Objectives:** Screening of metaclones for antibiofilm activity and characterization of antibiofilm agents.

**Methods:** Screening for antibiofilm activity was done through spectrophotometric assay, light microscopic observation and confocal laser scanning microscopy (CLSM). Mode of action was determined by bacterial adhesion to hydrocarbons assay and exopolysaccharide (EPS) analysis. Characterization of antibiofilm agents was done by heat inactivation, enzymatic treatment, solvent extraction, Thin Layer Chromatography (TLC), and High Performance Liquid Chromatography (HPLC).

**Results:** Recombinant fosmid clones ( $N = 10\ 200$ ) were propagated in *E. coli* DH10B and screened for antibiofilm activity, the metagenomic clone M8-70 disrupted the biofilm of almost all the pathogens tested. The spectrophotometric assay revealed that M8-70 inhibits the biofilm formation of all the test pathogens upto 61–89%. At the concentration of 2.5%, M8-70 exhibited 70% antibiofilm activity against *Proteus mirabilis*, *Escherichia coli*, *Shigella flexneri*, *Shigella boydii*, *Staphylococcus aureus*, *Streptococcus salivarius*, *Pseudomonas aeruginosa* PAO1 and *Serratia marcescens*. During the CLSM analysis, in the presence of M8-70, there was a decrease in the biofilm formation and a greater reduction in the biofilm thickness. The light microscopic observation also revealed M8-70's remarkable ability to disrupt biofilms. From the growth well diffusion and spectroscopic method it is clear that the supernatant of M8-70 is not having any antibacterial activity against Gram positive and Gram negative pathogens except *Vibrio* spp. Supernatants of M8-70 and reduced the cell surface hydrophobicity and EPS production in all the Gram positive and Gram negative pathogens. The result from biochemical tests yet again clearly shows that the antibiofilm activity of M8-70 is indeed an enzymatic activity. The results of the ring closure assay and the sequence analysis of the PCR products from M8-70 revealed that its activity as lactonase activity (GenBank accession number HQ876560). **Conclusion:** This is the first report wherein a metagenomic clone is shown to possess antibiofilm activity against a wide range of bacterial pathogens.

#### P2163 Simulation of *Methylobacterium* biofilm formation in endoscope channels in a novel in vitro biofilm model of endoscope reprocessing

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**Objective:** The last 2 years repeated contaminations of bronchoscopes with *Methylobacterium* are reported in the University Medical Center Groningen, The Netherlands. *Methylobacterium* is a slowly growing

Gram-negative rod that forms pink colonies on agar plates and is a common contaminant in water. Nevertheless it has been described to be able to cause colonization and infections in immunocompromised patients. A novel in vitro biofilm model of endoscope reprocessing was used in order to study the ability for *Methylobacterium* to form a biofilm in endoscope channels.

**Methods:** The *Methylobacterium* biofilms were prepared in sterile tissue culture polystyrene 96-well microtiter plates. After 7 days biofilm formation in R2A broth, biofilms were treated for 10 minutes with the disinfectant Neodisher Septo PAC (DrWeigert, Germany) at concentrations conform the minimal bactericidal concentration (MBC) of the strains tested and at 1% concentration, recommended for disinfection of flexible endoscopes by the company. In order to mimic the biofilm formation and to establish a possible regrowth of biofilms inside the endoscope channels after reprocessing, we developed the in vitro biofilm model which underwent the different steps of the disinfection and drying procedures applied for endoscope reprocessing (Figure 1). The viability of the biofilm was quantified by using the tetrazolium salt (MTT) reduction assay and by counting colony-forming units (CFU) of 10-fold serial biofilm dilutions on R2A agar plates.

**Results:** A low MTT formazan signal was demonstrated in *Methylobacterium* biofilms directly after 10 minutes treatment with the MBC and 1% disinfectant. A total inhibition of microbial growth in biofilms on agar plates occurred after treatment with 1% disinfectant. MBC caused a inhibition of microbial growth of all biofilms but not a 100% bactericidal efficacy. Regrowth of biofilms occurred following 7 day incubation with R2A broth directly after treatment with the MBC and 1% disinfectant if the drying procedure was skipped. No microbial growth and a low formazan signal was found in biofilms after the disinfection procedure followed by drying for 3 and 7 days.

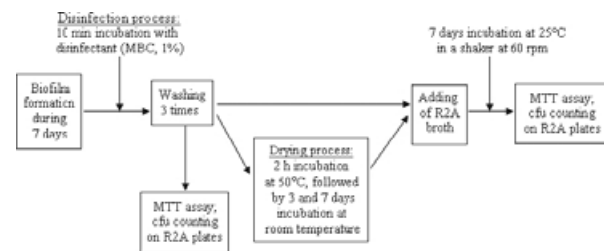


Figure 1. In vitro biofilm model mimicking the different steps of the disinfection procedure without and with the drying process in a flexible endoscope.

**Conclusion:** Routine cleaning procedures do not remove biofilm reliably from endoscope channels. This study demonstrated the high efficacy of the drying procedure after the disinfection step against *Methylobacterium* in biofilms. Failure of decontamination endoscopes can be explained by an invalid drying procedure.

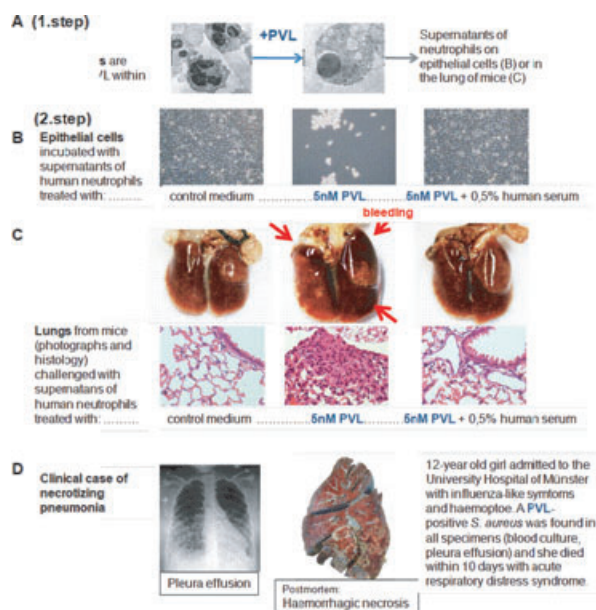
#### P2164 The combined action of influenza virus and *Staphylococcus aureus* Panton-Valentine leukocidin provokes severe lung epithelium damage

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**Objectives:** Necrotizing pneumonia is a severe disease, which is almost exclusively caused by Panton-Valentine Leukocidin (PVL)-producing *Staphylococcus aureus* strains and is often preceded by influenza infection. Whereas epidemiological data and pneumonia models in rabbits clearly support a crucial role of PVL in necrotizing infections, the precise pathogenic mechanisms of PVL and a possible contribution of influenza virus remains to be elucidated.

**Methods:** In this study, we perform costimulation with influenza virus and PVL in different human cell types (neutrophils, A549 cells) and evaluate the consequences of the elicited inflammatory process on the epithelium in vitro and in vivo.

**Results:** We found that PVL induced rapid cell death in neutrophils, which was further augmented by coinfection with influenza virus. Epithelial cells were not affected by PVL but they strongly up-regulated chemokine expression in response to influenza infection. Incubation of epithelial cells with supernatants from neutrophils either treated with PVL or with PVL-producing *S. aureus* resulted in a dose-dependent cell detachment and disruption of the epithelial monolayer. Furthermore, intranasal instillation of mice with supernatants from PVL-damaged human neutrophils caused extensive airway epithelial exfoliation and tissue damage with signs of necrotizing pneumonia. The devastating effect on lung epithelium was completely prevented by adding a protease inhibitor cocktail or human serum, indicating that destruction is caused by uncontrolled release of massive amount of neutrophil proteases in the respiratory space. Additionally, human serum conferred protection against PVL-induced cytotoxicity in neutrophils, which was mediated by PVL-neutralizing antibodies. The dual protective role of serum can explain why PVL-mediated tissue damage preferentially occurs in serum-free spaces, such as the pulmonary alveoli.



**Conclusion:** Taken together, we propose a model where influenza virus and superinfection with PVL-producing *S. aureus* lead to fatal necrotizing pneumonia. Modulation of PVL-induced neutrophil cytotoxicity as well as the uncontrolled release of proteases may represent possible targets for therapeutic interventions.

**P2165** Cerium nitrate: anti-*Candida albicans* activity in planktonic cells and biofilms

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**Objectives:** Cerium is a member of the lanthanides or rare earth elements. Several medical applications have been addressed to cerium compounds, based upon its antiemetic, antineoplastic, antiseptic and immunomodulatory properties. All these applications are still controversial, with the exception of its topical use for the treatment of burn wounds. Nevertheless studies addressing its antimicrobial effect produced contradictory results. Our objective was to clarify its antimicrobial activity against *Candida albicans*. We also tested its ability to impair biofilm formation and its potential use in biofilm treatment.

**Methods:** Six clinical isolates of *C. albicans* and the type strain ATCC 90028, were tested accordingly to the protocol M27-A3. Serial concentrations of cerium nitrate (ranging from 1 to 0.244 M) were used and the minimal inhibitory (MIC) and minimal lethal (MLC)

concentrations determined. A cytometric kinetic study was also performed by incubating *C. albicans* ATCC90028 with each of three concentrations (0.03, 8 and 50 mM) during 30 minutes, 90 minutes, 3, 6 and 24 hours. At each time point yeast cells were stained with FUN-1 0.5  $\mu$ M (a metabolic marker) or with Propidium iodide 1  $\mu$ g/mL (a membrane injury marker) and 20 000 events were analyzed in a flow cytometer (FACSCalibur BD Biosciences, Sydney). From each suspension, yeasts were plated on Sabouraud dextrose agar for viability assessment.

Biofilms growth was promoted in the presence of three cerium concentrations (0.03, 8 and 50 mM) for 24 hours. The biofilm metabolic activity and total biomass were quantified colorimetrically with XTT and crystal violet assays, respectively. Biofilm susceptibility was also assessed (tested concentrations ranging from 0.5 mM to 1 M).

**Results:** Cerium MIC and MLC were 0.03 and 8 mM, respectively. Flow cytometry showed a considerable decrease in cellular metabolic activity, after 3 hours of incubation with 8 and 50 mM, accompanied by a definitive membrane injury.

Cerium nitrate was able to impair efficiently biofilm formation at 8 mM. Treatment of pre-formed biofilms with cerium resulted in 90% reduction in the biofilm metabolic activity with 500 mM, and 92% of total biomass disaggregation with 16 mM.

**Conclusions:** Cerium nitrate exhibits not only antimicrobial but also anti-biofilm activity against *C. albicans*, being a promising agent for prevention or treatment of candidosis associated with medical indwelling devices.

## Pathogenesis of Gram-positive bacteria

**P2166** Use of a model of experimental endocarditis induced by continuous low-grade bacteraemia, mimicking human-like low-grade bacteraemia, to study the role of *Staphylococcus aureus* adhesins, platelet aggregation and inflammatory response in early endocarditis

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**Objectives:** Animal models of infective endocarditis (IE) induced by a bolus injection of large inoculum of bacteria, resulting in artificial high-grade bacteremia, has revealed the role of surface adhesins, platelet aggregation and inflammation in the pathogenesis of *S. aureus* IE. However, in humans, *S. aureus* IE likely occurs through repeated bouts of low-grade bacteremia from a colonized site. Here, we used a newly developed rat model of IE induced by continuous low-grade bacteremia, to mimic human IE and to explore further the contribution of adhesion to fibrinogen (Fg) and to fibronectin (Fn), platelet aggregation and cytokine production in IE initiation.

**Methods:** Rats with sterile aortic vegetations were inoculated with  $10^6$  CFU of non-pathogenic *Lactococcus lactis* pIL253 or recombinant *L. lactis* expressing individual *S. aureus* surface proteins, i.e., ClfA, FnbpA, BCD or SdrE, conferring different adhesive and platelet aggregation characteristics. The inoculum was given by continuous i.v. infusion at a rate of 0.0017 mL/minute over 10 hours. Vegetation infection was assessed 24 hours later. Plasma collected at 0, 2 and 6 hours post-inoculation was used to quantify TNF, IL-6 and IL-1b by Luminex assay. Platelet aggregation was monitored in an aggregometer for a maximum of 10 minutes.

**Results:** In vitro phenotypic traits and in vivo rates of vegetation infection of the different *L. lactis* are summarized in the Table. Conferring binding to Fg to *L. lactis* increased the infection rate of vegetations (pIL253: 11%; ClfA and ClfB: 50–54%;  $p = 0.03$ ), which further increase with adhesion to Fn (FnbpA: 75%,  $p < 0.001$ ). Expression of Fn-binding domains alone was not sufficient to induce IE (BCD: 10%). Platelet aggregation alone increased infection rate (SdrE: 30%) but not significantly. Conferring adhesion to Fg and Fn favoured cytokine production.

**Conclusion:** Our study extends, in a human-like model of IE by low-grade bacteremia, the essential role of Fg-binding to initiate IE, as shown for both ClfA and FnbpA. Expression of Fn-binding alone was

Strain	Adhesion to Fg	Adhesion to Fn	Platelet aggregation	TNF (2h/6h)	IL-6 (2h/6h)	IL-1b (2h/6h)	Infected/total vegetations
<i>L. lactis</i> pIL253	-	-	-	-/-	-/-	-/-	2/19 (11%)
<i>L. lactis</i> ClfA	+	-	+	-/-	-/-	-/-	8/12 (67%)*
<i>L. lactis</i> FnbpA	+	+	+	-/-	-/-	-/-	8/12 (75%)*
<i>L. lactis</i> ClfB	+	-	-	ND	ND	ND	8/11 (84%)*
<i>L. lactis</i> BCD**	-	+	-	ND	ND	ND	1/10 (10%)
<i>L. lactis</i> SdrE	-	-	+	ND	ND	ND	3/10 (30%)

\*,  $P < 0.05$  vs *L. lactis* pIL253

\*\* BCD is a truncated form of FnbpA expressing only fibronectin-binding domains.

ND: not determined

not sufficient to induce IE, but contributed to disease severity when associated to Fg-binding, as shown by early triggering of systemic inflammatory response by FnbpA. Triggering of platelet aggregation alone may also contribute to infection, as shown with SdrE. These results, confirming the critical role of dual Fg and Fn binding in IE induction, may have implications to design appropriate prevention strategies for *S. aureus* IE in humans.

#### P2167 PVL-positive MSSA are more prone to cause superficial skin and soft tissue infections

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**Objectives:** *S. aureus* is a major cause of purulent infections. Panton – Valentine leukocidin (PVL) is an extracellular pore forming *S. aureus* gamma toxin. PVL is encoded by lukSF-PV genes and carried on a bacteriophage. Although Panton-Valentine leukocidin has been strongly associated with community acquired methicillin – resistant *S. aureus* (CA – MRSA), luk-PV genes can be carried also by methicillin susceptible *S. aureus* isolates.

**Methods:** Antibacterial susceptibility was determined according to CLSI standards (M2-A9, M100-S16). The luk-PV gene was detected by PCR. The data was analyzed using SPSS version 17.0 for Windows.

**Results:** A retrospective observational study was conducted in the Children Clinical University Hospital in Riga, Latvia. During a period of 16 months from November 2006 to March 2008 224 *S. aureus* isolates were collected (eight blood isolates, 206 – from pus obtained by aspiration or operative procedures, 10 – other source.). Of all obtained isolates 218 were methicillin susceptible. PCR investigations of all 224 *S. aureus* isolates showed that 168 (75.0%) carried genes for PVL synthesis.

To calculate the association of PVL-positive isolates with types of staphylococcal infection all *S. aureus* isolates were categorized in four groups according to clinical details provided – superficial abscesses, superficial skin and soft tissues infections, bone and joint infections and other infections (including pneumonia and bacteremia). PVL gene – positive isolates were more likely to cause all types of infections ( $p = 0.014$ ) than isolates that were PVL gene-negative. The obtained results of odds risk calculations revealed that if isolated *S. aureus* is PVL positive, the risk of superficial abscesses development increases 2, 49 times. The risk of the development of bone and joint infections, and other infections remains equal in both groups – PVL positive/PVL negative.

**Conclusion:** Our study revealed that PVL genes are carried by a high number of *S. aureus* isolates obtained from children hospitalised in the Children Clinical University hospital. Most of these isolates were associated with abscesses and other skin and soft tissue infections.

#### P2168 Methicillin-susceptible *Staphylococcus aureus* bone and joint infections: chronic evolution is associated with bacterial ability to invade and persist within bone cells

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**Objectives:** Methicillin-susceptible *Staphylococcus aureus* (MSSA) is the leading cause of bone and joint infections (BJIs). Although the bacterial invasion of non-phagocytic cells has been shown to contribute to the pathogenesis of BJIs, the interaction of clinical strains of MSSA with osteoblasts has not been studied. We assessed bone cell invasion and cytotoxicity induced by BJIs MSSA clinical isolates in an ex vivo model of intracellular infection.

**Methods:** In a gentamicin protection assay, human osteoblastic MG63 cells were infected for 2 hours with 8325-4 *S. aureus* (control) and 94 BJIs clinical strains isolated from acute (time from initiation of symptoms to diagnosis lasting for  $\leq 4$  weeks,  $n = 63$ ) or chronic ( $n = 31$ ) BJIs. After selective killing of extra-cellular bacteria with gentamicin, internalized bacteria after 24 hours were quantified by plating cell lysates. Cell damage induced after 24 hours was assessed using a lactate dehydrogenase (LDH) release assay. All strains were characterized using spa sequence typing. Results are expressed as mean percentage  $\pm$  standard deviation of values obtained with 8325-4.

**Results:** Bacterial internalization in osteoblasts was significantly higher for chronic BJIs strains ( $240.7 \pm 189.4\%$  vs.  $174.3 \pm 154.3\%$ ,  $p = 0.047$ ). Moreover, bacterial intracellular persistence rate was correlated with BJIs delay of evolution (Pearson coefficient 0.326,  $p = 0.001$ ). LDH release rate was not significantly different between the two groups of strains although cytotoxicity induced by acute BJIs strains ( $86.4 \pm 27.8\%$ ) tended to be higher than chronic ones ( $79.9 \pm 22.0\%$ ) ( $p = 0.108$ ). Spa sequence typing of all strains revealed a great diversity, showing 50 different spa types grouped in 10 spa clonal complexes (spa-CC). One of these spa-CC was significantly more represented among strains isolated from chronic BJIs ( $p = 0.014$ ), and corresponded to the spa-CC with the highest rate of internalization in osteoblasts.

**Conclusion:** The intracellular persistence of MSSA within bone cells is correlated with the evolution delay of BJIs and could partially depend on bacterial genetic background. The ability of MSSA strains to invade bone cells could be an explicative mechanism of MSSA BJIs chronicity, a finding consistent with what is observed with MRSA strains in the same model.

#### P2169 Production of phenol soluble modulins by community- and hospital-associated MRSA strains correlates with in vivo virulence in a *Galleria mellonella* (caterpillar) model

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**Objectives:** To evaluate in vitro production of PSMs and in vivo virulence using a *Galleria mellonella* (caterpillar) model for UK healthcare-associated (HA) MRSA clones and local and internationally established community-associated (CA) MRSA clones.

**Methods:** Representative isolates of CA-MRSA that carry (+) or don't carry (-) Panton-Valentine leukocidin were selected: ST1-IV(+) (USA400), ST1-IV(-) (a local CA-MRSA clone), ST8-IV(+) (USA300), ST22-IV(+), ST30-IV(+), ST59-IV(+) and ST80-IV(+) (European clone). Two healthcare-associated clones were also included: ST22-IV(-) (EMRSA-15) and ST36-IV(-) (EMRSA-16). For the *Galleria mellonella* model,  $10^6$  bacteria were inoculated into groups of 12 caterpillars per isolate. Caterpillars were scored dead or alive at 16, 40, 64 and 96 hours. Survival times were compared using Kaplan-Meier analysis with a log rank test to assess statistical significance. Electrospray liquid chromatography and a multiple reaction monitoring method of mass spectrometry was used to measure relative production of PSM alpha1-4, beta 1-2 and gamma haemolysin.

**Results:** PSMs were identified in supernatants of all community and healthcare strains, although gamma haemolysis was not detected in 6/9 strains and PSM beta2 was not detected in 3/9 strains. Mean caterpillar survival time ranged from 32.8 hours for ST22-IV(+) to 88.2 hours for ST1-IV(+) (USA400) (Table). ST22-IV(+) was significantly more effective and ST1-IV(+) (USA400) significantly less effective at killing caterpillars than any other strain. Surprisingly, ST22-IV(-) (EMRSA15), the most common healthcare-associated MRSA clone in the UK, had the 3rd highest PSM production and was the second most effective caterpillar killer. There was a significant negative correlation between mean PSM production and mean caterpillar survival time ( $r^2 = 0.63$ , Pearson's Correlation  $p = 0.01$ ).

Table. Mean and median survival times, and matrix of p values generated from Kaplan-Meier plots comparing survival times between strains.

	Mean survival time / hours	Std. error	Median survival time / hours	P values generated from Kaplan-Meier plots comparing survival times									
				ST22 PVL+	ST22 (E15) PVL-	ST1 (E127) PVL-	ST8 USA300 PVL+	ST59 PVL+	ST36 (E16) PVL+	ST30 PVL+	ST80 PVL+	ST1 USA400 PVL+	
ST22 PVL+	32.8	3.6	16	-	0.004	0.018	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
ST22 (E15) PVL-	41.9	1.7	40	-	-	3.754	0.005	0.021	<0.001	<0.001	<0.001	<0.001	<0.001
ST1 (E127) PVL-	43.8	3.8	40	-	-	-	0.050	0.081	<0.001	<0.001	<0.001	<0.001	<0.001
ST8 (USA300) PVL+	56.2	4.7	40	-	-	-	-	0.516	0.203	0.153	0.014	<0.001	<0.001
ST59 PVL+	57.0	4.2	40	-	-	-	-	-	0.03	0.026	0.001	<0.001	<0.001
ST36 (E16) PVL+	61.9	2.0	64	-	-	-	-	-	-	0.016	0.016	<0.001	<0.001
ST30 PVL+	66.1	3.9	64	-	-	-	-	-	-	-	0.222	<0.001	<0.001
ST80 PVL+	73.0	4.1	96	-	-	-	-	-	-	-	-	-	<0.001
ST1 (USA400) PVL+	88.2	3.4	96	-	-	-	-	-	-	-	-	-	-

**Conclusion:** The *G. mellonella* virulence model is potentially a useful high throughput model to assess *S. aureus* virulence. The ability to kill caterpillars was not obviously linked to PVL carriage; however, there was a statistically significant negative correlation between overall production of PSMs and caterpillar survival time. Further work is required to correlate virulence in *G. mellonella* with human disease phenotypes.

#### P2170 Effect of antibiotics on virulence expression by community-acquired methicillin-resistant *Staphylococcus aureus*

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**Objectives:** To examine the effect of subinhibitory concentrations of anti-staphylococcal drugs on Pantone-Valentine leukocidin (PVL), alpha-hemolysin (HLA) and protein A (Spa) expression by community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA).

**Methods:** Five clinical isolates representing the main CA-MRSA clones were grown in presence of subinhibitory concentrations of five antibiotics (clindamycin, daptomycin, linezolid, tigecyclin and vancomycin). After 4 and 6 hours incubation, pellets of cultures grown with 1/8, 1/4 and 1/2 MIC of antibiotics were used for relative quantitative RT-PCR with specific *lukS-PV*, *hla*, *spa* and *gyrB* primers. The PVL and HLA concentrations were measured in the supernatant using a specific ELISA assay.

**Results:** The effect of subinhibitory concentrations on virulence expression depended on the antibiotics for each virulence factor. For all strains tested, clindamycin and linezolid dramatically reduced PVL mRNA expression both after 4 and 6 hours incubation, whereas tigecyclin induced a decrease in PVL mRNA levels for three of the five strains tested, mainly after 4 hours incubation. By contrast, daptomycin and vancomycin had no significant effect on PVL mRNA expression. PVL dosage was consistent and showed strong concentration-dependent inhibition of PVL release by clindamycin, linezolid and, in a lesser extent, tigecyclin.

Of all antibiotics tested, clindamycin only decreased HLA mRNA expression, whereas linezolid, tigecyclin and daptomycin showed heterogeneous results depending on the strain tested. Vancomycin had no significant effect whatever the strain tested. HLA dosage revealed strong concentration-dependant inhibition of HLA release by clindamycin, while linezolid only reduced HLA release when used at 1/2 MIC. By contrast, tigecyclin did not modify HLA release.

Clindamycin and linezolid both lead to concentration-dependent Spa mRNA decrease, while no effect was observed with the three other antibiotics tested.

**Conclusion:** These data support that clindamycin and linezolid suppress different virulence factor expression by CA-MRSA. This pleiotropic effect suggests that the mechanism triggered might involve global regulators of *S. aureus* virulence. Moreover, our observations promote clindamycin and linezolid to be used to target CA-MRSA virulence during severe infection. Concerning recent anti-MRSA agents, tigecyclin may specifically suppress PVL release, whereas daptomycin seems to be neutral on toxins expression by CA-MRSA.

#### P2171 Antiseptic dressing disrupts microcolonies of *Staphylococcus aureus* and prevents biofilm formation on human skin

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**Objectives:** An intravenous entry site dressing containing 2% by weight chlorhexidine gluconate (3M™ Tegaderm™ CHG Chlorhexidine Gluconate IV Securement Dressing) is used to reduce the density of skin microorganisms as part of the effort to minimize the risk of nosocomial infections including *Staphylococcus aureus*, which can invade via skin or mucosal surfaces. A human skin model of methicillin-resistant *S. aureus* (MRSA) biofilm formation was developed to determine the effect of this active dressing compared to a placebo control dressing (without CHG) and aqueous chlorhexidine gluconate (aq. CHG) on biofilm formation.

**Methods:** Explants of normal human skin (~5 mm<sup>2</sup>, full-thickness stratified, keratinized, squamous epithelium), (obtained from University of Minnesota Biological Materials Procurement Network; Institutional Review Board [IRB] exempt status), were infected with biofilm-producing MRSA (Xen30) (~1 × 10<sup>6</sup> CFU) or left untreated and incubated at 37°C for 24 – 96 hours. Following infection and microcolony development (72 hours), explants were treated with the active dressing (Tegaderm™ CHG), placebo dressing (without CHG) or aq. CHG (2%) for 24 hours or left untreated. Formation of MRSA biofilm was evaluated by confocal microscopy and LIVE/DEAD staining (Molecular Probes®/Invitrogen™). Tissue viability was monitored using the MTT assay.

**Results:** Human skin remained highly viable (70% relative to fresh skin) throughout 4 days of culture. By confocal microscopy, the stroma remains present and largely intact in uninfected skin throughout the course of the experiment. In contrast, when infected with MRSA, the stroma is degraded over time. Live, individual cocci are visualized at 24 hours post infection. By 96 hours post infection, mature biofilm is evident as are dead epithelia. Application of the active dressing, applied at 72 hours post colonization and allowed to remain in place for 24 hours disrupted and killed MRSA microcolonies/biofilm whereas aq. CHG treatment was not as effective. Application of a placebo dressing had no effect. Viability of the skin was not significantly affected by any treatments.

**Conclusion:** MRSA biofilms can form on normal healthy human skin. An antiseptic containing active dressing (Tegaderm™ CHG) prevented MRSA biofilm formation and disrupted microcolonies more effectively than aq. CHG or placebo dressing. The mechanism remains unresolved, but implies a synergistic effect of the dressing with the antiseptic.

#### P2172 Efficacy of N-chlorotaurine against *S. aureus* biofilms grown on TMZF® and CrCo discs

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**Objectives:** Many orthopedic surgeons consider surgical irrigation and debridement (I + D) and prosthesis retention as a treatment option for post operative infections. Among the surgeons who reported knowledge of existing literature on this topic, the majority of academic and community surgeons did not use antimicrobial agents but just saline



solution for irrigation. Since there are novel antiseptics with very good tolerability, why not associate the irrigation process with such a solution? To address this question, we investigated the activity of N-chlorotaurine (NCT) against *S. aureus* biofilms in vitro.

**Methods:** *Staphylococcus aureus* (ATCC 29213) biofilms were grown on titanium alloy (TMZF<sup>®</sup>) and cobalt-chromium (CrCo) discs for 48 hours. Susceptibility tests with NCT were carried out using different concentrations. Colony forming units (cfu) counting and scanning electronic microscopic (SEM) analysis were performed for the evaluation of NCT efficacy against biofilms.

**Results:** The CFU counting showed reduced growth during the first hour for all NCT concentrations. Killing by at least 6 log<sub>10</sub> steps was observed from three until 7 hours for 0.5%, 0.25%, 0.1% and 0.05% NCT (Fig. 1). SEM showed the presence of amorphous material over the group of bacteria which can be related to the extracellular polymeric substance (EPS). The SEM images of biofilms treated with NCT in all concentrations showed a disturbance of the biofilm architecture by rupture of the extracellular matrix and numerous dead cells.

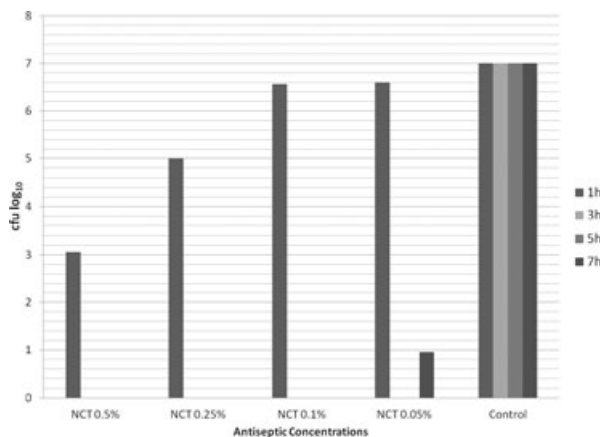


Fig. 1. Effect of NCT against *S. aureus* (ATCC 29213) biofilms in vitro.

**Conclusion:** NCT showed high efficacy against *S. aureus* biofilms in vitro. Once efficient to remove biofilms from the TMZF<sup>®</sup> and CrCo surfaces, NCT could be conceived as irrigation substance aiming the prevention and treatment of infections during surgeries of I + D and retention of joint replacement elements. In case of tolerability (which can be expected in view of the previous preclinical and clinical studies and the endogenous nature and mild activity of the substance), advantages of NCT would be the microbicidal activity against all kinds of pathogens without development of resistance.

#### **P2173** Activity of XF-70, a novel porphyrin antimicrobial against biofilms of *Staphylococcus epidermidis*

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**Objectives:** *Staphylococcus epidermidis* is the leading cause of infections involving indwelling medical devices. The formation of *S. epidermidis* biofilms on the surfaces of the devices is postulated to be why treatment of such infections is so difficult. There is therefore a significant need for novel treatments to eliminate such infections. This study aimed to investigate the activity of XF-70, a novel porphyrin antimicrobial, against *S. epidermidis* biofilms.

**Methods:** Planktonic MICs for *S. epidermidis* ATCC35984 was determined by broth microdilution according to British Society for Antimicrobial Chemotherapy (BSAC) specifications. The Calgary biofilm device was used to establish biofilm MICs (bMICs) and minimum biofilm eradication concentrations (MBECs). In addition to

XF-70, daptomycin, mupirocin, rifampicin and vancomycin were also investigated, as well as the polycyclic membrane active antibacterial peptide nisin.

**Results:** XF-70 is highly active against planktonic *S. epidermidis* ATCC35984 (MIC 0.5 µg/mL) but also against *S. epidermidis* ATCC35984 contained within biofilms (bMIC 2 µg/mL, MBEC 4 µg/mL). The increases in the bMICs compared to MICs for daptomycin, mupirocin, rifampicin, vancomycin and nisin were modest (4, 1, 1, 2 and 16-fold increases respectively), but the MBEC values were found to be 256, 256, 8000, 128 and >128-fold greater than the MICs.

**Conclusions:** XF-70 eradicates *S. epidermidis* ATCC35984 within biofilms at concentrations similar to the planktonic MIC. This attribute is not shared by the other comparator antibacterial agents tested in this study, including daptomycin and nisin, which were included in the study as both are membrane-active agents, the mechanism of action proposed for XF-70. These results support the concept that XF-70 could be utilized to treat *S. epidermidis* biofilms such as those found on indwelling medical devices and should be investigated further.

#### **P2174** The PavA-like fibronectin-binding protein of *Enterococcus faecalis*, EfbA, is important for virulence in a mouse model of ascending urinary tract infection

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**Objectives:** *Enterococcus faecalis* is an established nosocomial pathogen, yet the pathogenesis of enterococcal infections remains to be fully elucidated, particularly of urinary tract infections (UTIs). We report the first characterization of EfbA (enterococcal fibronectin-binding protein A), which is encoded by the enterococcal orthologue of *Streptococcus pneumoniae* pavA, and its involvement in UTI.

**Methods:** To construct efbA mutant and complemented strains from *E. faecalis* JH2-2, a molecular strategy based on plasmid pMAD, a thermosensitive pE194ts-based delivery vector system, was used. Full-length recombinant x6His N-tagged EfbA protein was purified from *Escherichia coli* and assayed for the adherence to extracellular matrix (ECM) components by enzyme-linked immunosorbent assay (ELISA) and whole-cell ELISA. Quantitative real-time RT-PCR analysis was performed to test efbA gene expression, whereas immunofluorescence and immunoelectron microscopy analyses were carried out to detect EfbA surface location using a specific mouse antiserum. Finally, JH2-2 and Delta efbA deletion mutant were compared for virulence using a mouse UTI model.

**Results:** We showed that, similar to PavA, the anchorless EfbA protein was localized to the enterococcal cell outer surface and bound to immobilized human fibronectin. In addition to abrogate EfbA expression, deletion of the efbA gene eliminated EfbA from the cell surface and drastically reduced the enterococcal cell binding to immobilized fibronectin. Again, the Delta efbA deletion mutant was highly attenuated vs. wild-type in the murine ascending UTI model.

**Conclusion:** Our data expand the knowledge of *E. faecalis* adherence to individual ECM proteins, and provide the first evidence that EfbA fibronectin-binding protein plays a role in *E. faecalis* UTI, probably contributing to the pathogenesis in this site.

#### **P2175** The autolysin LytA of *Streptococcus pneumoniae* and the toxin pneumolysin enhance pneumococcal invasive disease by a complement-depend mechanism

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**Objectives:** The pneumococcal capsule is one of the most important virulence factor of *Streptococcus pneumoniae*, although there are several proteins that play important roles in pathogenesis. One of these proteins is the cell wall hydrolase LytA. This protein is suggested to be

involved in virulence by releasing pneumolysin (Ply) and cell wall fragments that are markedly pro-inflammatory. Ply is a toxin partially responsible for immune response evasion by interacting with the C1q complement component. The complement system is one of the main host defence mechanisms against invading pathogens such as *S. pneumoniae*. Activation of the three complement cascades leads to the formation of the key component C3b that is essential for opsonisation of microorganisms, phagocytosis and inflammation. The objectives of this study were to investigate the role of both proteins in complement immunity, phagocytosis and in pneumococcal sepsis.

**Methods:** To explore their role in virulence, we have constructed isogenic, single (lytA; ply) or double (lytA ply) mutants on a serotype two pneumococcal background. We analyzed C3b deposition on the bacterial surface by using a flow cytometry assay and studied phagocytosis mediated by neutrophils and alveolar macrophages using two different cell lines. A mouse pneumococcal sepsis model was employed.

**Results:** C3b deposition increased on the lytA or ply mutants compared to the wild-type strain, confirming that LytA and Ply avoid complement mediated immunity. When the double lytA ply mutant was investigated, C3b deposition was markedly increased, suggesting that both proteins might have a synergistic effect in complement evasion. Phagocytosis of lytA or ply mutants was higher than of the wild-type strain whereas the phagocytosis of the double mutant was much higher either using neutrophils or alveolar macrophages. This confirmed that LytA and Ply together are very efficient in evasion of pneumococcal phagocytosis. Using a mouse sepsis model, we were able to demonstrate that both ply and lytA mutants were attenuated and that the virulence of the double mutant was greatly impaired demonstrating that both proteins play an important role in the establishment of pneumococcal sepsis.

**Conclusions:** Our study shows that LytA and Ply are very important virulence factors of *S. pneumoniae* that divert complement-mediated immunity and phagocytosis, and allow the bacterium to efficiently disseminate through the systemic circulation.

#### **P2176** Activity of macrolides, ketolides, and fluoroquinolones against *S. pneumoniae* in an in vitro pharmacodynamic model of biofilm

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**Objectives:** Persistent infections by *S. pneumoniae* like chronic sinusitis or otitis are associated with in situ formation of biofilms. This makes eradication difficult due to the protective role of the matrix in which bacteria are embedded. Our objective was to develop an in vitro model of young and aged biofilms of *S. pneumoniae* to study the effect of antibiotics on biofilm mass and intra-matrix bacterial survival.

**Methods:** Biofilms were obtained by culture of *S. pneumoniae* (capsulated [ATCC49619] and non capsulated [R6] strains) in 96-well plates for 2, 4, 7 and 11 days. Antibiotic activity was evaluated after 24 h of incubation at concentrations ranging from 0.0001 to 1000-fold the MIC in broth. Total biofilm mass (matrix + bacteria) was quantified by staining with crystal violet (CV) followed by OD measurement at 570 nm, and bacterial viability using the redox indicator resazurin (reduced in situ to fluorescent resorufin [RF] by living cells; [Lett Appl Microbiol 2008, 49:249–254]). An Hill equation was fitted to the data to calculate maximal relative activity ( $E_{max}$  [infinitely large antibiotic concentration]).

**Results:** In the absence of antibiotic, CV OD increased from 0.6–0.9 to 32–33 with both strains between day 2 and day 11. The Table shows that the activity of most antibiotics against bacteria (survival) was globally lower with R6 than with ATCC49619 in young biofilms, and markedly decreased for ATCC49619 in mature biofilms (with both strains reaching globally similar low values). For biofilm mass, activity was similar for macrolides/ketolides but reduced for fluoroquinolones in young and aged biofilms for ATCC49619 but globally lower in aged biofilms for R6. The most constant decreases amongst all antibiotics

were observed with moxifloxacin and amongst macrolides/ketolides, with solithromycin.

strain	Antibiotic*	MIC (µg/ml)	Viability (% decrease from control value)				Biofilm mass (% decrease from control value)			
			Day 2		Day 11		Day 2		Day 11	
			$E_{max}^{\dagger}$	$E_{max}^{\ddagger}$	$E_{max}^{\dagger}$	$E_{max}^{\ddagger}$	$E_{max}^{\dagger}$	$E_{max}^{\ddagger}$	$E_{max}^{\dagger}$	$E_{max}^{\ddagger}$
ATCC 49619	CLR	0.032	-25	-73	-10	-13	-13	-7	-19	-20
	AZM	0.064	-34	-80	-5	-20	+2	-15	-7	-15
	TEL	0.016	-0	-80	-11	-40	-10	-30	-26	-62
	SOL	0.032	-67	-70	-25	-38	-47	-65	-39	-65
	MXF	0.125	-90	-90	-51	-45	-76	-75	-25	-30
	L VX	1	-45	-75	-25	-35	-27	-65	-9	-10
R6	CLR	0.063	-22	-35	-5	-50	-26	-35	-3	-10
	AZM	0.5	+4	-35	+4	-45	-14	-20	-9	-10
	TEL	0.008	-15	-45	-5	-40	-23	-40	-9	-12
	SOL	0.004	-31	-45	-15	-35	-35	-50	-7	-0
	MXF	0.063	-91	-90	-17	-25	-75	-70	-40	-40
	L VX	0.5	-20	-65	-31	-5	-65	-60	-10	-35

\* CLR: clarithromycin; AZM: azithromycin; TEL: telithromycin; SOL: solithromycin (CEM-101); MXF: moxifloxacin; L VX: levofloxacin  
<sup>†</sup> values extrapolated from the Hill equation of the sigmoidal dose-response for experiments following crystal violet absorbance or resazurin fluorescence as a function of the drug concentration, with all data expressed as percentage of control values.  
<sup>‡</sup> values extrapolated from the Hill equation of the same sigmoidal dose-response, for an infinitely large antibiotic concentration

**Conclusion:** Biofilm production is independent of the non-capsulated or capsulated phenotype, and is accompanied over aging by a global decrease of antibiotic activity that, in proportion, affects more the strain (capsulated) for which a better activity was observed in young biofilms (perhaps related to differences in biofilm composition). The constantly higher activity of moxifloxacin and, to a lesser extent, of solithromycin, are perhaps due to combination of a low MIC and a bactericidal activity and may be worth further exploration.

#### **P2177** Transplantation of neural stem- and precursor-cells in hippocampal brain injury after experimental pneumococcal meningitis

S. Hofer, S.L. Leib\* (Berne, CH)

**Background:** In pneumococcal meningitis (PM), up to 50% of survivors suffer from long-term sequelae including impairment in learning and memory function. The neurofunctional deficits are associated with the occurrence of apoptotic brain injury to stem- and precursor-cells in the hippocampal dentate gyrus in experimental PM.

**Aim:** To assess survival, migration and differentiation of transplanted fetal neural stem and precursor cells (NPCs) after transplantation into injured hippocampus in vitro and in vivo, in an infant rat model of PM.

**Methods:** In vitro, NPCs from fetal rat hippocampus constitutively expressing green fluorescence protein (GFP) were grafted into the hilus of dentate gyrus organotypic hippocampal slice cultures which were previously injured by challenge with live *Streptococcus pneumoniae* (serogroup 3,  $n \geq 9$ ). The migration and differentiation of grafted stem- and precursor cells were assessed by immunohistochemistry.

In vivo, NPCs were stereotactically transplanted into the hilus of the hippocampus of rats 1 week after cured PM. At 1, 2 and 4 weeks following transplantation, survival, migration and differentiation of transplanted NPCs were evaluated by immunohistomorphometry.

**Results:** NPCs grafted in hippocampal slices injured by pneumococcal challenge, migrated to, and differentiated at the site of injury in the granular layer of the dentate gyrus. In rats after cured PM ( $n = 14$ ), GFP-expressing NPCs migrated from the injection site in the hilus to the injured granular layer of the hippocampal dentate gyrus and expressed markers of neuronal differentiation at 1 ( $n = 5$ ) and 2 ( $n = 3$ ) and 4 weeks after transplantation ( $n = 6$ ).

**Conclusions:** Fetal NPCs transplanted into the hippocampus after PM survived and migrated to the area of brain damage in the granular layer of the dentate gyrus where they differentiated into neurons in vitro and in vivo. The transplantation of NPCs may hold promise for cell replacement therapies aimed at repair of brain damage after PM.

**P2178** Adjuvant dexamethasone impedes hippocampal cell proliferation in experimental pneumococcal meningitis

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**Objectives:** Despite effective antibiotic therapies, pneumococcal meningitis (PM) causes neurological sequelae in up to half of the surviving patients. Neuronal damage associated with poor outcome is mediated in part by the elicited inflammatory host response against invading bacteria. Anti-inflammatory dexamethasone (dex) is used as adjuvant therapy. However, clinical usefulness of adjuvant dex to prevent neuronal damage is debated and empirical evidence is inconclusive. A recently published transcriptome analysis suggests that adjuvant dex impairs pro-neurogenic signalling during experimental meningitis. Moreover there is evidence from animal studies that in the developing brain, dex decreases hippocampal cell proliferation in the absence of inflammation. The aim of this study was to investigate whether in infant rat pneumococcal meningitis hippocampal proliferation is affected by adjuvant dex.

**Methods:** Eleven days old nursing Wistar rats (n = 97) were intracisternally infected with *Streptococcus pneumoniae* to induce experimental meningitis. In addition to antibiotics (ceftriaxone, 100 mg/kg, i.p, bid), animals were randomized for treatment with dex (0.7 mg/kg q8h s.c, n = 49.) or saline (n = 48). Treatment was continued until the time of sacrifice. Clinical parameters were documented and proliferative capacity of hippocampal neural precursors was assessed by the in vivo incorporation of BrdU at 3 days after infection and by an in vitro system promoting neurosphere formation at 4 days after infection.

**Results:** Dex treated animals exhibited a significantly more pronounced weight loss at 42 (p < 0.001), 72 (p < 0.0001) or 94 (p < 0.01) h after infection and a significantly lower survival rate (p < 0.05). Dex treated animals showed a significantly lower density of BrdU positive cells in the hippocampal dentate gyrus (p < 0.001). Accordingly the number of neurospheres formed from the hippocampus of animals receiving dex treatment was significantly decreased (p < 0.01).

**Conclusions:** Our findings document an antiproliferative effect of adjuvant dex on hippocampal neural precursors in experimental pneumococcal meningitis. In line with a recent gene expression analysis using the identical experimental set up, these results provide further evidence that adjuvant dex impedes neuroregenerative processes in acute pneumococcal meningitis of infant rats and should therefore be used with caution in the developing brain.

**P2179** *Streptococcus tigurinus* sp. nov., a novel species responsible of invasive infections in humans, is highly virulent in a rat model of experimental endocarditis

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**Objectives:** A novel species within the *Streptococcus mitis* group, for which the name *S. tigurinus* sp. nov. is proposed, was found to cause severe infectious diseases in humans, such as infective endocarditis (IE) (Zbinden et al. Abstract O213, ECCMID 2011). Here, we tested whether the virulence observed in humans correlates with increased infection in a rat model of IE. Three different *S. tigurinus* sp. nov. strains (AZ-3a, AZ-4a and AZ-8), isolated from patients with IE, were used to study (i) their capacity to adhere in vitro to immobilized fibrinogen (Fg) and fibronectin (Fn), which play a crucial role in the establishment of IE, (ii) the presence of ply gene, reported to be a virulence factor in streptococci, and (iii) the ability to colonize aortic vegetations in rats.

**Methods:** The adherence to surface-adsorbed Fg and Fn was tested on 96-well plates. The presence of ply was assessed by PCR using described primers. Rats with catheter-induced aortic vegetations were

inoculated with 10<sup>4</sup> CFU of the test strains. This inoculum was used because it allowed determination of increased bacterial virulence based on previous studies in this model. Animals were sacrificed 24 hours post-inoculation and the vegetations were removed, cultured, and the infectivity rate was recorded. Infection of 80–90% of vegetations was adopted as virulence parameter.

**Results:** No significant level of adherence to Fg or Fn was observed for any *S. tigurinus* strains in vitro. The ply gene was not detected. In rats challenged with 10<sup>4</sup> CFU, strains AZ-3a, AZ-4a, and AZ-8 infected 6/7 (86%), 7/9 (78%) and 5/9 (56%) aortic vegetations, respectively. For the strains AZ-3a and AZ-4a, the inoculum of 10<sup>4</sup> CFU, which infects 80–90% vegetations, is 10–100× lower than that needed by other streptococci to induce comparable infection rate in the same model (Glauser et al. JID 1983; Entenza et al. JID 1997), and similar to that of the major IE pathogen *Staphylococcus aureus* (Entenza et al. AAC 1995; Entenza et al. AAC 1997). For strain AZ-8, the infection rate obtained with such inoculum was similar to that of other streptococci causing IE.

**Conclusions:** The increased virulence of *S. tigurinus* sp. nov. in humans was confirmed in a rat model of IE. The infectivity of these strains was neither due to a greater ability to bind Fg or Fn in vitro, nor to the presence of the virulence determinant ply. The research for other putative virulence determinants in *S. tigurinus* sp. nov. is under investigation.

## Pathogenesis of Gram-negative bacteria

**P2180** Real-time qRT-PCR evaluation of HeLa cells apoptosis induced by *P. aeruginosa* quorum-sensing molecules

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**Objectives:** The purpose of this study was to evaluate by quantitative real-time RT-PCR the influence of different *Pseudomonas aeruginosa* cultures' fractions (whole bacterial cells, soluble molecules accumulated in the culture supernatants) on the expression of apoptosis genes effect in HeLa cells. N-3-oxododecanoyl homoserine lactone (3-oxo-C12-HSL) is the *P. aeruginosa* quorum-sensing (QS) molecule synthesized by las QS system, that plays critical roles in the pathogenesis of *P. aeruginosa* infection, not only in the induction of bacterial virulence factors but also in the modulation of host response.

**Methods:** The study was performed on 10 *Ps. aeruginosa* strains isolated from different clinical specimens. HeLa cells have been cultivated with three types of bacterial samples: standard bacterial cultures, whole bacterial cultures/culture supernatants grown in the presence of 100 μM 3-oxo-C12-HSL. The relative quantification of apoptosis genes expression in HeLa cells treated with each type of *P. aeruginosa* cultures comparatively with HeLa cells as control was performed by real-time qRT-PCR using the instrument Mx3005 (Stratagene). In addition, the expression of each pro-apoptotic (caspase 3, 8, 9 and Bax), respectively anti-apoptotic (Rel-A and Mcl-1) target gene in the control vs. experimental samples was normalized using the GAPDH reference gene.

**Results:** The results of the real time qRT-PCR assays showed an increase in the level of pro-apoptotic genes expression, simultaneously with the decrease of anti-apoptotic genes expression level in experimental samples comparatively with control.

**Conclusion:** Different fractions of *P. aeruginosa* cultures induced the overexpression of four pro-apoptotic genes, accompanied by the decrease of two anti-apoptotic genes expression in HeLa cells. Taking into account that the most significant pro-apoptotic effect was registered for bacterial cultures grown in the presence of 100 μM 3-oxo-C12-HSL, we could conclude that the quorum sensing molecules including 3-oxo-C12-HSL, present both in the cultures supernatants as well as in the *P. aeruginosa* integral cultures are responsible for this specific effect on the host cells.

**P2181** Characterisation of biofilm formation and extracellular polysaccharide production by *Acinetobacter baumannii* in response to environmental signals linked to initial host colonisation

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**Objectives:** We investigated the ability of an *Acinetobacter baumannii* clinical isolate (SMAL clone) to form biofilm and to produce extracellular polysaccharides (EPS) in environmental conditions mimicking host colonization. In particular, we tested microaerophilic conditions, growth at 30°C vs. 37°C, iron availability and growth either in the absence or in the presence of glucose. Glucose is only present in the blood and might represent an important signal in bacterial septicemia. Finally, we determined global transcriptional response to glucose by transcriptome sequencing.

**Methods:** Biofilm formation was determined by crystal violet assay. Production of EPS and cell surface-exposed structures was assessed by Congo red and calcofluor binding assays, immunoblot, and by treatment with EPS-degrading enzymes. Transcriptome sequencing was performed using the Illumina's Solexa sequencing technology.

**Results:** Biofilm formation by *A. baumannii* SMAL was strongly increased by growth in microaerophilic conditions. Dot blot analysis with antibodies against poly-N-acetylglucosamine (PNAG) showed that this EPS is overproduced in oxygen-limiting conditions, suggesting that it might be the factor responsible for increased biofilm formation. However, transcript levels of PNAG-related genes were not altered in anoxic conditions, suggesting that PNAG production is regulated at post-transcriptional level. Growth in glucose-supplemented medium did not significantly affect biofilm formation, but resulted in strong production of a yet unidentified EPS. Transcriptome analysis of *A. baumannii* SMAL grown either in the presence or in the absence of glucose did not show any significant difference in expression of EPS-related genes, suggesting that glucose-dependent EPS production is not mediated by transcriptional regulation.

**Conclusions:** Our results show that environmental conditions that might act as signal of interaction with the host, i.e., anoxia and presence of glucose, strongly impact EPS production in *A. baumannii*, resulting in increased production of PNAG and at least another unknown polysaccharide. Our observations suggest that modulation of EPS in response to these signals might play an important role in host colonization and virulence in *A. baumannii*.

**P2182** Biofilm-formation ability and diversity of globally spread *Escherichia coli* ST131 isolates resistant: 1991–2010

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**Objectives:** The diversity of a broad representative sample of ST131 isolates and the factors driving its persistence are poorly understood. We aim to characterize ST131 isolates from different origins/settings, and to assess their ability to adhere and form biofilm on abiotic surfaces.

**Methods:** Thirty-two representative isolates of ST131 *E. coli* (31 ST131, one ST1035) from eight EU countries (n = 21), the USA (n = 3) and Korea (n = 2) were studied (1991–2010). They were ESBL (CTX-M, TEM) and non-ESBL producers from nosocomial (62.5%) or community outbreaks (9.4%) but also from healthy volunteers (12.5%), animals (9.4%) and environmental samples (6.3%). Clonal relatedness was established by phylogroups identification, PFGE and MLST. Screening for 38 ExPEC virulence factors (VFs) was performed by PCR. Plasmid content was analyzed by S1-PFGE and further identification of the Inc groups (PBRT, sequencing). Biofilm production was tested in isolates from different lineages by a modified quantitative biofilm production assay and ultramicroscopical analysis (CLSM and FESEM).

**Results:** Six clusters (I–VI, 69.8%) were identified. Clusters I–IV (82.8–90.9%) comprise most isolates (n = 27, 84%; 2002–2010),

including widespread UK and Spanish clones. Most (59.2%) of them showed a common VFs profile (fimH-ihA-traT-usp-sat-malX-fyuA-iatA-ompT-kpsMTII-K5) with variable presence of K1 (50%), papEF or afa/draBC (both detected occasionally), or absence of kpsMTII-K5 (37.5%). They contained IncFIIpC15-1a/FIA-blaCTX-M-15 (56%), IncFIIpRSB107 + FIA + FIB (40%) or IncI1pEK204-blaCTX-M-3 (4%) plasmids. Cluster V (n = 4, 81.1%) included early ST131 isolates (1991–2003) with different genetic backgrounds (presence of ibeA; absence of malX, fyuA, fimH, iha and/or sat) and variable plasmid types (IncN-blaCTX-M-1, IncI-blaTEM-4, IncA/C-blaTEM-24, FIIUTI89). Cluster VI (69.8% homology) was minority. ST131 isolates were classified as weakly (n = 17, 0.16 < OD < 0.21) or moderately adherent strains (n = 3, 0.25 < OD < 0.26), but none of them was able to develop a mature biofilm.

**Conclusions:** The presence of a common VF profile in highly related clusters (I–IV) suggests expansion from a common ancestor, which evolved by loss/acquisition of specific VFs. Particular ST131 backgrounds were able to acquire different plasmid types. The low ability to adhere and grow as biofilm detected under the conditions tested suggests unknown mechanisms for the persistence of this lineage in humans which need to be explored.

**P2183** Diversity and biofilm-production ability of widespread *Escherichia coli* phylogroup D lineages (ST69, ST393, ST405)

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**Background:** Extra-intestinal pathogenic *E. coli* (ExPEC) clones belonging to phylogenetic group D (ST69, ST393, ST405) have greatly contributed to the global spread of extended spectrum beta-lactamases (ESBL). The diversity of a broad representative sample and the ability to adhere and form biofilm on abiotic surfaces is investigated.

**Methods:** Thirty-six widespread D- *E. coli* (13 ST69, 12 ST393, 11 ST405) isolates from four EU countries (n = 24), the USA (n = 8), Korea (n = 2), Kuwait (n = 1) and Brazil (n = 1) were analysed (1980–2010). They include ESBL (CTX-M, TEM) or AmpC (CMY-2) producers, non-ESBL producers causing outbreaks (67% nosocomial, 17% community) and also strains from healthy volunteers or food products (8% each). Clonal relatedness was established by PFGE and MLST. Screening for 38 ExPEC virulence factors (VFs) was performed by PCR and analysed statistically by the Fisher's test. Biofilm production was investigated by a modified quantitative assay.

**Results:** Most isolates (73%, mainly ST393 and ST69) were classified as ExPEC, but all non-ExPEC isolates (ST405) also caused extra-intestinal infections. ST69 and ST393 showed high virulence scores (median 14/range 9–15 vs. median 13/range 3–15, respectively), in contrast with ST405 (median 6/range 2–14). ST69/ST393 were enriched in pap (69–92%), iha (77–83%), kpsMTII-K5 (92–100%) and ompT (50–92%) (p < 0.001) while ST405 isolates contained more frequently kpsMT III (46%), PAI (64%) and fyuA (100%) (p < 0.04). Most ST69 isolates (humans and animals) were highly related (71.4%; 1999–2007) and classified as weakly (n = 10, 0.17 < OD < 0.23) or moderately (n = 1, OD = 0.45) adherent. Only two ST69 isolates from ready-to-eat salads and unrelated to the above cluster (2010) were strongly adherent (OD = 0.5). Most ST393 isolates (n = 12; 1980–2007) from either hospital or community humans shared 11 VFs but only three of them were moderately adherent (0.35 < OD < 0.47). Similarly, among four ST405 clusters (68.9% homologous, 2000–2008), only two isolates were classified as moderately (OD = 0.3) or strong (OD = 0.6) adherent.

**Conclusion:** The lack of correlation between non-ExPEC and the ability to cause extraintestinal disease indicate other traits may contribute to the pathogenicity of these strains. Particular ST69, ST393 and ST405 lineages (congruent PFGE/virulence profiles) were identified in different settings throughout time but only a few isolates were potential biofilm producers.

**P2184** The fimbriae of enteroaggregative *Escherichia coli* induce epithelial inflammation in a human intestinal xenograft model

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**Background:** Enteroaggregative *Escherichia coli* (EAEC) is an important agent of inflammatory diarrhea. EAEC pathogenesis is believed to be initiated by adherence to the ileal intestinal mucosa which requires expression of aggregative adherence fimbriae (AAF). A biofilm is then formed and subsequent release of toxins leads to mucosal damage. We have previously demonstrated that EAEC prototype strains promote transepithelial migration of neutrophils in vitro. The objective of this study was to investigate in vivo pro-inflammatory properties of EAEC using human small intestinal xenografts, and to identify the virulence factor(s) responsible for triggering inflammation.

**Methods:** EAEC wildtype and mutant strains as well as clonal constructs of AAFs were tested for their ability to induce transepithelial migration of neutrophils in vitro and for their ability to induce inflammation and tissue damage in an in vivo model of human intestinal xenografts in severe-combined immunodeficient (SCID-HU-INT) mice. These intestinal grafts become extensively vascularised, secrete mucus and develop into morphologically normal human intestine.

**Results:** Plasmids encoding AAF gene clusters are sufficient for triggering transepithelial migration of neutrophils in vitro. Moreover, employing SCID-HU-INT mice, severe tissue damage and inflammation is observed in the human tissue following infection with EAEC prototype strain 042. These pathological marks are strongly related to AAF expression.

**Conclusion:** Our data demonstrate extensive pro-inflammatory properties of EAEC and a key role for AAF fimbriae in triggering these events.

**P2185** Virulence potential of commensal *Escherichia coli* isolates encoding colicins and microcins

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**Objectives:** *Escherichia coli* (*E. coli*) strains commonly produce bacteriocins, colicins and microcins, to inhibit growth of related bacteria. Among commensal *E. coli* the following colicins/microcins are known to be the most prevalent: microcin H47 (MicH47), microcins M (MicM), colicin Ia (ColIa), colicin Ib (ColIb), colicin E1 (Col E1), microcin V (MicV), colicin M (ColM), colicin E7 (ColE7) and colicin B (ColB). Colicinogenic strains are also often found among pathogenic *E. coli* strains and it is believed that colicinogeny is enhancing the pathogenicity potential of *E. coli* strains. It is assumed that the intestinal microbiota is the reservoir of the so called extraintestinal pathogenic *E. coli* (ExPEC) strains that can due to specific virulence factors instigate an impressive variety of extraintestinal infections. However, to our knowledge it has not been investigated yet whether there is any correlation between encoding colicin/microcins genes and genes for virulence determinants among commensal *E. coli* in the intestinal microbiota.

**Methods:** A total of 90 commensal *E. coli* isolates from our collection that were previously screened for the presence of virulence (related) factor genes was investigated for the carriage of colicin/microcin genes. Using the method of PCR amplification with specific primers all isolates were screened for the following colicin/microcins genes: cba (ColB), cE1a (ColE1), cE7a (ColE7), claa (ColIa), clba (ColIb), cma (ColM), mchIB (MicH47), mcmM (MicM) and cvaC (MicV). Further, a statistical analysis (Fisher's exact test and Bonferroni correction) was used to reveal statistically significant associations of colicin/microcins genes and genes for virulence (related) factors. The threshold for statistical significance after Bonferroni correction was set at p values of <0.05.

**Results:** Our results showed that the most prevalent bacteriocin genes were cvaC (16, 17%), claa (14, 16%) as well as mcmM and mchIB (13, 14%). The cba, cE1a, and cma genes were found in 7 (8%) of studied isolates, cE7a gene in 6 (7%) and clba gene in 2 (2%). The mcmM and mchIB genes were statistically significantly associated with the following virulence related genes cnf1, hlyA, papGIII, sfaDE and tcpC, the gene claa with iro and the gene cvaC with iucD and iro.

**Conclusions:** Our results indicate that among the commensal *E. coli* the mcmM and mchIB encoding bacteria possess the highest virulence potential to instigate extraintestinal infections.

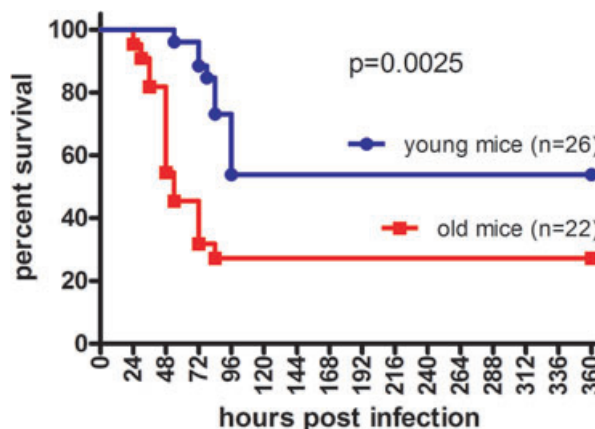
**P2186** Decreased resistance of the aged brain to infections: higher mortality and impaired elimination of bacteria in aged mice after intracerebral infection with *E. coli* K1

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**Objectives:** The prevalence of bacterial infections of the central nervous system (CNS) in adults increases with age, and the clinical outcome in elderly individuals is worse than in young persons. Death in the acute phase of the infection and neurologic or neuropsychologic deficits are common complications. We compared the resistance of young and old mice to an intracerebral infection with *E. coli* and the phagocytic potential of primary microglial cells from young and old mice, in order to elucidate causes for the decreased resistance of the aged brain to infections.

**Methods:** C57BL/6N mice at the age of 26.2 ± 2.3 months (old mice, n = 22) and 2.2 ± 0.3 (young mice, n = 26) were infected by injection of 10<sup>5</sup> CFU *E. coli* K1 into the right frontal brain and monitored regularly by a clinical score, the tightrope test, and weighing. Mice that were unable to walk or survived 15 days post infection (p.i.) were sacrificed. *E. coli* concentrations in cerebellum and spleen homogenates were determined by quantitative plating on blood-agar-plates. Primary microglial cells prepared from 2 and 18 months old mice were compared concerning their ability to phagocytose *E. coli* upon stimulation with agonists of TLR 2, 4, and 9 (n = 6–11).

**Results:** Mortality after intracerebral infection with *E. coli* was significantly higher in old mice (73%) than in young mice (46%; p = 0.0025; see figure). Bacterial concentrations (median [25/75 percentile] CFU/mL) were significantly higher in surviving old mice than in surviving young mice 15 days p.i. (cerebellum: 20 000 [775/92 500] vs. 99 [99/175]; p = 0.0094/spleen: 2500 [775/7250] vs. 99 [99/200]; p = 0.0059) – seven of 14 young mice, but only one of six old mice were sterile. In vitro, phagocytosis of *E. coli* K1 was significantly increased after stimulation with TLR agonists in microglial cells from young mice (p < 0.05 for LPS [TLR4] and CpG [TLR9]), but not in microglial cells from old mice.



**Conclusion:** Reflecting the observation in aged patients with CNS infections, mortality after intracerebral infection with *E. coli* was higher in old mice than in young mice. The ability to eliminate bacteria and to

clear the infection was impaired in old mice, possibly due to the reduced ability of aged microglia to phagocytose bacteria upon activation. Strategies to improve the phagocytic potential of aged microglial cells without increasing microglia-induced neuronal injury appear promising for prevention and treatment of CNS infections in elderly patients.

**P2187** **The inherent role of Hfq for pathogenicity of several *Escherichia coli* pathotypes towards *Caenorhabditis elegans***

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**Objectives:** *Escherichia coli* are a major cause of intestinal and extraintestinal diseases worldwide. Due to the high morbidity and the increasing problem of antibiotic resistance further studies on virulence mechanisms and future antimicrobial targets are needed. The RNA chaperone Hfq is a key regulator of bacterial posttranscriptional regulation and evidence is increasing that Hfq riboregulatory functions are involved in virulence of diverse bacterial pathogens. A role for Hfq in the distinct pathotype adherent-invasive *E. coli* (AIEC) in virulence towards the nematode organism *Caenorhabditis elegans* was recently reported. Here we address the possibility that Hfq play a role for pathogenic *E. coli* in general.

**Methods:** Deletion of hfq in prototype verocytotoxin-producing (VTEC), enteroaggregative (EAEC) and uropathogenic (UPEC) *E. coli* was obtained by lambda-Red-mediated recombination. Pathogenicity of wildtypes, mutants and their hfq-complemented derivatives was assessed by feeding *C. elegans* (sek-1, glp-4) on bacterial lawns and scoring of dead worms daily. Non-pathogenic *E. coli* OP50 was included as a control. Furthermore, nematode colonization was evaluated by fluorescence microscopy of worms feeding on GFP-marked bacteria.

**Results:** Killing assays showed that worms feeding on VTEC, EAEC and UPEC strains die by a slow-killing mechanism over the course of several days, however, significantly faster than worms subjected to the negative control only. Deletion of hfq significantly extends the lifetime of the worms (LT50<sub>delta</sub>hfq-LT50<sub>WT</sub> ≥ 3 days) for all pathotypes. Fluorescence microscopic visualization did, however, not reveal a significantly reduced ability of mutants to colonize the nematode intestinal tract.

**Conclusion:** Hfq is required for full virulence of several *E. coli* pathotypes towards *C. elegans* suggesting that riboregulation is involved in pathogenicity of *E. coli* in general. Future studies may reveal whether attenuation is caused by disruption of shared regulons or if loss of pathogenicity remain pathotype-specific. Lack of hfq is known to cause pleiotropic phenotypes in bacteria and the increase in lifetime of the nematode might be a simple result of a lowered physiological fitness of *E. coli*. However, the fact that hfq mutants do colonize the nematode suggests that attenuation is related to more specific virulence traits. Modulation of Hfq function or riboregulation in general is an attractive target for future antimicrobial therapy against pathogenic *E. coli*.

**P2189** **Role of immune pathways in *Caenorhabditis elegans* during *Serratia marcescens* infection**

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**Objectives:** *Serratia marcescens* is an opportunistic pathogen and produce a nuisance for public health. *Caenorhabditis elegans* is a free-living soil nematode used as a model organism due to its accessibility to genetic, molecular, and behavioral analyses. This study aims to analyze the immune regulation of *C. elegans* against *S. marcescens* (clinical and reference strain) using two *C. elegans* knockout mutants. Mitogen-activated protein kinase (MAPK) signaling pathways play a key role in *C. elegans* and required for pathogen defense. pmk-1 and sek-1 functions as the downstream of MAPK pathway. In addition, DAF-2/

DAF-16 pathways also analyzed which include few antimicrobial genes such as lys-7, spp-1 spp-12 and thaumatin homologues.

**Methods:** *S. marcescens*-mediated infection assays were performed to study the physiological response of the host (wild-type [N2] and mutants [KU25, AU37]). Semi-quantitative RT-PCR analysis was performed to kinetically analyze the expression and regulation of host specific candidate antimicrobial genes (lysozymes, C-type lectins) during the infection course of *S. marcescens*.

**Results:** *S. marcescens* kills *C. elegans* after colonizing the nematode's intestine. N2 worms exposed to *S. marcescens* (clinical strain) and IGX2 (reference strain) showed complete killing at 75 ± 2.6 and 97 ± 1.5 hours, respectively. Whereas, KU25 (pmk-1 (km25)) exposed to *S. marcescens* and IGX2 showed complete killing at 63.3 ± 2.9 and 76.7 ± 1.2 hours, respectively. AU37 (sek-1 (km4)) exposed to *S. marcescens* and IGX2 showed complete killing at 67.5 ± 2.3 and 88.9 ± 3.5 hours, respectively. RB1285 (lys-7 (ok1384)) exposed to *S. marcescens* and IGX2 showed complete killing at 63.3 ± 2.9 and 93.3 ± 1.2 hours, respectively.

**Conclusions:** The worms exposed to *Serratia* showed reduced rate of survival, pharyngeal pumping and egg laying indicated the possible role of MAPK (pmk-1, sek-1) and DAF-2/DAF-16 (lys-7) pathways in host immune responses during *S. marcescens* infection. The mRNA levels of candidate antimicrobial genes lys-7, clec-60 and scl-2 were altered in both wild-type and mutant worms upon exposure to the pathogen.

**P2190** **Lipopolysaccharide O1 antigen contributes to the virulence in *Klebsiella pneumoniae* causing pyogenic liver abscess**

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*Klebsiella pneumoniae* is the common cause of a global emerging infectious disease, community-acquired pyogenic liver abscess (PLA). Capsular polysaccharide (CPS) and lipopolysaccharide (LPS) are critical for this microorganism to be able to spread through the blood and to cause sepsis. CPS type K1 is an important virulence factor in *K. pneumoniae* causing PLA. However, the role of LPS in PLA is not clear. Here, we characterize the role of LPS O antigen in the pathogenesis of *K. pneumoniae* causing PLA. NTUH-K2044 was an LPS O1 clinical strain, which was identified by chemical structure analysis as 1,3-galactan, and sequence alignment with the wb gene cluster. Serologic analysis of *K. pneumoniae* clinical isolates, the O1 serotype was more prevalent in PLA strains than that in non-tissue-invasive strains (38/42 vs. 9/32, p < 0.0001). O1 serotype isolates had a higher frequency of serum resistance, and mutation of the O1 antigen changed serum resistance in *K. pneumoniae*. O1-deletion mutant of O1:K2 PLA strain was profoundly attenuated in virulence compared to that of the wild-type strain in two mice models of septicemia and liver abscess. Immunization of mice with the K2044 magA-mutant (K1-O1) against LPS O1 provided protection against infection with O1:K2 PLA strain, but not O1:K1 PLA strain. Our findings indicate that the O1 antigen of PLA-associated *K. pneumoniae* contributes to virulence by conveying resistance to serum killing, promoting bacterial dissemination and colonization to internal organs after the onset of bacteremia and could be a useful vaccine candidate against infection by O1:K2 PLA strain.

**P2191** **Effects of erythropoietin and mesenchymal stem cells combination in an experimental sepsis model in rats**

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O. Kuzovlev (Moscow, Obninsk, RU)

Last years, several studies have demonstrated the effectiveness of transplantation of mesenchymal (MSC) and embryonic stem cells in experimental models of sepsis. Recently found that MSCs have receptors for erythropoietin (EPO) on their surface. We hypothesized that EPO may enhance the action of MSCs in their joint application.

**Objective:** To study the effects of allogeneic MSCs and human erythropoietin combination in rats with endotoxemia induced by bacterial lipopolysaccharide.

**Methods:** Fifty Wistar rats were randomized into five groups of 10 animals each. The first group used as healthy controls. Animals of 2–5 were injected by intraperitoneal LPS 20 mg/kg. After 2 hours 400 000 allogeneic MSCs were transplanted to the rats of Group 3, animals Group 4 received 8.5 mg of EPO-beta, and group 5 was treated with combination of MSC and EPO in the same doses. The Group 2 was considered as the sepsis controls. The animals were euthanased on the 4th day of the experiment by intravenous injection of Nembutal. The morphological changes of the liver, spleen, thymus, lung, heart tissues and blood leukocytes level we evaluated.

**Results:** In LPS groups there was a significant increase of white blood cells count (maximum in LPS + MSC + EPO group –  $8150 \pm 1045$  cells/mm<sup>3</sup>) compared with controls ( $2150 \pm 424$  cells/mm<sup>3</sup>). Histologically in Group 5 we observed considerable hyperplasia of spleen white pulp (up to 64.9%) and thymus cortex (up to 69.7%) which were significantly different from other groups, whereas in group 2 (control LPS) marked atrophy of the appropriate areas. In animals of group 3 and 5 lower degree of leukocyte infiltration of alveolar inflammatory cells into alveolar interstitium and degeneration kidneys tubular apparatus recorded. No differences in mortality between 2 and 5 groups were found.

**Conclusions:** The addition of erythropoietin to the systemic transplantation of MSC in an experimental model of sepsis leads to hyperplasia organs of immunity and increase systemic leukocyte reaction.

## Biomarkers of inflammation

### P2192 Diagnostic and prognostic value of procalcitonin and C-reactive protein: systematic review

G. Dornikova\* (Cavan, IE)

**Objectives:** The current review aimed to assess diagnostic and prognostic value of C-reactive protein and procalcitonin as inflammatory markers.

**Methods:** There was performed search on PubMed for all studies on humans in English language, published from January 2004, to January 2010. Twenty-eight articles and more than 14 108 patients were included into analysis.

**Results:** Diagnostic value of procalcitonin for diagnosis of sepsis, severe sepsis and septic shock was 80% for sensitivity and 73% for specificity, whereas CRP yielded 87% for sensitivity and 45% for specificity only. Prognostic value for adverse medical outcome – death and/or ICU admission, was higher in procalcitonin (85%/73%) than in CRP (76%/74%).

**Conclusion:** PCT is a potential new marker for prediction of adverse medical outcomes of sepsis. Use of PCT as diagnostic and monitoring tool may improve management and the survival of patients with severe sepsis and septic shock. The use of both inflammatory variables, PCT and CRP is limited in patients with haematology–oncology disorders due to the T-cells directed immunomodulatory treatment, granulocyte support and graft-versus-host-disease.

All three inflammatory markers: WBCC, CRP and procalcitonin are recommended to be monitored by international guidelines for management of sepsis, severe sepsis and septic shock.

### P2193 Nosocomial outbreak of *Corynebacterium striatum* infection in a surgical intensive care unit

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**Objective:** *Corynebacterium* spp. are widely disseminated in the environment and found as colonizers of skin and other tissues of human. Except some strains like *Corynebacterium jeikeium* and diphtheriae the role of these bacteria as a causative agent of diseases

is not well understood. In this study we presented an outbreak of *Corynebacterium striatum* infection in the cardiothoracic surgical intensive care unit of a Turkish University hospital.

**Methods:** All isolates included in the study were detected in routine diagnostic cultures of patients and some environmental samples were performed. For the diagnosis of infection CDC criteria were used. Isolates were grown on blood agar plates and further characterized by gram stain morphology and the API Coryne (bioMerieux, FR) kit.

**Results:** From December 2010 to October 2011, 17 *C. striatum* strains were isolated from 15 patients. Ten of the strains were isolated from respiratory samples, four from blood and catheter, and one from wound aspiration. All strains were found to be identical by ERIC PCR, and this outbreak strain was resistant to penicillin, daptomycin, gentamicin, trimethoprim-sulphamethoxazole, quinolones, and was susceptible to erythromycin, vancomycin, and linezolid. Patient characteristics were given in the table. The outbreak strain was also isolated from one of the environmental samples.

Table. Patient characteristics infected or colonized with *Corynebacterium striatum*

Patients	Age/Gender	Sample	Diagnosis	Therapy	Prognosis
1	51/Male	Respiratory sample	Colonized	No	Died (3 months later)
2	69/Male	Respiratory sample	Pneumonia	Vancomycin	Survived
3	70/Male	Wound aspiration	SSI*	Vancomycin	Died
4	60/ Female	Catheter tip	BSI**	Daptomycin/ Vancomycin	Died
5	68/Female	Catheter tip	BSI	Vancomycin	Survived
6	60/Male	Respiratory sample +blood	Pneumonia	Linezolid	Survived
7	63/Male	Respiratory sample	Colonized	No	Survived
8	49/Male	Respiratory sample	Pneumonia	Vancomycin	Died
9	53/Male	Respiratory sample	Pneumonia	Linezolid	Died
10	69/Female	Respiratory sample	Pneumonia	Linezolid	Died
11	69/Male	Respiratory sample	Colonized	No	Survived
12	67/Female	Respiratory sample +blood	Pneumonia	Vancomycin	Died
13	1/Female	Blood	SSI	Teicoplanin	Survived
14	3 days/Male	Catheter tip	SSI	No	Died
15	74/Female	Respiratory sample	Colonized	No	Survived

\* SSI: Surgical site infection

\*\*BSI: Blood stream infection

**Conclusion:** Although unusual, *C. striatum* has caused an outbreak of opportunistic infections in the cardiothoracic surgical intensive care unit. After the outbreak was recognized, it was controlled by strict adherence to hand-washing and environmental cleaning procedures.

### P2194 Procalcitonin levels as an inflammatory marker for rapid and appropriate choice of empirical antibiotic therapy

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**Objective:** This study is undertaken to determine whether concentrations of procalcitonin (PCT) depend on the type of pathogen and correlate with the microbial load and if is useful for the rapid and appropriate choice of empirical antibiotic therapy.

**Material and methods:** We reviewed the medical records of 428 patients admitted in the ICU between January 2009 and October 2011. C-reactive protein (CRP), PCT on the onset of bacteremia and time to positivity (TTP) of blood cultures were measured. One episode of bacteremia was defined as the recovery of any bacterial species, in one or more blood cultures. Patients with polymicrobial cultures were not eligible.

**Results:** Two hundred and fifty-two negative blood cultures and 176 episodes of either Gram negative bacteremia (GN) (n = 110) or Gram

positive bacteremia (GP) (n = 66) were included. CRP values >0.5 mg/dL and PCT values >0.5 ng/mL were considered abnormal. A PCT level of 0.5 ng/mL yielded a 81.63% positive predictive value (PPV) and a 92.17% negative predictive value (NPV), while a CRP level of >0.5 mg/dL yielded a 43.61% PPV and a 72.55% NPV for bacteremia (Area under the curve for PCT = 0.938 (95%CI, 0.915–0.962 while area under the curve for CRP = 0.689 95%CI, 0.636–0.742). PCT levels were found to be statistically significant higher in patients with GN bacteremia (median = 16.7) than in those with GP bacteremia (median = 4.7) (Mann-Whitney *U* test *p* value <0.0005). The diagnostic ability of PCT discriminating GN from GP bacteremia as reflected by the area under the curve of the receiver operating characteristic (ROC) was 0.839 (95%CI, 0.799–0.899). A PCT level of 1.65 ng/mL yielded a 84.63% PPV and a 65.75% NPV. Patients with PCT > 1.65 ng/mL had 10.2 (95%CI, 4953–20 694). The TTP of blood cultures was linearly correlated with the PCT serum levels ( $r = 0.435$   $R^2 = 0.19$   $p < 0.0005$ ). The PCT levels were statistically significant higher in oxidase negative GN microorganisms (median = 12.43) than oxidase positive microbes (median = 3.78) ( $p < 0.005$ ) and lower in coagulase negative Staphylococci (CoNS) (median = 1.26) than other GP cocci (median = 2.51) ( $p = 0.035$ ).

**Conclusions:** Our findings suggest that baseline PCT elevation could be greater when bacteremia is caused by GN bacteria in comparison with GP bacteria and lower when caused by CoNS than other gram positive cocci. Since PCT measurement is available sooner than the Gram stain result, its value could be considered when discussing the choice of first line antibiotics in critically ill patients with clinical sepsis.

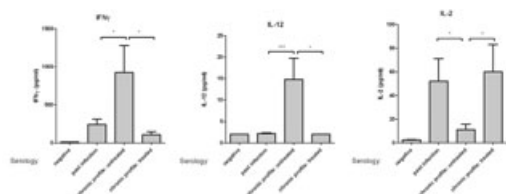
#### P2195 Interferon-gamma, interleukin (IL)-12 and IL-2 release in *Coxiella burnetii* stimulated whole blood for the diagnosis of chronic Q fever

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**Objectives:** Infection with *Coxiella burnetii* may lead to serious life-threatening chronic disease like endocarditis and vascular infection. In daily practice it is difficult to discriminate, based on serology alone, between patients with these chronic infections and those with a previous infection without chronic disease. In the current study we investigated whether the in-vitro cytokine production in whole blood after stimulation with *C. burnetii* can differentiate between chronic and past infection.

**Methods:** The production of interferon-gamma (IFN-g), interleukin (IL)-12p70, IL-1beta, IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-10, and tumor necrosis factor (TNF)-alpha, induced in-vitro by inactivated *C. burnetii* Nine Mile phase I in whole blood, was measured by a multiplex cytokine assay. The production in patients with proven or probable chronic Q fever (according to the Dutch guidelines for chronic Q fever), either untreated (n = 9) or on treatment for >3 months (n = 5), was compared with the production in individuals with a past infection (phase 1 IgG-antibody titer <1024) (n = 17) and individuals without any evidence of previous infection (no anti-*Coxiella* antibodies and a negative skin test) (n = 13).

**Figure 1.** The production of IFN-gamma, IL-12 and IL-2 in *C. burnetii* stimulated whole blood. Production of IFN-gamma and IL-12 was significantly higher in individuals with chronic infection than in individuals with past infection based on serology. IL-2 was significantly lower in individuals with chronic infection. IFN-gamma, IL-12 and IL-2 production in patients with chronic Q fever treated for > 3 months was similar to that in individuals with previous infection without chronicity. Median values  $\pm$  SE are presented of 13 individuals with negative serology, 17 individuals with a past infection (phase 1 IgG-antibody titer < 1024) and 14 patients with proven or probable chronic Q fever, either untreated (n=9) or on treatment for > 3 months (n=5).



**Results:** The production of IFN-g, IL-12, IL-1beta, IL-2, IL-6 and TNF-alpha was significantly higher in individuals with either chronic or past infection than in naïve individuals. The production of IFN-g and IL-12 was significantly higher in individuals with chronic infection than in individuals with a past infection. Interestingly, IL-2 was significantly lower in individuals with chronic infection (Figure 1.). Overall, a high IFN-g, high IL-12 and a low IL-2 production could distinguish cases of chronic Q fever from those with a past infection. Cytokine profiles in patients with chronic Q fever treated for >3 months were similar to those in individuals with previous infection without chronicity.

**Conclusion:** IFN-g, IL-12 and IL-2 production distinguishes patients with chronic Q fever from individuals with a past infection. Chronic patients on treatment >3 months had a cytokine profile similar to individuals with a past infection. Measurement of IFN-g, IL-12 and IL-2 production is helpful for diagnosing chronic Q fever. Its use for clinical monitoring during treatment of chronic Q fever needs further study.

#### P2196 Comparison of human cytomegalovirus QuantiFERON and IFN-g ELISPOT: prediction, specificity and accuracy of the tests in anticipating symptomatic CMV viraemia in a cohort of kidney transplant recipients

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**Background:** Monitoring post-transplant antiviral immune recovery is an appealing strategy to detect kidney transplant recipients (KTRs) at risk of developing episodes of post-transplant CMV infection and disease. Assessing the antiviral immune response in transplant recipients may also represent a clinical helpful guide. At the present several methods may be employed in detecting patients antiviral T-cell response. Notably we compared CMV QuantiFERON (QTF) and ELISPOT.

**Methods:** We employed both Interferon gamma (IFN-g) ELISPOT and cytomegalovirus (CMV) QTF tests to detect CMV specific T-cell immune recovery in a cohort of 279 KTRs and 11 healthy subjects.

**Result:** Both ELISPOT and QTF tests are able to specifically detect cytomegalovirus (CMV) specific T-cell responses in KTRs. None of the cytomegalovirus (CMV) seronegative subjects displayed significant levels of T-cell responses. Both in transplant patients and healthy subjects, ELISPOT and QTF tests displayed a concordance of 65% and discordance of 35%. Receiver Operator Characteristics (ROC) analysis revealed that both ELISPOT and QTF displayed a moderate ability in anticipating CMV viraemia (AUC > 0.76 ELISPOT; 0.6 QTF). ELISPOT test displayed a significant higher ability in detecting clinical significant viraemia >20 000 copies of cytomegalovirus (CMV) DNA/mL of whole blood and duration of cytomegalovirus (CMV) viraemia compared to CMV QTF test. On the other side QTF test is more practical than ELISPOT and requires minor sample handling and elaboration.

**Conclusion:** Both ELISPOT and QTF tests possess predictive value for assessing patients at risk of CMV infection.

#### P2197 Detection of T cell receptor Vbeta expansion allows the diagnosis of toxic shock syndromes from septic shocks

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**Objectives:** Point out of the interest of an in vitro biological test for the diagnosis of toxic shock syndromes in septic patients: the expansion measurement of Vbeta repertoires of T cell receptor (VB).

**Materials and methods:** Between 2006 and 2011, we determined the 64 main VB expansions of 45 patients admitted for suspected toxic shock syndrome (TSS) clinically defined by the minimal association of fever, hypotension and cutaneous signs at the admission. Based on



published standardized clinical criteria, the final diagnosis of 45 patients encompassed nine menstrual staphylococcal toxic shocks (M-SaTS), nine non menstrual staphylococcal toxic shocks (NM-SaTS), 11 streptococcal toxic shocks (SpTS), and 16 septic shocks (SS). The first VB expansions were measured from 24 to 48 hours after admission to ICU by flow cytometry. If results were doubtful, a second measurement was realized up to 5 days later in order to conclude. Concomitantly, adapted microbiological samples, including blood cultures, were analyzed by conventional methods. If *Staphylococcus aureus* (Sa) or *Streptococcus pyogenes* (Sp) isolates were obtained, their toxic gene profiles were determined by molecular methods (French National Reference Center for Staphylococcal or Streptococcal).

**Results:** The 9 M-SaTS patients showed VB expansions corresponding to Toxic Shock Syndrome Toxin 1 (TSST-1) profile. In each case, except one, a TSST-1 gene positive-Sa strain was isolated from the vaginal pad. The 9 NM-TSS cases showed VB expansions related to staphylococcal enterotoxin B (n = 2) or TSST-1 (n = 7). A Sa strain with a toxin gene profile corresponding to VB expansions was isolated in each case except one. Characteristic VB expansion profiles were measured for 11 StTS (10 with streptococcal pyrogenic exotoxin [Spe] A and 1 with SpeC), with a corresponding Sp strain toxin gene profile. No VB expansion was measured in the 16 SS cases, notably in the seven SS and three SS finding respectively Sa and Sp strains.

**Conclusion:** VB measurements appear to be a powerful diagnostic tool for M-SaTS, NM-SaTS and SpTS. This test seems to be predictive of superantigen involvement and to allow the toxin type characterization. Contrary to the clinical diagnosis, which is based on partial or late clinical signs, it allows the rapid administration of anti-toxic therapies and help to diagnose toxic shock syndromes.

#### **P2198** Is hypophosphataemia associated with lymphopenia are predictive of Gram-negative *Bacillus* bacteraemia?

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**Background:** Gram Negative *Bacillus* (GNB) bacteriemia are potentially severe conditions that need early appropriate diagnosis and management to avoid complications. Early diagnostic tools are mandatory. Among them, biological abnormalities, present at admission, might help pointing to this diagnosis. It has been suggested that hypophosphatemia and lymphopenia might be associated with such infections

**Objective:** To determine, in a series of blood cultures coupled with serum analysis, the relevance of the association of lymphopenia and hypophosphatemia with the diagnosis of Gram negative bacillus bacteriemia.

**Methods:** A 6 months retrospective study carried out in Jolimont hospital from March to September 2010. Full data were available for 153 patients, older than 18 years old. Eighty (52%) presented gram negative bacillus bacteriemia, 73 (48%) bacteriemia from other origin. The association between lymphopenia (under 1000/ $\mu$ L), hypophosphataemia (under 2.5 mg/dL) and gram negative bacillus bacteriemia was assessed.

**Results:** The phosphates levels were significantly lower in case of bacteriemia with GNB, as compared to bacteriemia from other origin (2.82 mg/dL vs. 3.34, p = 0.00369), as well as the lymphocytes levels (830/ $\text{mm}^3$  vs. 1177, p = 0.01), even if total leucocytosis was quite the same among the two populations (p = 0.8). In case of bacteriemia associated with lymphopenia and hypophosphatemia, the probability of identifying a GNB was 75% (with a specificity of 84%, a sensibility of 40.74%).

**Conclusions:** In case of bacteriemia, hypophosphatemia associated with lymphopenia is predictive of identification of a gram negative bacillus.

## Clinical experience with antibiotics

#### **P2199** Trimethoprim and ciprofloxacin resistance and prescribing in *E. coli* associated urinary tract infection: a multilevel model

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**Objective:** To examine the individual (patient) and group (practice) level factors associated with the probability of antimicrobial resistance of *E. coli* isolated from patients with suspected UTI by means of multilevel statistical modelling.

**Methods:** All adult patients consulting with a suspected UTI in one of the 22 general practices over the 9 months supplied a urine sample. An opt-out methodology was applied. All urine samples with laboratory confirmed *E. coli* were included and antimicrobial susceptibility testing was performed. All *E. coli* isolates from two general practices were evaluated using Pulse Field Gel Electrophoresis (PFGE) to assess the similarity between isolates within and between practices. Data on antimicrobial exposure in the previous 12 months and other patient characteristics were recorded.

**Results:** Six hundred and thirty-three patients with a laboratory confirmed *E. coli* UTI and a full record for all variables were included. Of the *E. coli* isolates, 36% were resistant to trimethoprim and 12% to ciprofloxacin. The odds that an *E. coli* UTI was resistant increased with an increasing number of prescriptions over the previous year. For trimethoprim resistance the odds increased from 1.4 (0.8–2.2) for one previous trimethoprim prescription, to 4.7 (1.9–12.4) for 2 and 6.4 (2.0–25.4) for 3/> prescriptions in the previous year. For ciprofloxacin resistance the odds ratios were 2.7 (1.2–5.6) for one and 6.5 (2.9–14.8) for 2/> ciprofloxacin prescriptions in the previous year. The probability a patient was diagnosed with a resistant *E. coli* UTI showed important variation between practices and a difference of 17% for trimethoprim and 33% for ciprofloxacin was observed for an imaginary patient moving from a practice with low to a practice with high probability. Twenty clusters of two or more isolates with >85% homology were identified including four (of two isolates each) that were indistinguishable. The two practices were in roughly the same geographical area but patients would generally not be shared.

**Conclusions:** The more trimethoprim or ciprofloxacin prescribed in the year previous to an episode of UTI, the higher the odds that an episode of *E. coli* UTI was resistant to this agent. This was particularly important for ciprofloxacin emphasising that this antimicrobial should be used very carefully. The practice attended can also influence this chance that an *E. coli* UTI was resistant even though the transmission of organisms was suggested to transcend practice boundaries.

#### **P2200** Antibiotic use in cystitis

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**Objectives:** To show the pattern of antibiotic use in cystitis and calculate some disease-specific quality indicators developed by the ESAC (1).

**Methods:** The aggregated regional-level reimbursement data on systemic antibiotic prescriptions were purchased from the National Health Fund Administration. The study period was between 2007 January and June. All prescriptions claimed in the pharmacies of the region (n = 445 pharmacies) during this half year were included in the analysis. Antibiotic use was evaluated by means of the ATC/DDD methodology (version 2008). The registered ICD (International Classification of Diseases version 10) codes enable us to evaluate antibiotic by indication (in this case: cystitis). Quality indicators of antibiotic prescribing proposed by the ESAC (usage rate of recommended antibacterials and usage rate of quinolones) were determined.

**Results:** For cystitis we registered 1.61 DDD per 1000 inhabitant-days (DID) antibiotic use which accounts for 7.6% of all systemic antibacterial use. The top five agents used in cystitis were:

norfloxacin (0.49 DID, 30.2%); ciprofloxacin (0.26 DID, 16.4%); sulfamethoxazol-trimethoprim combination (0.21 DID, 13.3%); nitrofurantoin (0.12 DID, 7.7%); and ofloxacin (0.11 DID, 6.8%). The ESAC recommended antibacterial agents (J01XE-nitrofurans derivatives; J01EA-trimethoprim and derivatives; J01XX-other antibacterials) which should be used in cystitis (ideally in 80–100%) were used only in 22.63% while the cumulative use of quinolones were 54.5%, which is well above the ESAC recommended acceptable fluoroquinolone use range (0–5%).

**Conclusion:** Cystitis was mainly treated with fluoroquinolones and ESAC recommended agents were responsible only for one-fifth of antibiotic use in this indication. Comparison to national guideline is wanted and may partly explain the present findings.

**Reference:** 1. Adriaenssens N, Coenen S, Tonkin-Crine S, et al. *BMJ Qual Saf* (2011).

#### **P2201** Emergence of carbapenem-non-susceptible extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* isolate after meropenem therapy

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**Objectives:** To investigate the resistance mechanisms of carbapenem susceptibility isolate recovered from one patient after treatment with meropenem.

**Methods:** Since November 2010, an infection control program for ESBL-producing Enterobacteriaceae was introduced in an acute-care private hospital in Lisboa, Portugal. Seven *Klebsiella pneumoniae* isolates were recovered from one ICU inpatient. Cultures of rectal swabs and clinical samples were performed on a chromogenic medium (chromID™ ESBL; bioMérieux). The isolates were studied by Etest MBL, PCR for blaCTX-M, blaIMP, blaVIM, blaKPC, blaOXA-48 genes and ERIC fingerprinting. Experiments were performed to detect synergy between meropenem or other antimicrobials and the efflux pump inhibitor reserpine.

**Results:** A 79 years old female patient was admitted to H.SAMS with large bowel angiodysplasia leading to hemicolectomy. She had a long recovery period in ICU with prolonged ventilatory support. She was colonized with *K. pneumoniae* K137 recovered from a rectal swab prior to meropenem therapy. After 27 days in ICU the patient had ventilator-associated pneumonia and urinary tract infection and two *K. pneumoniae* strains K138, K139 were identified from bronchial secretions and urine, respectively. The patient was subsequently treated with meropenem, after which, during 1 month, three *K. pneumoniae* isolates were once again isolated from rectal swab, bronchial secretions and urine. Thirty-seven days after meropenem therapy one *K. pneumoniae* K168 isolate was recovered from a rectal swab with different antibiotics susceptibility, for imipenem and meropenem MIC values of 4 and 1.5 mL/L, while MIC values to previous strains were 0.25 and 0.125 mL/L, respectively.

From ERIC electrophoresis profiling, all strains exhibited identical banding patterns and all were ESBL producing CTX-M-15 enzyme. They gave negative results in the MBL Etest and lacked genes encoding carbapenemases. Only the *K. pneumoniae* K168 exhibited synergy between meropenem, ertapenem and reserpine, indicating that efflux pump activity could contribute to less susceptibility to carbapenem.

**Conclusions:** The carbapenem phenotype observed in *K. pneumoniae* K168 was attributable to a combination of ESBL CTX-M-15 enzyme and an up-regulated efflux pump.

The clonal relationship observed between the initial and subsequent *K. pneumoniae* strains may explain the emergence of resistance under meropenem selective pressure.

#### **P2202** First cases of urinary tract infection cured by linezolid

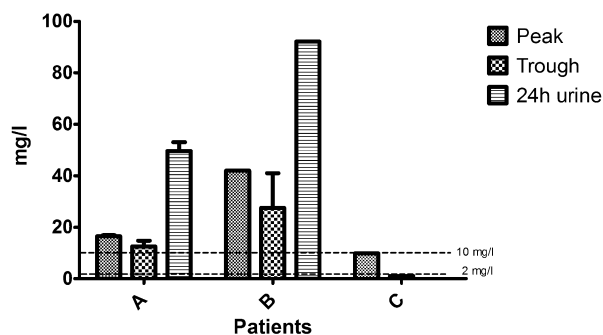
G. Beraud\*, N. Venisse, M. Puyade, S. Delbes, C. Chagneau-Derrode, F. Roblot (Poitiers, FR)

**Objectives:** Cocci gram positive pathogens account for up to 30% of complicated urinary tract infection (UTI). Linezolid is an area under the

concentration curve (AUC) dependant antibiotic with urinary elimination, for which efficient AUC have been correlated to trough concentration. Linezolid has been proposed for treatment of UTI due to Cocci gram positive pathogens but no clinical reports exist. We report the first cases of Cocci gram positive UTI treated by linezolid.

**Methods:** Patient A had UTI with  $10^6$  *Enterococcus faecium* resistant to ampicillin. His past medical history was notable by a kidney transplant 1 month earlier and a persistent chronic renal failure, which contraindicated vancomycin. Patient B had UTI with  $10^5$  *Staphylococcus aureus* penicillin resistant. Her past medical history was notable by alcoholic cirrhosis (child-pugh C) and frequent hepatic decompensation with edema and ascites. Patient C had UTI with  $10^7$  *Enterococcus faecalis* sensible to amoxicillin. His past medical history was marked by severe polyarthritis with difficulties regarding infusion. Linezolid was administered orally at 600 mg twice a day during 3 weeks. Peak samples obtained 2 hours after ingestion and trough concentration just before ingestion were measured at steady state. Urinary concentrations were determined on 24 hours urine. Trough concentration target in plasma are usually 2–10 µg/mL.

**Results:** Median (min-max) peak concentration was 16.5 (9.9–42.0) µg/mL while trough concentration was 12.0 (1.1–41.0) µg/mL. Median (Min-Max) urine concentrations were 50.8 (32.6–92.1) µg/mL. Minimum inhibitory concentrations (MIC) for linezolid were 1, 2 and 2 µg/mL, respectively. All the patients were cured, and didn't experience relapse during the 1 month follow-up. No patient experienced anaemia, neutropenia, thrombopenia or neuropathy.



**Conclusion:** Urinary concentrations were elevated and far superior to MIC, which is consistent with the urinary elimination of linezolid. Linezolid could be an interesting alternative for UTI due to cocci gram positive uropathogens.

#### **P2203** Impact of empirical ceftriaxone on acute pyelonephritis caused by ESBL-producing *Escherichia coli*

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**Objectives:** Ceftriaxone is one of most common empirical option for acute pyelonephritis (APN) with increasing quinolone-resistance. Recently, Infections due to extended-spectrum beta-lactamase (ESBL)-producing organism is increasing even in the community onset infections. However, there is few data about the impact of empirical ceftriaxone treatment on acute pyelonephritis caused by ESBL-producing organisms.

**Methods:** A retrospective case-control study was performed from January 2009 to September 2011 in a secondary care hospital (Daegu, South Korea). During study periods, all cases whose urine culture were requested on the 1st day of hospitalization and yielded *E. coli* were detected through review of microbiology data base. Among them, cases that fulfilled following criteria were included. Inclusion criteria were that (i) one's body temperature on admission was more than 38°C, (ii) one's imaging study was consistent with APN, and (iii) ceftriaxone was chosen as initial empirical antibiotics. Medical records and laboratory data of included cases were reviewed retrospectively. ESBL group included ESBL positive cases, while non-ESBL group included ESBL negative cases.

**Results:** A total of 171 cases, 24 in EBSL group and 147 in non-EBSL group, were enrolled in this study. Mean age was  $62.4 \pm 15.5$  years and 93% were female. One hundred and fifty-three cases (90.1%) were community acquired infection. The demographic and clinical characteristics were comparable between groups except healthcare association (25% in EBSL group vs. 7.5% in non-EBSL group,  $p = 0.02$ ). Median to defervescence (IQR) was 4 (3–5.75) days in EBSL group and 2 (2–3) days in non-EBSL group ( $p < 0.01$ ). ICU-care was necessary for 16.7% (4/24) in EBSL group and 2.7% (4/145) in non-EBSL group ( $p = 0.01$ ). 72.2% (13/18) in EBSL group and 100% (116/116) in non-EBSL group achieved microbiological resolution within 5 days after antibiotic treatment ( $p < 0.01$ ). Rates of complete symptom relief at 2 weeks were 75% (18/14) in EBSL group and 93.8% (136/145) in non-EBSL group. Relapse rates at 12 weeks were similar in each group (4.2% vs. 6.8%,  $p > 0.99$ ).

**Conclusion:** Empirical ceftriaxone therapy can deteriorate APN caused by EBSL producing *E. coli*. Delayed response to ceftriaxone is one of useful marker of EBSL-producer in treating APN.

**P2204** **Temocillin for treatment of urinary tract Infections due to extended-spectrum beta-lactamase producing coliforms**

*H. Habayeb\* (Chertsey, UK)*

Resistance in gram negative bacteria is increasing in the UK and Europe leaving only Carbapenems dependently active against some coliforms. Temocillin (6-alpha-methoxy-ticarillin) like all penicillins inhibits the synthesis of the peptidoglycan bacterial cell wall. It irreversibly binds to the active site of specific transpeptidases and carboxypeptidases known as penicillin binding proteins (PBP). It's stable to nearly all Amp C and Extended spectrum beta-lactamases (ESBLs).

In those patients who have a urinary tract infection (UTI) or bacteraemia due to an ESBL producing coliform, temocillin could be a very good choice. It could also be a good choice as de-escalation from carbapenems due to its narrower spectrum being active only against gram negative bacteria except *Pseudomonas* and *Acinetobacter*.

**Objectives:** To assess the clinical efficacy of temocillin in treatment of UTI due to an ESBL producer or previous history of ESBL producer.

**Methods:** The use of temocillin was studied in a 450 bedded NHS Trust for treatment of UTI caused by ESBL producing coliform (14 patients) or history of ESBL (five patients) retrospectively over a period of 18 weeks from June to October 2011. The study excluded patients who had temocillin in combination with other antibiotics and/or who had both UTI and chest infection.

**Results:** Total patients: 19; in one of them the effect of temocillin was indeterminate. Overall 17 (94%) out of 18 patients recovered on temocillin. In two out of the 17 who recovered, temocillin was used as de-escalation from meropenem (which had caused diarrhoea) after 3 and 4 days respectively. The duration of temocillin courses varied from 5 to 7 days in most patients except one patient who had it for 14 days for epididymo-orchitis plus UTI. The one patient who had treatment failure also had cellulitis and flucloxacillin but his blood culture grew ESBL *E. coli* which he had temocillin for. He was switched from temocillin to meropenem and recovered.

**Conclusion:** Temocillin seems to be a very good option in the treatment of UTI due to an ESBL producing coliform or when there is history of ESBL. Since it is also useful for de-escalation from carbapenems or piperacillin/tazobactam it helps to reserve these valuable agents, potentially prolonging their useful lives.

**P2205** **Wound dehiscence and infection after amputation: risk factors and associations with peri-operative antibiotic administration**

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**Background:** Clinical risk factors for wound breakdown and infection and the ideal duration of antibiotic prophylaxis after major forefoot and leg amputations are contested.

**Objective:** We assess the epidemiology and risk factors of stump infections and wound break-down after transmetatarsal and leg amputation with an emphasis on the antibiotic prophylaxis.

**Methods:** Prospective cohort at Geneva University Hospitals, January 1995–June 2010, including all adult patients with major amputations in the foot and leg.

**Results:** A total of 289 amputation episodes in 270 patients (199 males, median age 70 years) were included. Stump dehiscence occurred in 47 episodes (16.7%) after a median of 24 days; stump infection in 63 episodes after a median of 14 days post amputation. The median durations of antibiotic administration pre- and post-amputation were 10 and 6 days, respectively. In multivariate Cox regression analyses, no clinical variable was significantly associated with outcome infection. Regarding stump dehiscence, only diabetes mellitus and an age more than 80 years was associated with dehiscence.

The duration of antibiotic administration post-amputation showed no association with infection. Compared to 1 day post-amputation, 2–3 days (odds ratio 1.1, 95%CI 0.4–3.3), 4–5 days (1.3, 0.5–3.6) or >5 days (OR 1.0, 0.4–2.7) did not enhance prevention of infection. This was equally true for the duration of pre-operative antibiotic therapy and when analysis was performed with outcome dehiscence.

**Conclusions:** Infection and stump dehiscence after foot and leg amputations are frequent, but the few identified associations are not modifiable in terms of prevention. The duration of antibiotic administration before or after amputation does not change the epidemiology of stump complications. Provided there is no concomitant infection, antibiotic agents can be stopped the day of amputation.

**P2206** **Antibiotic therapy for intra-abdominal infections: reported success rates for commonly proposed regimens**

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**Background:** Many studies have been performed to determine the efficacy of antimicrobial agents in treating complicated IAI, but overall efficacy of antimicrobials has not been reported, except for individual drugs. The aim of this study was to analyze the clinical efficacy of different drugs that have been proposed for the treatment of cIAI.

**Methods:** A PubMed search was conducted to identify studies on the treatment of complicated IAI with antimicrobial agents that were published from May 1949 through March 2010. From these studies, success rates for interventional antimicrobial therapy and the comparator (when applicable) were retrieved from the studies. From these data, the reported success rates were combined for each antibiotic or antibiotic scheme that have been proposed for the empirical treatment of cIAI by the Infectious Disease Society of America (IDSA); the obtained results were then compared.

**Results:** Seventy-four studies were identified, and from 53 studies success rates of on one of more antibiotic (or antibiotic scheme) proposed by the IDSA could be retrieved. Imipenem and piperacillin/tazobactam were studied most frequently (16 and 11 studies respectively), whereas for levofloxacin/metronidazole, cefazoline/metronidazole and ceftazidime/metronidazole no data were available. The number of patients studied varied from 75 (ticarcillin/clavulanic acid) to 1474 (imipenem/cilastatin) Overall, 7296 patients were treated with any of the proposed antibiotic combinations, and overall reported success rate was 85.3% (range 61.33–94.09%). In RCTs that directly compared regimens covering enterococcus vs. regimens that did not, success rates were comparable (83.83% vs. 85.66%).

**Conclusions:** Clinical experience with antibiotic schemes as proposed in the IDSA guidelines is limited or absent for some of the treatment regimens, and reported success rates are highly variable.

**P2207** Impact of a multidisciplinary approach to the antibiotic management of inpatients with diabetic foot infections

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**Objectives:** With the development of a local hospital antibiotic guideline for diabetic foot infection (DFI) and recent UK NICE guidance on the management of such patients, we have assessed the impact of a multidisciplinary approach to optimising the antibiotic management of inpatients with DFI.

**Methods:** A multidisciplinary diabetes foot team (MDFT) was established consisting of a diabetologist, podiatrist, clinical microbiologist and pharmacist. All inpatients referred to the podiatry services due to diabetic foot problems were assessed by the MDFT on a regular weekly ward round. Data was collected prospectively using a standard proforma and antibiotic advice given on all patients reviewed over a 7 month period (January–July 2011).

**Results:** Fifty-seven patients were reviewed (mean age 66.9 years, range 32–91 years) of whom 40 had a DFI. Seven patients (17%) had an unrecognised DFI and had antibiotic therapy initiated by the MDFT. Fifteen patients (37%) had evidence of bone infection. Only 40% of patients (16/40) were already known to the clinical microbiologists and only 37% of patients (21/57) were nursed in a designated ward for diabetes. In total 216 patient assessments were undertaken. Initial antibiotic prescriptions were altered or stopped in 30% of cases (10/33). On follow up, antibiotic therapy was again altered or stopped in 31% of cases (12/38). The most frequent reasons for an alteration to antibiotic therapy were the availability of laboratory sensitivity data and clinical progress allowing a switch from the intravenous to oral route.

**Conclusion:** The MDFT ward rounds had a significant impact on antibiotic management of DFI and resulted in improved patient outcomes and safety.

**P2208** Oral levofloxacin at standard dosage for treatment of bone and joint infections is efficacious and safe. A cohort study

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**Objectives:** Assessment of benefit/risk ratio of Levofloxacin by oral route at a standard dosage (500 mg/day) for the treatment of bone and joint infections (BJI). This fluoroquinolone is usually prescribed since it is marketed, without specific recommendations for BJIs.

**Methods:** Retrospective analysis of all cases of BJI treated with levofloxacin 500 mg od by oral route (cohort). The patients were selected among adult patients admitted in the orthopaedic septic unit of a large French teaching hospital over a period of 1 year (2009). A multidisciplinary team : surgeons, anaesthesiologists, microbiologists, hospital pharmacist, infectious diseases practitioner and hospital infection control pharmacist, has taken all the decisions regarding diagnosis and treatment. All clinical and microbiological data were prospectively recorded on a standardised form. The outcome was assessed at the end of antibiotic treatment, and after 1 year.

**Results:** Among 211 BJI, 76 patients were treated with levofloxacin during 13 weeks (median, p25-p75: 6–13), with a follow-up of 12 months (median, p25-p75: 4–17). These infections involved prosthetic joints for 29% (n = 2), were post-traumatic infection for 37% (n = 28), infection of rachis instrumentation for 12% (n = 9), or others BJIs for 22% (n = 17). Most of them were due to cocci gram positive (CGP), including MSSA for 47% (n = 28) coagulase negative staphylococci for 13% (n = 8), and others CGP for 13% (n = 8). Gram negative bacilli (10%, n = 6) and anaerobes (12%, n = 7) were less often identified. All patients were surgically treated by change or removal of infected device. During treatment, three cases of toxicity (5%) were recorded, leading to stop levofloxacin. At the end of treatment (n = 62), the outcome was considered as favourable (total or partial recovery, including orthopedics aftermath) in 89% of cases.

After 1 year of follow-up (n = 38), only one case of failure was recorded, with a modification of the susceptibility of the strain to fluoroquinolone.

**Conclusion:** Levofloxacin 500 mg once day could be a well-tolerated and efficacious treatment in BJI. There was no clinical study demonstrating the efficacy of levofloxacin in BJIs treatment before its marketing, nevertheless this antibiotic is widely used in this indication. Our approach, following up the cohort of all treated patients, is a way to validate this prescription practice.

**P2209** The clinical impact of empirical antimicrobial therapy on outcomes in patients with non-fermentative Gram-negative bacteraemia

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**Objectives:** The infection caused by non-fermentative gram-negative bacilli (NFGNB) has been one of great concerns in recent years, due to carbapenem-resistance and the limited potent antimicrobial agents against them. We studied the characteristics of patients with NFGNB bacteremia, that is, those with *Pseudomonas aeruginosa* or *Acinetobacter baumannii* bacteremia, carbapenem-resistant isolates of which might require urgent use of colistin, and we investigated the impact of empirical therapy with colistin or other antimicrobial agents on clinical outcomes.

**Methods:** Between January 2006 and September 2011, 88 patients with *P. aeruginosa* bacteremia and 71 patients with *A. baumannii* bacteremia were identified at Chung-Ang University Hospital and Chung-Ang University Yongsan Hospital. Clinical data were collected from the medical records at each institution.

**Results:** Of the 157 patients, 44 died within 30 days (28.0%). Patients with 30-day mortality had the following characteristics more frequently than those without 30-day mortality: chronic lung disease, ICU care, neutropenia, dialysis, mechanical ventilation, indwelling urinary catheter, central venous catheterization, septic shock, pneumonia as a primary source of infection, and nonsusceptibility to ceftazidime, cefepime, fluoroquinolone, piperacillin-tazobactam, aminoglycosides, and carbapenem. Concordant empiric antimicrobial therapy was less frequently performed in patients with 30-day mortality than in those without (36.4% vs. 54.0%, p = 0.047). Patient who received carbapenem or colistin as empirical therapy had 30-day mortality more frequently than those who did not (51.4% vs. 20.8%, p < 0.001; 66.7% vs. 24.8%, p = 0.004). Independent risk factors for 30-day mortality were neutropenia (aOR = 7.63, 95% CI = 1.28–45.35, p = 0.025), central venous catheterization (aOR = 5.88, 95% CI = 1.74–19.89, p = 0.004), ICU care (aOR = 5.36, 95% CI = 1.37–20.95, p = 0.016), septic shock (aOR = 15.91, 95% CI = 4.16–60.86, p < 0.001), nonsusceptibility to carbapenem (aOR = 6.21, 95% CI = 1.10–35.05, p = 0.038), and empiric therapy with colistin (aOR = 44.15, 95% CI = 3.95–494.09, p = 0.002). The only independent preventive factor for 30-day mortality was concordant empiric antimicrobial therapy (aOR = 0.07, 95% CI = 0.01–0.36, p = 0.002).

**Conclusion:** Concordant empiric antimicrobial therapy prevented fatal outcomes in patients with NFGNB bacteremia. However, empiric use of colistin was not helpful in reducing fatal outcomes.

**P2210** Clinical safety of Moxifloxacin: an analysis of “Valid for Safety” data from controlled phase II to phase IV studies performed between 1996 and 2010

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**Objectives:** Clinical Safety of Moxifloxacin (MXF) is approved for treatment of respiratory tract, skin, pelvic and intra-abdominal infections. Its safety profile is considered favourable in most reviews but has been challenged with respect to rare but potentially fatal adverse reactions. Our objective was to compare the MXF safety profile to that of comparators using the clinical trial database.

**Methods:** Source of data: Double-blind and open-label, actively controlled, Phase 2–4 trials (valid for safety patients: n = 14 981 [MXF] vs. 15 023 [comparators; standards of care and/or agreed upon with authorities]) (i) completed between 1996 and 2010 for both approved and other indications, (ii) using the recommended MXF dosage (400 mg), administration route (oral, IV-only, or IV/oral), and precautions of use, and (iii) including patients at risk ( $\geq 65$  years, diabetes, renal and hepatic impairment, cardiac disorders, BMI  $< 18$  kg/m<sup>2</sup>). Patients with known contraindications were excluded from enrolment by design but any patient having entered a study, even if inappropriately, was included in the analysis.

**Analysis:** Crude incidences and relative risk estimates (Mantel-Haenszel analysis) of patients with any adverse events (AEs), drug-related adverse events (ADRs), serious adverse events (SAEs), drug-related serious adverse events (SADRs), treatment discontinuation due to AEs and ADRs, fatal outcomes related to AEs and ADRs. Analyses were exploratory in nature and included systematic comparisons between groups and treatments.

**Results:** Overall incidence rates of adverse events were similar in MXF and comparator groups, except for AEs and SAEs in IV-only double-blind studies, AEs, ADRs, SADRs in PO, SADRs in IV/PO, and premature discontinuation due to AEs in IV-only open-label studies, which were slightly more frequent in MXF-treated patients (mainly gastrointestinal disorders and “changes observed during investigations” such as asymptomatic QT prolongation). No medically-relevant differences in rates of AEs were seen between MXF and comparators in patients at risk. Incidence rates of hepatic disorders, tendon disorders, surrogates of QT prolongation, serious cutaneous reactions and *Clostridium difficile*-associated diarrhea were similar with MXF and comparators.

**Conclusions:** No higher safety risk for MXF compared to standard therapies was seen in patients receiving the registered MXF dosage and for whom contra-indications and precautions of use (as in the product label) were taken into account.

#### **P2211** Outcome of initial piperacillin-tazobactam treatment for bloodstream infections caused by extended-spectrum beta-lactamase (ESBL)-producing *E. coli* and *K. pneumoniae*

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**Objectives:** The Purpose of the study was to evaluate the treatment outcome of initial piperacillin-tazobactam for patients with bacteraemia caused by ESBL-producing *E. coli* or *K. pneumoniae*.

**Methods:** From the database of a nationwide surveillance program (from October 2006 to April 2009) for bacteraemia, we analyzed the clinical data of 1647 patients with bacteraemia due to *E. coli* or *K. pneumoniae*. Patients with initial piperacillin-tazobactam treatment for bacteraemia due to ESBL-producing *E. coli* or *K. pneumoniae* were compared with those with non-ESBL-producing bacteraemia.

**Results:** Of total 1647 patients with bacteraemia, 238 (14.5%) were ESBL-producing group (153 in *E. coli*, and 85 in *K. pneumoniae*), and 1409 (85.5%) were non-ESBL-producing group (968 in *E. coli*, and 441 in *K. pneumoniae*). And of all, 6.6% (110/1647) patients were given initial piperacillin-tazobactam without combination (33.1% in ESBL group, and 2.2% in non-ESBL group). Piperacillin-tazobactam was susceptible in 49.6% of ESBL group, whereas 62.7% was susceptible in non-ESBL group.

Thirty-days mortality rate was significantly lower in non-ESBL-producing group (9.1% [129/1409]) than in ESBL-producing group (15.1% [36/238]) ( $p < 0.001$ ). However, of patients with initial piperacillin-tazobactam treatment, 30-days mortality rate was not significantly different (32.9% [26/79] in ESBL group, and 22.6% [7/31] in non-ESBL group) ( $p = 0.287$ ).

By univariate analysis, older age ( $\geq 65$ ), presence of healthcare-associated factor, higher PITT score ( $> 4$ ), and severe sepsis ( $p < 0.05$  for all) were the risk factors for 30-day mortality in initial piperacillin-

tazobactam treatment group for bloodstream infections caused by *E. coli* or *K. pneumoniae*. By multivariate analysis, only higher PITT score ( $> 4$ ), and severe sepsis ( $p < 0.05$  for all) were the common independent risk factors for mortality. Whereas ESBL production was a risk factor in all patients with bloodstream infection caused by *E. coli* or *K. pneumoniae* (OR 1.768, 95% CI 1.0188–2.633,  $p = 0.005$ ), it was not found to be an independent factor for mortality in initial piperacillin-tazobactam treatment group (OR 1.816, 95% CI 0.440–7.494,  $p = 0.410$ ).

	ESBL (+) (n=238)	ESBL (-) (n=1409)	P value
Age, year (mean $\pm$ SD)	60.4 $\pm$ 17.2	62.3 $\pm$ 32.4	0.361
Male	139 (58.4)	641 (45.5)	<0.001
Healthcare-associated infection	43 (18.1)	195 (13.8)	<0.001
Underlying disease			
Solid tumor	66 (27.7)	324 (23.0)	0.004
Hematologic malignancy	35 (14.6)	121 (8.5)	0.005
Cardiovascular disease	35 (14.6)	238 (16.9)	0.337
Liver disease	43 (18.15)	183 (13.0)	0.006
Renal disease	21 (8.8)	129 (9.1)	0.093
Neurologic disease	31 (13.0)	118 (8.4)	0.004
Diabetes Mellitus	50 (21.0)	270 (19.2)	0.068
Comorbid condition			
Recent operation	19 (8.0)	46 (3.3)	<0.001
Immunosuppressant use	34 (14.3)	152 (10.8)	0.011
Corticosteroid use	25 (10.5)	95 (6.7)	0.003
Neutropenia	32 (13.4)	137 (9.7)	0.006
Indwelling urinary catheter	49 (20.6)	162 (11.4)	<0.001
Prior receipt of antibiotics	88 (37.0)	189 (13.4)	<0.001
Site of infection			
Primary bacteraemia	104 (43.7)	413 (29.3)	<0.001
Pneumonia	17 (7.1)	76 (5.5)	<0.001
Urinary tract infection	44 (18.5)	401 (28.5)	<0.001
Intraabdominal infection	57 (23.9)	381 (27.1)	<0.001
Susceptible to Piperacillin-tazobactam	118 (49.6)	884 (62.7)	<0.001
Severe sepsis	54 (22.8)	272 (19.5)	<0.001
High Pitt score ( $> 4$ )	15 (14.7)	116 (16.4)	0.667
30-day mortality			
In all group	36 (15.1)	129 (9.1)	<0.001
In Piperacillin-tazobactam treatment group	26/79 (32.9)	7/31 (22.6)	0.287

**Conclusion:** In bloodstream infection caused by ESBL-producing *E. coli* or *K. pneumoniae*, initial piperacillin-tazobactam treatment was not associated with higher mortality. Other factor such as severe sepsis rather than initial piperacillin-tazobactam use should be considered as an important prognostic factor.

#### **P2212** Prospective evaluation of fosfomycin in intensive care unit patients

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**Background:** Prevalence of hospital- and Intensive Care Unit (ICU)-acquired infections caused by multidrug resistant (MDR) bacteria such as carbapenem-resistant *K. pneumoniae* (CR-KP) has rapidly increased, creating a new interest in old antibiotics. Fosfomycin (FOS) has shown promising in vitro activity against MDR bacteria, however, clinical data is lacking, especially for severe ICU patients.

**Methods:** Between July 2010 and September 2011 we performed a prospective descriptive study to record the efficacy and safety of FOS for CR-KP bacteremias. All ICU patients who received FOS were included. Clinical records including demographical data, severity scores, microbiological data and ICU outcome were analyzed.

**Results:** Eight patients were included. Values were recorded as mean  $\pm$  sd (range). Age was 63.6  $\pm$  10.3 years (43–79), 63.3% were men. APACHE II and SAPS II on ICU admission were 21.4  $\pm$  5.8 and 47.1  $\pm$  8.9, respectively. Total ICU stay was 40  $\pm$  19 days (18–65) and ICU stay after infection 19.7  $\pm$  13.4 days (6–40). CR-KP infections were diagnosed with a delay of 17  $\pm$  14 days (5–51) from ICU admission. Fifty percent of the patients underwent a surgical procedure

and all had received antibiotics in the past 5 days. In all patients FOS was administered, after strain identification and antibiotic susceptibility test, to treat severe sepsis or septic shock. Indications were bacteremias: five secondary (three due to catheter-related infections, one due to urinary tract infection, one due to ventilator-associated pneumonia and one secondary to peritonitis) and two bacteremias of unknown origin. FOS was administered for  $9 \pm 5$  days (4–17). All *K. pneumoniae* strains were carbapenemase-producers (KPC) with an MICFOS at 0.05 µg/mL. Resistance to colistin-COL, tigecycline-TIG and gentamycin-GEN was observed in 25%, 12.5% and 0%, respectively. The following antibiotic combinations were used: FOS-GEN 62.5%, FOS-COL 12.5%, FOS-COL-TIG 12.5%, FOS-GEN-TIG 12.5%. No adverse reactions were recorded. 5/8 patients died in the ICU (overall mortality 62.5%), 3/8 (37.5%) due to CR-KP infections and 2/8 (25%) for other reasons.

**Conclusions:** This is one of the few prospective studies showing the safety and usefulness of FOS against CR-KP bacteremias in ICU patients. However, FOS use was forced by the severity of our patients and the lack of alternative appropriate antibiotic regimen and should be interpreted with caution. Randomized control trials are needed to evaluate the real burden of efficacy and safety of the drug.

**P2213** Skin and soft tissue infection in a University teaching hospital: appropriateness of initial antibiotic choice and compliance with antimicrobial prescribing guidelines

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**Objective:** To evaluate appropriateness of initial antibiotic treatment for hospitalised patients with skin and soft tissue infection (SSTI) and to assess compliance with institutional prescribing guidelines.

**Background:** SSTI is a common condition requiring admission and IV antibiotic therapy. Management of SSTI is often inconsistent. Galway University Hospitals (GUH), a 750 bed tertiary care hospital, has developed antimicrobial guidelines recommending the following intravenous antibiotics; flucloxacillin for mild to moderate cellulitis, flucloxacillin plus clindamycin for severe cellulitis, co-amoxiclav for mild diabetic soft tissue infection and piperacillin/tazobactam for moderate to severe diabetic foot infection.

**Methods:** A prospective observational study of adults admitted to GUH with SSTI between 19 September and 14 October 2011 was conducted. Surgical site infections were excluded. Information on empiric antibiotic therapy administered within 24 hours of admission was collected from medical and nursing notes and prescription charts. This was assessed as appropriate if it complied with hospital guidelines or was recommended by an infection specialist.

**Results:** Forty-eight patients were admitted with SSTI during the study period. 39/48 (81%) had cellulitis, 8/48 (17%) diabetic foot infection and skin abscess in one (2%). Only 1/48 (2%) had severity documented and 46/48 (96%) were commenced on IV treatment. Eleven (23%) patients were assessed as having been prescribed appropriate initial antibiotics. Of the 37 patients (77%) on inappropriate therapy, 31/37 (84%) received additional antibiotics compared to the guidelines. Of these, 23/31 (74%) received IV benzylpenicillin in addition to IV flucloxacillin +/- clindamycin. 6/37 (16%) patients were undertreated, all had diabetic foot infections. 6/8 (75%) patients with diabetic foot infection did not receive adequate Gram negative and/or anaerobic coverage.

**Conclusion:** The majority of patients admitted with SSTI received inappropriate initial antibiotic therapy. There was significant overuse of benzylpenicillin, with nearly half the patients receiving IV penicillin in addition to either flucloxacillin or clindamycin. However, there was significant under treatment of diabetic foot infection. Compliance with hospital antimicrobial guidelines for SSTI was low. These prescribing patterns have significant implications for patient care, drug costs and nursing time.

**P2214** Underdosed colistin prescription in patients with multi-resistant Gram-negative bloodstream infections

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**Objectives:** To assess colistin prescription in hospitalized patients with multi-drug-resistant (MDR) or extensively drug-resistant (XDR) Gram-negative bloodstream infections.

**Methods:** Retrospective study (from January 2010 to September 2011) in a tertiary care hospital (1000 beds). Patients with blood cultures yielding MDR and XDR (by CDC and ECDC definition) Gram negative bacteria were enrolled. Clinical records were reviewed for demographic characteristics, ward of admission, antibiotic prescription and clinical outcome. Colistin dosing was judged as appropriate in adult patients with normal renal function when it was dosed 3 MUI (240 mg) every 8 hours and with a loading dose.

**Results:** A total of 67 multi-resistant gram negative pathogens in 66 patients were identified. Fifty-two (78%) patients were males, mean age (SD) was 57 years old (19) and 26 (39%) were admitted to ICUs. Mean time (SD) to positive blood culture was 27 (28) days since admission. *Pseudomonas aeruginosa* accounted for 21 (31.3%), *Klebsiella* for 22 (32.8%), others Enterobacteriaceae for 18 (25.9%) and *Acinetobacter* for 6 (8.9%) of total pathogens. Overall, 97% of them were resistant to fluoroquinolones, 53.7% to Beta-lactams, 30% to aminoglycosides, 37% to carbapenems, 88% to cotrimoxazole, 55% to tygecycline, 73% to aztreonam and 2.9% to colistin. A total of 47 (70%) and 20 (30%) pathogens were MDR and XDR, respectively. A total of 19 patients were prescribed iv colistin, in 12 patients as to ID specialist's prescription. For nine patients, colistin dosing was adjusted to renal failure. For the remaining 10 patients, colistin dosing was appropriate in three subjects and underdosed in seven patients (with range from 3 to 6 MUI daily). Only two patients received a loading dose (4 and 4.5 MUI, respectively). Colistin was always prescribed in combination with 1, 2 or 3 other antimicrobial agents in 8, 10 and 1 patients, respectively. The mean time (SD) of colistin therapy was 14 days (9.7) and eight patients were successfully treated. A total of 19 patients (28.7%) died, eight of them (42%) were treated with colistin.

**Conclusions:** Colistin was underdosed in 7/10 (70%) of patients with normal renal function. The loading dose was not prescribed in 80% subjects. Consensus over colistin prescription and adherence to appropriate dosing should be implemented given the paucity of antimicrobials for Gram negative organism.

**P2215** Worldwide colistin and polymyxin usage, the "last" Gram-negative antibiotics: results from an online global survey

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**Background:** Colistin belongs together with polymyxin B to the polymyxin antibiotic group. Colistin is a bactericidal antibiotic with a broad Gram-negative spectrum. Since the emergence of multi drug resistant Gram-negative infections, colistin is being used more commonly. However, it is unclear what the optimal dosage is for colistin and what drug combinations are optimal. Therefore, it is relevant to know how colistin is being used worldwide regarding indications and dosage.

**Methods:** A structured online questionnaire was developed consisting of 24 questions covering the background of the respondent and his/her facility, indications and dosage of colistin use for adult patients. Various networks of groups working on infectious diseases were invited to complete the questionnaire. The survey was open from June to November 2011. Data were analysed using SPSS 15.0 software using descriptive statistics as appropriate.

**Results:** The survey was completed by 297 respondents. The majority represented large tertiary care and teaching hospitals. 50.3% was an ID doctor/microbiologist and 16.8% ICU doctor. Responses were from 56 countries from all continents: Europe (38.7%), South America (21.6%), Oceania (11.8%), North America (11.1%), Asia (8.4%), and Africa

(3.7%). 14/56 countries reported that colistin was not available in their country. Majority (75.4%) used polymyxin drugs for adults. 47.5% used colistimethate sodium, 15.4% colistin sulfate, and 1.3% polymyxin B, remainder did not know. 80.1% used intravenous formulation, 41.4% aerosolized, 12.1% oral for selective gut decontamination. Indications were ventilator associated pneumonia, sepsis and catheter related infections. Most common bacteria for which colistin was used was *Acinetobacter baumannii* followed by *Pseudomonas aeruginosa*. 21.9% of respondents used a colistin loading dose and 25.9% adjusted the dose in obese patients. Fifty-seven percent used colistin in combination with another antibiotic, usually a carbapenem. Dosing regimens highly varied between centers and underdosing occurred. Patients are treated for a median of 14 days. **Conclusion:** Colistin is variably used in different settings. As poor dosing may lead to colistin resistant infections, clear guidance need to be provided how dosing should be done and which antibiotic combinations are appropriate. Colistin/polymyxin should be considered a last resort drug and its use needs to be strictly controlled. It is recommended not to use colistin in agriculture.

**P2216** Evaluation of risk factors for nephrotoxicity due to colistin use

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**Objectives:** Infections due to resistant gram negative bacteria are increasing especially in intensive care units (ICU). Treatment of carbapenem resistant *Acinetobacter* and *Pseudomonas* infections is the most prominent problem recently. An old antimicrobial colistin use became an alternative choice of treatment in such cases. In this study, patients receiving colistin therapy were evaluated for nephrotoxicity and related risk factors.

**Methods:** All of the patients who received colistin and who had normal renal functions prior to colistin use in our hospital in the last 1 year period were included into the study. The study was retrospectively performed by evaluation of patient records. The dosage of colistin was 2 × 150 mg/day colistimethate sodium (equivalent to 150 mg colistin base activity) according to the manufacturer's instructions (Colimycin, Kocak). Patients receiving colistin <5 days were excluded. Renal functions after colistin use, the causative agent, site of infection and ICU stay were noted. SPSS 15.0 packet programme was used for statistical analysis.

**Results:** Totally 58 patients (26 female, 32 male) with the mean age of 59.9 (SD: 17.7) were evaluated. The mean age of patients was 59.9 ± 17.7 years. Nephrotoxicity had been developed in 55% of the patients. Female gender and older than 60 years age were found statistically significant factors for risk of nephrotoxicity (p < 0.05 and p < 0.001 respectively). Nephrotoxicity risk was increased 3.9 times in female gender and 10.2 times in 60 years and older patients.

**Conclusion:** Female gender and older than 60 years age gave rise to nephrotoxicity more frequently. Side effect of nephrotoxicity may be related with the dosage adjustment problem in colistin use. Standardization of dosage is needed for all colistin formulas and all colistin including products.

**P2217** The risk factor analysis of breakthrough bacteraemia developed during tigecycline therapy

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**Objectives:** Tigecycline was approved for complicated skin and soft tissue infection (CSSI) and complicated intra-abdominal infection (CIAI). However, many case reports and meta-analyses reported that tigecycline was not fully effective in serious infections by multidrug-resistant pathogens. Herein, we analyzed the emergent breakthrough bacteremia cases during the tigecycline treatment.

**Methods:** We retrospectively searched the cases which had been treated with tigecycline for at least 3 days from April 2009 to October 2011 in an 850 bed University Hospital. All the episodes of bacteremia were collected if they developed during the tigecycline treatment. The characteristics of patients, status of medical devices, previous antibiotics use, pathogen, the point of breakthrough bacteremia, and outcomes were recorded. The risk factors of breakthrough bacteremia during the tigecycline treatment were also analyzed.

**Results:** Total 72 tigecycline-treated cases were identified, which were 27 cases of CSSI, 19 cases of CIAI and 28 cases of pneumonia. We experienced 11 (15%) breakthrough bacteremia events during the treatment. They consisted of cases of five MRSA bacteremia, five MDR acinetobacter bacteremia, and one MRCNS bacteremia. The causes of diseases for tigecycline treatment were composed of five cases of CSSI, four cases of CIAI and two pneumonia cases. Breakthrough bacteremia was more likely to be associated with old age, ICU stay, intra-abdominal infection, hypertension, central catheter insertion, urinary catheter insertion, and ventilator application. Multivariable analysis revealed that hypertension (OR: 2.9 95% CI 2.38–7.39) and intra-abdominal infection (OR: 1.83, 95% CI 1.43–4.85) were independent risk factors of breakthrough bacteremia.

**Conclusions:** We identified breakthrough bacteremia cases developed during the tigecycline treatment, which were more common in patients with hypertension and in patients with CIAI. This result suggests that tigecycline could not eradicate the invasive bacteremia and it should be cautiously used for serious infections.

**P2218** Prediction of residual disease using F18 FDG PET/CT in patients with staphylococcal implant-associated spine infection requiring long-term suppressive therapy

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**Objective:** Long-term suppressive antimicrobial therapy is a treatment option in patients with a high risk of relapse during staphylococcal implant-associated bone and joint infection. The goal of suppressive treatment is to control clinical manifestations rather than eradicate infection. However, suppressive therapy is binding for the patients and not always safe.

**Method:** We prospectively used F18 FDG PET/CT in a cohort of patients with staphylococcal implant-associated spine infection requiring long-term suppressive therapy, to evaluate residual disease at the site of infection. PET/CT was performed 6 weeks to 6 months after the surgical therapy and then during the follow-up (once to twice in a year). Persistent residual disease was defined as a  $SUV_{max}$  of infected-spine >  $SUV_{max}$  of non-infected spine ( $\Delta SUV_{max+}$ ). The suppressive therapy was stopped if both  $SUV_{max}$  were similar.

**Result:** Five patients (mean age of 54 ± 21 years) with chronic staphylococcal (three infected with *S. aureus*, one infected with coagulase-negative staphylococci and one infected with both) implant-associated spine infection were included (four patients with instrumented posterior lumbar fixation and one patient with vertebral cementoplasty). Surgery, which was considered non optimal to obtain a microbiological cure (implant retention), was performed in four of the five patients. Ten PET/CT were performed during the suppressive therapy. Similar  $\Delta SUV_{max}$  was obtained in two patients at 6 month and in one patient at 12 month, allowing the discontinuation of the suppressive therapy. No relapse was observed during a follow-up of 18, 23 and 24 months.  $\Delta SUV_{max+}$  was still observed in the two other patients, 9 and 21 months after the initiation of suppressive therapy, respectively. Suppressive therapy is still ongoing in these patients.

**Conclusion:** F18 FDG PET/CT may facilitate the discontinuation of long-term suppressive therapy in patients with staphylococcal implant-associated spine infection.

**P2219** Switching to an oral antibiotic regimen after 2 weeks is safe for treatment of spinal osteomyelitis without endocarditis

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**Background:** Spinal osteomyelitis may lead to disabling neurologic complications. Prompt surgical drainage is essential if epidural or paraspinal abscess is present. Little is known about the optimal antibiotic management.

**Methods:** Ten-year retrospective analysis of all patients (pts) with primary spinal osteomyelitis in a tertiary hospital between 2001 and 2010. We excluded pts with endocarditis, immunodeficiency, spinal implants and surgical site infection following spine surgery. Treatment failure was defined as persistence of clinical or radiological signs of vertebral osteomyelitis at 1 year. Logistic regression was used to estimate the odds ratios (ORs) of switch to an oral regimen after 2 weeks.

**Results:** Sixty-nine pts were included. Characteristics: 40 males (58%), median age 66 years (IQR 52–79), cardiovascular comorbidity in 51% and diabetes in 14% of pts. Clinical presentation included fever in 29%, back pain in 93%, neurological deficits in 20% of pts. The median leucocytes count was  $9.9 \times 10^9$  cells/L (IQR 7.6–12.3) and C-reactive protein 100 mg/L (IQR 62–180). Epidural or paraspinal abscess were found in 36 (52%) pts. Coagulase-negative staphylococci and *Staphylococcus aureus* were the most frequently isolated microorganisms (38%), followed by gram negative bacteria (20%), streptococci (16%) and *Propionibacterium acnes* (4%). All pts were treated with antibiotics, being amoxicillin-clavulanic acid the most frequent empirical treatment. Among 36 pts with epidural or paraspinal abscess, 30 (83%) underwent surgical debridement and 3 (8%) CT-controlled drainage. The median antibiotic treatment duration was 60 days (IQR 44–88). Switch to an oral antibiotic regimen was performed in 74% of pts after a median intravenous therapy of 18 days (14–27). During the follow-up, two pts experienced treatment failure, i.e. the 1-year success rate was 97%. In univariate and multivariate analysis, after adjustment for microorganism, epidural or spinal abscess, surgery and laboratory parameters, lower C-reactive protein at 2 weeks was the only independent predictor of switching to an oral antibiotic regimen after 2 weeks (OR 0.7, 95% CI 0.5–0.9,  $p = 0.028$ , per 10 mg/L increase). Infections due to gram negative bacteria tended to be treated longer than 6 weeks, but these differences were not statistical significant.

**Conclusions:** Our results suggest that switching to an oral antibiotic regimen after 2 weeks intravenous therapy is safe for vertebral osteomyelitis without endocarditis.

**P2220** Evaluation of safety and efficacy of daptomycin therapy in elderly: results from a patient registry in Europe

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**Objective:** Gram-positive infections are a leading cause of death in the elderly ( $\geq 65$  years of age). There are only limited data on daptomycin use in this population in randomized clinical trials. The objective of this analysis was to evaluate the safety profile and clinical outcomes of daptomycin in older adults in clinical practice in Europe.

**Methods:** Data were collected from the European Cubicin® Outcome Registry and Experience (EU-CORE), a non-interventional multicenter study on patients who received at least one dose of daptomycin for the treatment of a serious Gram-positive bacterial infection between January 2006 and June 2011. Treatment success was defined by investigator as cured, improved, failure or non-evaluable following daptomycin therapy.

**Results:** Out of 4592 patients enrolled in EU-CORE, a total of 2126 patients aged  $\geq 65$  years received daptomycin. Forty-eight percent were  $\geq 75$  years and 58% were male. At initiation of therapy, 9% patients were on dialysis and 17% patients had CrCl  $< 30$  mL/minute. Ninety-four percent patients had underlying diseases such as cardiovascular

disease (72%) and diabetes (34%). The most frequently reported pathogen was *S. aureus* (30%), including MRSA (17%). The majority of patients received 6 mg/kg (46%) and 4 mg/kg (28%) daptomycin as initial dose and median duration of therapy was 12 days (range 1–300 days). The most common infections were complicated skin and soft tissue infection (cSSTI) (34%), bacteraemia (19%) and uncomplicated skin and soft tissue infection (uSSTI) (11%). Most common concomitant antibiotics were carbapenem (20%), penicillin (13%) and fluoroquinolones (12%). The overall clinical success rate (cured + improved) with daptomycin therapy was 79% (comparable to the rate seen in patients  $< 65$  years, 82%). Success rates by infection type were: uSSTI 90%, cSSTI 83%, endocarditis 79%, osteomyelitis 78%, and bacteraemia 70%. Success rates in *S. aureus* were high (81%; 80% in MRSA). Overall, 15% patients had adverse events (AEs), of which 3% were possibly related to daptomycin (including 1% with CPK elevation). Serious AEs were reported in 11% patients with 1% possibly related to daptomycin.

**Conclusion:** Daptomycin was effective and well tolerated achieving high success rates against a wide range of infections in patients aged  $\geq 65$  years.

**P2221** Daptomycin, elevated creatine phosphokinase and rhabdomyolysis – is there a co-relation? Clinical experience from the UK EU-CORE<sup>SM</sup> registry, 2006–2011 (RP 1–4)

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**Background:** Baseline and regular monitoring of creatine phosphokinase (CPK) is standard requirement during Daptomycin (DAP) therapy. The rationale comes from early studies with DAP and association with rhabdomyolysis. Literature suggests CPK elevation in patients may be related to several other causes including surgical procedures, diabetes mellitus, statin use, etc. We present a review of 5-year data on elevations in creatine phosphokinase (CPK) levels during DAP use from the UK EU-CORE<sup>SM</sup> database.

**Methods:** Data were collected retrospectively from January 2006 to June 2011. The analysis included patients treated with daptomycin (DAP) with elevations in CK recorded either at baseline or during DAP therapy.

**Results:** Since the beginning of the registry, 590 patients from 15 participating institutions in the UK were entered in to the database. Baseline CPK measurements were recorded for 193 patients (32.7%): of which 166 (%) were  $< 1 \times$  upper limit of normal (ULN); 15 (%)  $> 1$  to  $2 \times$  ULN. Twelve patients had CPK levels  $> 2 \times$  ULN prior to commencing DAP: seven patients  $> 2$  to  $5 \times$  ULN; two patients  $> 5$  to  $10 \times$  ULN; three patients  $> 10 \times$  ULN.

During DAP therapy CPK measurements were recorded for 218 patients. CPK:  $< 1 \times$  ULN for 179 patients.  $> 1$  to  $2 \times$  ULN for 22. CPK

Patients with CPK recorded at $> 2 \times$ ULN during DAP therapy (n=17)		
	$> 2$ to $5 \times$ ULN n=5 (%)	$> 5 \times$ ULN n=12 (%)
Age (Mean)	54.8	57.4
Male	5 (100)	9 (75)
Weight kg (Mean)	99.1	88.0
HMG-CoA reductase inhibitor	1 (20)	3 (25)
Prior Antibiotics	4 (80)	11 (92)
Concomitant antibiotics	3 (60)	7 (58)
Days of therapy (Mean)	19.4	11.2
Baseline CPK		
<1xJLN	-	4 (33)
>2 to 5xULN	1 (20)	1 (8)
>5xJLN	-	4 (33)
Not measured	4 (80)	3 (25)



>2 to 5 × ULN during therapy was reported for five patients, >5 × ULN, 12 (Table). Of the 17 patients with elevated CPK, 10 had levels in excess of >2 × ULN at the start of DAP therapy. Day of highest CPK ranged from 1 to 32 days. CPK elevations or high CPK levels were reported as an adverse event (AE) for nine patients and asymptomatic CPK elevation for one patient. An AE of myalgia was reported in one patient where CPK was recorded as >10 × ULN at start of DAP therapy. Rhabdomyolysis was reported as an AE for two patients: CPK was not recorded for one and the other CPK was <1 × ULN during DAP therapy.

**Conclusion:** Of the patients reported with elevations in CPK in the registry, 10 had elevated CPK prior to DAP, suggesting that these elevations were related to other causes. Waddington et al. report no muscular symptoms and progressive reduction from >113 × ULN to >1 × ULN during successful treatment with DAP. BNF suggests discontinuing DAP only if both unexplained muscular symptoms and markedly elevated CK co-occur.

**P2222** **Observational, retrospective study to evaluate nephrotoxicity of daptomycin and vancomycin in the treatment of Gram-positive infections**

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**Introduction:** The progressive loss of sensitivity of *Staphylococcus aureus* (SA) to vancomycin (VAN) has led to an increase in dosage to maintain its effectiveness, which implies a higher risk of nephrotoxicity. Small increases in serum creatinine of hospitalized patients are associated with increased mortality, hospital stay and health costs. In clinical trials, daptomycin (DAP) has demonstrated to be less nephrotoxic than other anti-staphylococcal antibiotics. This study aims to evaluate the nephrotoxicity of VAN and DAP in the clinical practice.

**Methods:** We retrospectively evaluated two groups of hospitalized patients with Gram-positive infections who received DAP or VAN according to clinical practice in nine Spanish hospitals. Both groups were matched based on age, comorbidities (Charlson index) and renal function at treatment initiation.

**Results:** A total of 133 patients were included: 62 received DAP and 71 VAN. No significant differences were found between groups in age (mean (SD) of 68 (15)/69 (15) years for DAP/VAN, respectively), gender (71%/58% males), comorbidities (42%/44% of Charlson index  $\geq 3$ ), baseline glomerular filtration rate (57 (27)/65 (28) mL/minutes/1.73 m<sup>2</sup>), severity of infection (14%/10% of serious sepsis and 5%/3% of septic shock), pathogen (36%/24% of methicillin-resistant SA, 29%/35% of coagulase-negative staphylococci) and use of concomitant antibiotics or nephrotoxic drugs. Patients who received DAP had more endocarditis, skin and soft tissue/osteosarticular infections (19%, 26% and 31%, respectively vs. 6%, 17% and 17% in the VAN group). Nephrotoxicity was significantly higher with VAN compared with DAP ( $p = 0.0058$ ): 17/71 (23.9%) patients (from whom eight switched to another antibiotic), vs. 4/62 (6.5%) (one switched). In a multivariate logistic regression model, VAN remained as an independent predictor of nephrotoxicity (odds ratio [OR] 7.5 95%CI 1.9–30.3,  $p = 0.004$ ), along with age (OR 1.6, 95% CI 1.1–2.5 for each 10 years) and endocarditis as type of infection (OR 7.0, 95% CI 1.5–33.3). No significant effect of gender, comorbidities, severity of infection and use of concomitant antibiotics or nephrotoxic drugs was observed. Response rates were comparable (90% in the two groups). All-cause mortality was 12% in the two groups.

**Conclusions:** Compared with vancomycin, daptomycin treatment of Gram-positive infections in hospitalized patients showed a comparable overall response, but was associated with a much lower risk of nephrotoxicity.

**P2223** **Daptomycin MIC increase among patients with methicillin-resistant *Staphylococcus aureus* persistent bacteraemia treated with daptomycin. Prospective study in 22 Spanish hospitals.**

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**Objectives:** The aim of this study was to ascertain the evolution of daptomycin minimum inhibitory concentration (MIC) of subsequent isolates in Persistent Bacteraemia (PB) episodes from a large series of MRSA bacteremia.

**Methods:** Prospective study from 8 June to December 2009, in 22 Spanish hospitals. Epidemiology and clinical data were recorded. Methicillin-resistant *Staphylococcus aureus* (MRSA) strains were analyzed in a central lab, including susceptibility to antibiotics and molecular characterization. Vancomycin MIC (V MIC) and daptomycin (D MIC) were studied by E-test and microdilution methods. Microbiological failure: Long Persistent bacteremia (LPB): documented MRSA bacteremia during 7 days or more. Clinical failure: 30-day mortality.

**Results:** Five hundred and seventy-nine episodes were included, 67% male, mean age 69 years. Microdilution vancomycin MIC<sub>90</sub> and E-test daptomycin MIC<sub>90</sub> were 1 and 0.5 µg/mL, respectively. Definitive therapy was daptomycin in 124 episodes (22%). Among them, 23 (19%) episodes of LPB were observed. All the isogenic isolates from five LPB episodes were studied. Their main characteristics are summarized in Table 1.

Table 1: Summary of patients with MRSA LPB treated with daptomycin

PATIENT	MRSA STRAIN	THERAPY		OUTCOME			
		Antibiotic dose (days)	Sterile BC	30-day mortality			
Age/Gender	Source/Acquisition	Molecular Identification	Initial/Final D MIC	Initial/Final V MIC			
84M	Catheter/HCR	CC5agr2/Scorec IV	0.09/1	0.7/1.5	D 10mg/kg (18)	No	Yes
74F	Osteoarticular/HCR	ST22agr1/Scorec IV	0.2/1.5	0.7/0.7	D 10mg/kg (26)	Yes w/ fos+ms	No
65M	Catheter/ICU	CC5agr2/Scorec IV	0.12/0.38	0.7/0.7	D 10mg/kg (21)	Yes w/ fos+ms	No
83M	Endocarditis/HCR	ST8agr1/Scorec IV	0.09/1	0.7/1.5	D 10mg/kg (13)	No	Yes
76F	Catheter/ICU	ST22agr1/Scorec IV	0.12/1.5	0.5/1	D 10mg/kg (13)	No	Yes

D MIC: Daptomycin MIC; V MIC Vancomycin; M Male, F Female, HCR Health-care related, ICU Intensive Care Unit  
BC Blood cultures FOS: fosfomicin, IMI imipenem

**Conclusions:** Moderate increasing MIC and treatment failure may not be rare in patients with MRSA long persistent bacteremia treated with daptomycin. Our results support switching to other antibiotic options if sterile blood cultures are not early achieved.

**P2224** **The impact of a computerised, integrated antibiotic-authorisation-system in a medium-sized hospital**

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**Objective:** Overuse and abuse of antibiotics is a major promoter of microbial resistance. Within the hospital setting such overuse necessitates real-time supervision by infectious diseases (ID) specialists. We evaluated the impact of a computerized antibiotic authorization system (integrated with the patient medical record) on the pharmacy's expenses in a 400 bed university hospital.

**Methods:** Antibiotic requests placed via the computerized system were retrospectively analyzed. Successive 2 years, before and after introduction of the authorization system were compared for total and individual antibiotic usage and expenditure.

**Results:** During the first year of using this system, 7167 antibiotic requests were placed, of which 20% were rejected, mostly for improper indication (43% of the rejects). During the same period the antibiotic expenditure was curtailed by 17%. Of the 35 antibiotics under control, the use of seven was most probably curtailed by the supervision. Pareto analysis revealed that four drugs contributed >50% of the pharmacy's expenses. The death rates (per 1000 hospitalization days) during these 2 years fell from 4.0 to 3.8.

**Conclusion:** Computerized antibiotic control is a feasible and cost saving modality that may help reduce unnecessary antibiotic prescriptions.

**P2225** **Outpatient parenteral antimicrobial therapy for intra- and extra-cranial central nervous system infections**

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**Objectives:** Treatment of central nervous system (CNS) infections frequently requires prolonged parenteral anti-microbial therapy, which traditionally warranted an extended in-patient hospital admission. Recently, there has been a move to complete parenteral anti-microbial therapy in the community using outpatient parenteral antimicrobial therapy (OPAT) services.

Despite clear advantages, there are concerns over the potential difficulties and safety; alongside a paucity of published material regarding this service model in the management of CNS infections. We present our local experience of an OPAT service for intra- and extra-cranial CNS infections.

**Method:** We retrospectively reviewed 74 consecutive cases from 2008 to 2011 registered with the local OPAT service database. This included the broad range of CNS infections deemed suitable for OPAT, the types of antimicrobial agents used, duration of treatment and any adverse effects documented.

**Results:** Brain abscess, infected craniotomy, discitis and vertebral osteomyelitis were the commonest CNS infections treated with this service. The median duration of treatment was 29 days, with a range of 11–236 days. An adverse event occurred in 26 cases (35%), necessitating antimicrobial change in six cases (8%). The commonest problem encountered was elevated teicoplanin levels requiring dose modification in six cases (8%). There were no serious adverse events. Vascular access problems occurred in three cases (4%).

**Conclusion:** OPAT appears to be a safe and effective treatment strategy for CNS infections with few serious adverse effects, but requires specialist and multidisciplinary input for optimal infection management and close monitoring in the community.

**P2226** **10-year trends in a large outpatient parenteral antimicrobial therapy cohort based in western Scotland: retrospective observational findings**

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**Objectives:** Outpatient Parenteral Antimicrobial Therapy (OPAT) is an increasingly prevalent modality of infection treatment. Several observational cohort studies describing experience with OPAT in Europe have now been published. With some exceptions, studies have been small and limited to data collected in the initial set up phase of OPAT services. This study aimed to consider trends in patient, microbiological and logistical factors in a large OPAT cohort over a 10 year period.

**Methods:** A retrospective cohort analysis was performed by accessing the prospectively maintained electronic case database maintained at this OPAT service. For purposes of trend analysis, all cases were categorized by ordered 2 year groupings of date of OPAT referral (2001–2002, 2003–2004, 2005–2006, 2007–2008 and 2009–2010). Trends in binary outcomes were tested using Chi squared test for trend. Differences in continuous outcome variables were tested for trends with linear regression if parametric, or Spearman's rank correlation if non-parametric.

**Results:** This OPAT cohort included 2638 patient episodes comprising 39 035 days of patient care. Three-quarters of OPAT cases were Skin and soft tissue infections (SSTIs) or bone and joint infections (BJIs), but a wide range of other infections were also treated. Overall outcome variables for 2233 first OPAT attendances were assessed: 2063 (92.4%) resulted in cure or improvement; unplanned admission was observed in 9.1% (6.3 events per 1000 OPAT patient days). There was a low incidence of Healthcare Associated Infection (HAI): 14 intravenous line

infections (0.4 per 1000 OPAT patient days) and two cases of *Clostridium difficile* infection (0.05 per 1000 OPAT patient days).

Proportion of successful outcome and adverse events did not vary over time. However statistically significant trends were seen for the following variables over the 10 years: decrease in OPAT treatment time per case; increased incidence of comorbidity of OPAT referrals; and increased self or carer administration of antimicrobials. Decreased incidence of MRSA relative to MSSA was observed, while cases of resistant gram negative infection treated in OPAT increased over time.

**Conclusion:** This large cohort study adds to the observational data suggesting OPAT is a safe and effective model of care. The trends demonstrated over the 10 year study period demonstrate that this model of OPAT is sustainable and can adapt to changing clinical, microbiological and health system exigencies.

## Clostridium difficile

**P2227** **Clostridium difficile infection associated with primary and/or community settings in Northern Ireland: prevalence, ribotypes and risk factors**

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**Objective:** To describe the epidemiology of *Clostridium difficile* infection (CDI) in primary and community care settings in Northern Ireland.

**Methods:** A programme for enhanced surveillance of CDI in community and primary care settings was introduced in April 2010. Since January 2011, enhanced CDI proformas have been completed for all CDI specimens taken in care homes and requested from GP surgeries. All records were assigned a CDI ribotype using probabilistic matching. Explanatory variables included patient demographics, residence of case, prior exposure to antimicrobials and gastrointestinal drugs, previous hospitalisation and CDI ribotype. CDI cases were differentiated according to likely associations (healthcare onset or community onset). The period prevalence of CDI during January to June 2011 was calculated using the mid 2010 population estimates.

**Results:** From January to June 2011 there were 371 cases of CDI in acute and community settings. Of these, 273 were hospital onset, 96 were community onset (5.3/100 000 population) and two were from a hospice. Of the 96 community-onset CDI cases, 72% were females (69/96) and 86% were aged 65 years and over (83/96). Almost half of the cases were living in a care home at the time of sampling (47/96; 49%). Forty-six percent (44/96) of the community-onset cases had received antimicrobial therapy in the previous 1–4 weeks prior to CDI onset. Forty-five percent (43/96) had received GI therapy including proton pump inhibitors and H2 antagonists. 86.5% (83/96) had some form of healthcare contact prior to symptom onset. For both 'community onset-community associated' and 'community onset-hospital associated' cases – most prevalent ribotype was 078 (Table 1: 13/66; 20% and 6/24; 25% respectively).

**Table 1:** Ribotypes of community onset, community associated (CDI identified in the community where the patient has no history of admission to hospital) and community onset, hospital associated (CDI identified in the community less than 4 weeks following discharge from a hospital).

Ribotype:	Community onset, community associated		Community onset, hospital associated	
	n	%	n	%
001	1	2	2	8
002	2	3	3	13
012	2	3	2	8
014	3	5	0	0
015	6	9	2	8
023	3	5	1	4
027	4	6	1	4
078	13	20	6	25
193	4	6	0	0
Other	10	15	5	21
Not groupable	8	12	1	4
Not on ribotype list	5	8	0	0
Not isolated	5	8	1	4
Total*	66	-	24	-

\*6 community onset cases could not be differentiated further

**Conclusion:** Whilst the incidence of CDI in acute healthcare settings in NI has decreased significantly since January 2009, the incidence of CDI with onset of symptoms in the community setting has been increasing. Almost half of community-onset cases are identified as a resident in a care home. The use of antimicrobial and GI therapy and previous healthcare contacts are possible risk factors for community-onset CDI. Similar to the acute setting, ribotype 078 dominates among community CDI cases in NI. This programme of enhanced surveillance represents a unique data source, facilitating detailed analysis of the epidemiology of community CDI cases. This will guide effective methods of infection prevention and control and the management of outbreaks.

**P2228** **Outbreak of *Clostridium difficile* ribotype 027 in a hospital and a nursing home, Deventer, the Netherlands**

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**Background:** In December 2010–February 2011, 10 patients from a surgery/gastro-enterology department of the hospital and a neighboring nursing home developed *Clostridium difficile* infection (CDI) caused by one clone of hypervirulent PCR ribotype 027.

**Methods:** A bundle of interventions was implemented. An admission stop was decreed. Hospital personnel was advised to wash their hands, instead of disinfection with alcohol. The standard treatment of CDI was changed from metronidazole to vancomycin, and patients were nursed in contact-isolation for the full period of treatment. The use of 3rd generation cephalosporins and fluorochinolones was banned. All contaminated rooms were disinfected by aerosol-based H<sub>2</sub>O<sub>2</sub> (Nocolyse®).

To improve case-finding, a PCR-based detection method (GeneXpert® *C. difficile*, Cepheid) was added to the antigen test (ImmunoCard® *C. difficile* Toxins A and B, Meridian Bioscience). On all positive faecal samples a bacterial culture was performed. *C. difficile* isolates were characterized by PCR ribotyping and MLVA.

**Results:** After the intervention, eight new patients were found positive with the epidemic *C. difficile* strain within a period of 3 months. All of them had an epidemiological link to the surgery/gastro-enterology ward. In July the antibiotic regimes were changed to normal. Since then, two new cases of epidemic CDI occurred.

Mean age of infected patients was 77 years (64–88). Relapses occurred in 9/20 cases (1–4 relapses), most of which were treated outside the hospital.

From March until July 2011, 484 faecal samples were tested, from which 64 (13.2%) were PCR-positive; 32 were also antigen-positive. From 58 PCR positive faecal samples, 53 (91%) were culture positive. Twenty-two strains were ribotyped from which 11 (50%) was ribotype 027. Compared to ribotyping, the positive-predictive value of the GenExpert outcome ‘presumptive 027’ was 0.92 and negative-predictive value 1.00.

**Discussion:** After implementation of a bundle of measures, the incidence of new patients with CDI decreased quickly, which implies that application of a bundle approach is very efficient to combat CDI outbreaks. The use of GeneXpert® PCR doubled the amount of diagnosed patients with *C. difficile* as compared to the antigen-test. It is possible that a number of PCR diagnosed patients were in fact carrier of *C. difficile*. The role of carriers to the spread of CDI is unknown, but our experience favors active surveillance and isolation when an outbreak occurs.

**P2229** **First case of autochthonous *Clostridium difficile* PCR ribotype 027 detected in Spain**

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**Objectives:** Hypervirulent epidemic strains of *Clostridium difficile* belonging to ribotype 027 have caused outbreaks of severe infections in the United States and Canada since 2001. Outbreaks of *C. difficile*

ribotype 027 have also been described in several European countries. Data on the molecular epidemiology of *C. difficile* in Spain are scarce. In 2007, our laboratory implemented active surveillance of *C. difficile* ribotype 027 at our hospital. We report the incidence of ribotype 027 in our *C. difficile* isolates and describe its molecular characteristics.

**Methods:** Strains of *C. difficile* were cultured and identified using conventional microbiological methods. DNA was obtained from pure cultures using Chelex resin (Instagene matrix, BioRad). The *tcdA* gene (toxin A), *tcdB* gene (toxin B), and binary-toxin genes *cdtA* and *cdtB* were detected using multiplex PCR (modified from Persson, 2008). Binary toxin-positive isolates were subsequently characterized using PCR-ribotyping (Stubbs, 1999). Phylogenetic analysis of ribotyping profiles was conducted using Bionumerics software 5.0. Isolates belonging to ribotype 027 were characterized using multiple-locus variable-number tandem repeat analysis (MLVA, Van den Berg, 2007) and sequencing of *tcdC* gene (Rupnik, 1998 and Spigaglia, 2002).

**Results:** From 2007 to November 2011, we characterized 3209 strains of *C. difficile* and found that 376 isolates (11.7%) were toxin A+B+binary+. Of these, 269 (71.5%) were ribotype 078/126. Only eight ribotype 027 *C. difficile* strains were detected in four patients: two previously published cases of laboratory transmission (a Spanish patient residing in the UK and a pregnant laboratory technician), one case of a British tourist, and one case nonsevere diarrhoea in an elderly Spanish patient with no epidemiological criteria for acquiring *C. difficile* 027. The ribotype 027 isolates had three different MLVA types (*tcdC* gene identical).

**Conclusion:** Although we report the first finding of *C. difficile* 027 in Spain, these strains are not a problem in our area. However, ribotype 078/126 is the most frequent among our binary toxin-positive isolates. We detected one autochthonous case of nonsevere *C. difficile* ribotype 027 diarrhoea in a patient with no remarkable epidemiological history.

**P2230** **Are different ribotypes of *Clostridium difficile* present simultaneously in a patient with a *Clostridium difficile* infection?**

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**Objectives:** Infection with toxin-producing *Clostridium difficile* strains is a common cause of diarrhea and colitis. *Clostridium difficile* infection (CDI) has increased in frequency and severity in North America and Europe over the last decade largely due to the emergence of the epidemic PCR ribotype 027 strain. PCR ribotyping is based on a comparison of patterns of PCR products from the 16S-23S rRNA intergenic spacer region. Isolates are considered to be of a new PCR ribotype if the pattern is at least one band different from previously described patterns. The aim of this study was to investigate whether CDI is caused by multiple toxigenic strains or by a single strain.

**Methods:** Colonies of *C. difficile* isolates were collected from each stool sample of 28 *C. difficile* positive patients. The stool samples were cultured on TCCFA agar plates and five different colonies were subcultured on blood agar plates in anaerobic conditions for 48 hours. In total 140 isolates of *C. difficile* were included in the study. Toxin B was detected by cell culture neutralisation assay (CCNA). In addition, all isolates were tested with the Cepheid Xpert™ realtime PCR. PCR ribotyping was used to analyse the different isolates of *C. difficile*. Ribotyping PCR products were separated on 5% polyacrylamide gels by electrophoresis. The gels were scanned and analyzed by Bionumerics software version 6.5. PCR ribotyping patterns were compared to a database including *C. difficile* reference strains.

**Results:** Overall, 12 different ribotypes were found in the 28 samples. The most common ribotypes were 001, 002, 014 and 078, four samples each. All isolates from each individual sample showed the same ribotype except in one sample where four colonies were of the same ribotype and one colony was different. The colony that differed was shown to be non-toxicogenic by the CCNA assay.

**Conclusion:** The results from the ribotyping of the 28 stool samples indicate that CDI is usually caused by one particular *C. difficile*

ribotype. In this study it is shown that CDI is not caused by multiple toxigenic strains.

**P2231** **Characterisation of *Clostridium difficile* 018: an epidemic PCR-ribotype recently emerged in Italy**

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**Objectives:** To characterize *C. difficile* PCR-ribotype 018, a recent epidemic type cause of numerous outbreaks and sporadic cases in Italy, in comparison with other PCR-ribotypes predominant in the past.

**Methods:** Two hundred and forty-two toxigenic clinical strains, isolated from 1985 to 2011 and collected by the Istituto Superiore di Sanità, were typed by the PCR-ribotyping method. Two different strains PCR-ribotype 018, one strain 126 and one strain 012, were further investigated as representative of different hospital outbreaks occurred in different time periods. The MICs for erythromycin (ERY), clindamycin (CM) and moxifloxacin (MX) were evaluated by the Etest. ermB genes were detected by PCR assay. TcdC, the toxin negative regulator, and SlpA, the S-layer proteins precursor, were characterized by sequencing. In vitro adhesion was performed on Caco-2 monolayers at 3 and 15 days after seeding and adherent bacteria were counted on Blood agar plates after 48 hours of incubation. Different dilutions of filtered supernatants from overnight cultures of the strains were used for cytotoxicity assay on confluent Caco-2 cell monolayers.

**Results:** Results indicated PCR-ribotype 018 as the predominant type in Italy since 2006, while PCR-ribotypes 126 and 012 were those most frequently isolated in the previous years. All the representative strains analysed were resistant to ERY, whereas one of the two strains 018 and the strain 012 were also resistant to CM and ermB-positive. All strains were resistant to MX out of strain 012. Sequence analysis identified the SlpA of PCR-ribotype 018 as a new variant, with an identity of 89% and 57% compared to those found in strains 126 and 012, respectively. In all assays performed, strains 018 showed a number of adherent bacteria per cell significantly higher compared to the other strains. A mutated TcdC was observed only in strain 126. As far as cytotoxicity is concerned, strains 018 showed an earlier activity visible as cell damage at 6 hours.

**Conclusion:** PCR-ribotype 018 is a new epidemic *C. difficile* type showing peculiar characteristics, such as a new SlpA variant, an increased adhesiveness on Caco-2 cell monolayers and an earlier cytotoxic activity, which may play a role in the enhancement of virulence and in facilitating the spread of these strains.

**P2232** **Description of notifications of severe cases of *Clostridium difficile* associated diarrhoea in North Rhine-Westphalia**

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In Germany in November 2007 *Clostridium difficile* associated diarrhoea (sCDAD) has been classified as threatening disease with evidence of serious danger to the community. These cases are notifiable pursuant to § 6 paragraph 1 No. 5a Infection Protection Act.

At federal state level we descriptively analyzed the notifications of the 53 local health departments submitted from 2008 to 2010 in order to characterize the epidemiology of sCDAD in North Rhine-Westphalia (NRW) by trends in the number of reported cases, in occurring symptoms and criteria of the severe course of the disease, as well as by age, sex and regional distribution. Average hospital stay was taken from NRW hospital statistics. The average sCDAD incidence and the fatality rate were taken from the Yearbook of notifiable diseases of the Robert Koch-Institut.

From 2008 to 2010 in NRW 196 cases of sCDAD has been reported. The average incidence was 0.36 per 100 000 inhabitants, 1.54 cases per 100 000 admissions and 0.19 cases per 100 000 patient-days. From 2008 to 2010 in men 90 cases were reported (0.34 per 100 000 male

inhabitants) in women 101 (0.37 per 100 000 female inhabitants). One hundred and forty-nine subjects (78.0%) were aged over 70 years. The age-specific incidence in men of 80–89 years was 36.7% higher than that in the women in the same age. 96.6% of cases were treated in hospital in average for 21.6 days.

Regarding the criteria of severe course in 36% of cases CDAD caused or was implicated in the death, in 25% it came to admission on an intensive care unit for treatment of CDAD or its complications, 23% of notified cases were readmitted due to a recurrence, 7% underwent surgery (colectomy) and in 9% of the cases was detected ribotype O27. Detection of ribotype O27 in NRW has increased significantly from 2008 to 2010.

According to the notification data the incidence sCDAD in NRW in 2010 is at 0.42 per 100 000 inhabitants below the national average of 0.60. The higher age-specific incidence of males is probably due to the higher life expectancy of women. There are fewer fatalities of sCDAD-cases in NRW compared to the German average. This may indicate less under-reporting of non fatal sCDAD cases in NRW. The large hospitalization rate, the nearly threefold longer (21.6 vs. 8.1 days) in hospital stay and the high fatality rate (in 2010 46.7%) indicate the importance of surveillance, rational antibiotic use and implementation of infection control measures appropriate to the guidelines to prevent the nosocomial spread of the pathogen.

**P2233** **High prevalence of *Clostridium difficile* colonisation among nursing home residents in Hesse, Germany: comparison with the population outside healthcare facilities**

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**Objectives:** *Clostridium difficile* is the most common cause of antibiotic-associated diarrhoea in hospitals and other healthcare facilities. The elderly are particularly susceptible and at increased risk for adverse outcome as a result of *C. difficile* infection. The aim of this study was to determine the prevalence of *C. difficile* colonization among residents of nursing homes in Germany and to compare it with the prevalence in the population living outside long-term care facilities (LTCF). Furthermore, we evaluated possible risk factors for colonization by *C. difficile* and determined the genotype of circulating strains.

**Methods:** Using a cross-sectional design, we studied the prevalence of intestinal colonization by *C. difficile* among 240 nursing home residents and 249 volunteers living outside LTCF in Hesse. *C. difficile* was isolated by culture. All isolates were tested for production of *C. difficile* Toxin A and/or B by ELISA and for presence of the *cdtB* gene by PCR. Molecular typing was performed by PCR-Ribotyping.

**Results:** *C. difficile* was isolated from 11/240 (4.6%) nursing home residents and 2/249 (0.8%) individuals living outside LTCF ( $p = 0.02$ ). Ten of 11 (90.9%) isolates from nursing homes and one of two isolates from the population outside LTCF were toxigenic. The prevalence of *C. difficile* colonization varied from 0% to 10% in different nursing homes. Facilities with known actual or recent CDI cases were more likely to have colonized residents than facilities without known CDI cases. *C. difficile* PCR-ribotypes 014 and 001 were the most prevalent genotypes and accounted for 30% and 20% of toxigenic isolates in nursing homes, respectively. Interestingly, no individuals carried the epidemic strain PCR-ribotype 027.

**Conclusion:** Our results indicate that residents of nursing homes in Germany are at high risk for being colonized by virulent *C. difficile* strains. The high prevalence of *C. difficile* colonization in nursing homes underscores the importance of good adherence to standard infection control precautions even in the absence of a diagnosed infection. They also emphasize the need for specific programs to increase the awareness of healthcare professionals in LTCF for CDI.

**P2234** Incidence, outcome and ribo-typing of culture positive *Clostridium difficile* infection in a tertiary hospital in Norway from 2002 to 2010

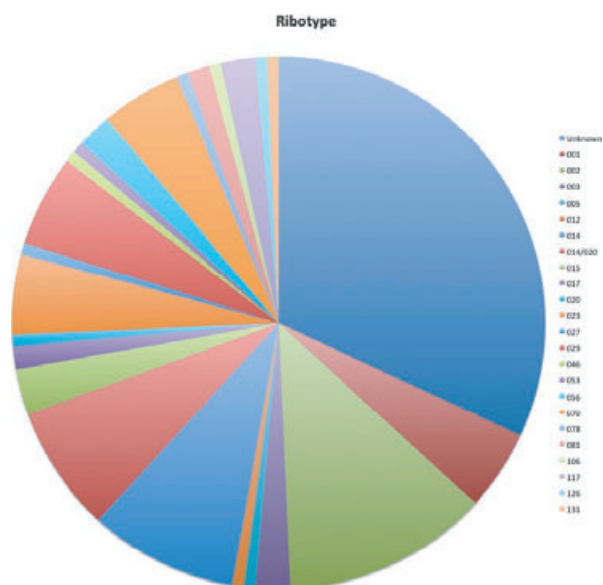
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**Objective:** Describe the incidence of toxin positive *Clostridium difficile* infection, culture findings, ribo-typing and predisposing factors and outcome in hospitalised patients the period 2002–2010.

**Methods:** All toxin tests performed 2002–2010 were reviewed and compared to culture findings. Culture positive *Clostridium difficile* isolates were characterized with ribo-typing and toxinotyping. Clinical data, morbidity, McCabe score, prior antibiotic usage, treatment and 3-month outcome were obtained from patient records.

**Results:** Toxin positive rate 11%. A little more than 50% were culture positive. Clinical data were obtained from 136 of these (the most recent) and are included in the study. Sixty-four percent of the infections were hospital acquired. Eighty-four percent of patients received antibiotics prior to *Clostridium difficile* infection; cefotaxime 30%, penicillins 21%, cefuroxime 10%, clindamycin 14% and ciprofloxacin 8%. Among the culture positive patients, 53% developed symptoms and were diagnosed during predisposing antibiotic treatment, 35% within the first week.

The crude 3 month mortality rate in *Clostridium difficile* infection were 23%, early mortality rate 10%. There were no clear association between ribo-type and hospital or community acquired disease, or between ribo-type and mortality. The most prevalent ribo-types were 002 (13%) and 014 (9%). Unknown/non-typable (33%). One wild type 027 was found, isolated in 2003. Ribo-type distribution is shown in Figure 1



**Conclusion:** An increasing number of toxin test were performed during the period, but the positive rate remained stable. This was also reflected by culture findings and discharge diagnoses. The crude mortality rate is high in this patient population and the majority received broad-spectrum antibiotics prior to *Clostridium difficile* infection. No association between ribo-type and disease were demonstrated, one wild type 027 was found.

**P2235** Clinical and molecular epidemiology of *Clostridium difficile*-associated diarrhoea at a university medical centre, Ljubljana, Slovenia

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**Objectives:** This is a cross-sectional study of epidemiological characteristics of *Clostridium difficile*-associated diarrhoea at the

University Medical Centre Ljubljana (UMCL) from the molecular and clinical perspective with aim to determine a possible connection between a ribotype and course of diarrhoea and to assess the role of routine ribotyping in patients with *C. difficile* infection (CDI).

**Methods:** Stool samples of the patients with suspicion of a CD infection hospitalized or admitted to the UMCL (from October 2007 to the end of April 2011) were analysed using the TechLab CDiff Quik Chek Complete Tox A/B (Inverness Biomedical). From February 2011 the samples were analysed using the Illumigene *C. difficile* (Meridian Biosciences) assay. Toxigenic culture was performed on all samples. All isolates were analysed using PRC-ribotyping. From October 2010 to April 2011 patients' clinical characteristics were also collected. Ribotypes were divided into three groups: 014/020, 'hypervirulent' ribotypes and other ribotypes. Proportions of ribotype groups were compared with other variables using the chi-square or Fisher exact test.

**Results:** Altogether 279 strains were submitted for toxinotyping and ribotyping and 70 different ribotypes were found, the most common was 014/020 (22%). The proportion of presumptive 'hypervirulent' ribotypes (027, 078, 017) was 9%, of which ribotype 027 represent (1.4%). The average age of the patients was 55.9 years and the highest proportion represented patients aged  $\geq 75$  years (32%). The rate of the infection was increasing during the study period (regression coefficient  $b = 0.125$ ). For 47 patients a questionnaire was completed, 64% of the patients had received antibiotics in the month preceding the onset of diarrhoea. There was an association between a ribotype and severity of diarrhoea ( $p = 0.029$ ). 'Hypervirulent' ribotypes as well as some other ribotypes caused more severe disease.

**Conclusions:** Ribotype 014/020 is the most common ribotype in the UMCL. We have established that in none of the observed clinics a higher probability for infection with a certain ribotype exists and also that there is no significant difference in odds for ribotype 014/020 among those clinics. Some ribotypes were associated with a severe course of diarrhoea. Given the diversity of ribotypes, routine ribotyping is reasonable only in case of an outbreak or in severe cases of CDI.

**P2236** Molecular epidemiology and resistance of *Clostridium difficile* in a tertiary care hospital in Spain

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**Objective:** To analyze the clonal epidemiology, population structure and resistance of toxigenic *Clostridium difficile* isolates as well as the incidence of *C. difficile* infection (CDI) in our institution.

**Methods:** A total of 100 toxigenic *C. difficile* clinical isolates collected from August 2007 to April 2011 from different patients selected by rapid ELISA detection of toxins A and B (Wampole®) were studied. All isolates were characterized through detection of *tcdA* (toxin A), *tcdB* (toxin B) and *cdtA* and *cdtB* (binary toxin) genes by PCR amplification and ribotyping by amplification of the 16S–23S rDNA.

In addition, Multilocus Sequence Typing (MLST) was performed according to described protocol (Griffiths et al. 2010) in ten selected strains. Antimicrobial susceptibility to metronidazole (MTR), vancomycin (VAN), erythromycin (ERY) and moxifloxacin (MXF) was determined by Etest.

**Results:** The estimated mean incidence of CDI in our hospital was 1.22 cases/1000 patient admissions between 2008 and 2010 with no significant variations between these years. Toxin A and B genes were detected in all isolates whereas binary toxin genes were detected in 20% of isolates. We found 52 different ribotype patterns with prevalence of 014 (30%), 078 (15%) and 001 (6%) ribotypes. Noteworthy that 71% of binary toxin producing isolates were ribotype 078. Nine different sequence types (STs) were identified by MLST including a newfound ST (ST130) belonging to clade 2. We have found an association between ribotypes 014, 078 and 001 and STs 2, 11 and 3, respectively. All isolates were susceptible to MTR and VAN whereas 18% and 19% were resistant to ERY and MXF, respectively. The highest rate of ERY resistance was observed in ribotype 078 (71%) and half of ribotype 001 strains (50%) were resistant to ERY and MXF.

**Conclusion:** Although there is a low incidence of CDI in our hospital, knowledge of its clonal epidemiology shows that our data are consistent with other European hospitals, being ribotype 014 the most frequently encountered as well as a considerable presence of the hypervirulent ribotype 078. Although we have observed an association between some ribotypes and STs, other STs could be related to these ribotypes. Finally, no antimicrobial resistance to usual treatments was found. Likewise, the overall rates of resistance to ERY and MXF are not high in our isolates, associating mainly ribotypes 078 and 001 with less susceptibility to these antibiotics.

**P2237 Persistence of toxigenic *Clostridium difficile* in the gut microflora of healthy Swedish infants**

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**Objectives:** *Clostridium difficile* is an important cause of diarrhoea and colitis in adults, whereas infants are frequently colonized without symptoms. The objective of the present study was to characterize in detail the longitudinal colonization pattern by *C. difficile* in healthy Swedish infants, and to identify factors promoting such colonization.

**Methods:** *C. difficile* were isolated from faecal cultures obtained from 184 healthy Swedish infants followed from birth for a period up to 3 years of age. In a subgroup of 42 colonized infants, individual strains of *C. difficile* were characterised by PCR ribotyping, toxin genes and toxin expression.

**Results:** Colonization by *C. difficile* increased steadily until 1 year of age, when 60% of the infants harboured these bacteria. Thereafter, colonization decreased to 30% by 1.5 years and to 6% by 3 years of age. Delivery by caesarean section and absence of elder siblings increased the risk of *C. difficile* colonization, while breastfeeding was protective.

In the subgroup of 42 colonized infants, 56 *C. difficile* isolates were identified. A majority of the isolates (73%) carried toxin A and toxin B genes and produced the toxin B in vitro. The most common PCR ribotypes were 001 (34%) and 014 (20%). Fourteen of the infants harboured *C. difficile* isolates that persisted for at least 6 months in the gut microflora, and 12 of these 14 strains (86%) were of the PCR ribotype 001 or 014.

**Conclusions:** Our results show that *C. difficile* is a common colonizer beyond the first year of life in Swedish infants, and indicate that certain toxigenic PCR ribotypes of *C. difficile* possess an increased capacity to persist in the infantile gut microflora, which may function as a reservoir for strains causing *C. difficile* infection in adults.

**P2238 Asymptomatic carriers of *Clostridium difficile* in a Serbian population**

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**Objectives:** The aim of the research was to determine the intestinal carriers of *C. difficile* in different human population groups in Serbia.

**Methods:** The research included 877 persons with formed stools (newborn children in maternity hospital for up to 2 weeks) (23), group A: children aged from 2 weeks to 2 years (121), group B: children aged 2–10 years (54), group C, healthy individuals aged 10 and over (516), group D: hospitalized patients in the hospital spent at least 48 hours (100), group E and staff of Clinical Center in Nis (doctors, medical staff with higher and secondary level of education, paramedical staff) (63), group F. Stool sample were seeded in the laboratory of selective CCF (cykloserin [500 µg/mL], cefoxitin [16 µg/mL], fructose) agar (Biomedics, Parg qe tehnicologico, Madrid, Spain). Identification of *C. difficile* isolates performed was using API system for anaerobic bacteria (API 20A BioMerieux, France) and agglutination Culture CDT Rapid Latex Test Kit (Becton Dickinson, USA). Colonies of *C. difficile* were subcultivated in 5 mL brain-heart infusion broth under anaerobic conditions during 4 days (1 day = 24 hours). After incubation, liquid

cultures of *C. difficile* were centrifugated at 3000 g for 15 minutes and filtered through 0.45 µm membranes. The toxins A and B of *C. difficile* were detected by ELISA-ridascreen *Clostridium difficile* Toxin A/B (R – Biopharm AG, Germany). *C. difficile* toxin A detected by ColorPAC Toxin A test (Becton Dickinson, USA).

**Results:** From the sample of the 70 (7.98%) persons cultivated *C. difficile*. Of the total number of persons (877), carriers of certain types of toxin-producing strains of *C. difficile* were 6.04% (A-B-), 1.83% (A+/B+) and 0.11% (A-/B+). Representation of persons with *C. difficile* by followed groups ranged from 1.75% to 47.83%. In most of the groups (5/6), was established the dominance of non-toxigenic (A-/B-) isolates with the rate of carriers 1.75–30.43% depending on the group. Toxigenic isolates only were prevalent in the group F in relation to non-toxigenic (7.94% vs. 4.76% of persons). In other groups, the carriers of toxigenic strains ranged from 1.65% to 17.45%, provided that in the group D, there were not carriers of toxigenic strains.

Production of certain toxin of <i>Clostridium difficile</i>	Find of Enterotoxin A/B test	Find of Clostridium Toxin A test	Study group						Total N = 79 (300%) n (%)
			A N = 23 (300%) n (%)	B N = 121 (300%) n (%)	C N = 54 (300%) n (%)	D N = 100 (300%) n (%)	E N = 63 (300%) n (%)	F N = 43 (300%) n (%)	
Enterotoxin A and B (A+/B+)	+	+	0 (0.0)	2 (1.6)	4 (7.4)	0 (0.0)	2 (2.0)	0 (0.0)	18 (22.6)
Enterotoxin B (A-/B+)	+	-	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.3)	1 (1.3)
non-toxigenic (A-/B-)	-	-	7 (28.4)	18 (14.8)	13 (24.1)	9 (1.7)	3 (3.0)	3 (3.7)	39 (47.7)
Total			11 (47.8)	20 (16.3)	17 (27.7)	9 (1.7)	7 (7.0)	4 (5.0)	
Control									
ATCC43046 (A+/B+)	+	+							
ATCC43025 (A+/B+)	+	+							

**Conclusion:** The presence of asymptomatic intestinal carriers of *C. difficile* in the human population, indicates the possible reservoirs and sources of infections caused by this bacterial species in and outside of the hospitals setting.

**P2239 *Clostridium difficile* prevalence, toxino-type and antibiotic sensitivity in a cohort of patients with inflammatory bowel disease in Northeastern Italy**

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**Objectives:** *Clostridium difficile*, an opportunistic pathogen causing antibiotic-associated diarrhea in hospitalized patients, is now recognized as a risk factor in disease reactivation in Inflammatory Bowel Disease (IBD) patients. The aim of the study was to determine the prevalence of *C. difficile* infection (CDI) in IBD according to clinical activity; characterize *C. difficile* strains isolated from IBD patients as regard to antibiotic sensitivity, toxino-type and adhesion to intestinal epithelium.

**Methods:** Stools were collected from IBD patients (n = 233) and healthy controls (n = 40). IBD patients (100 ulcerative colitis [UC] and 97 Crohn's disease [CD]) in remission or mild activity were enrolled during a routine follow-up visit while patients with severe activity (19 UC and 17 CD) were enrolled at hospital admission. Stools were cultured after ethanol shock and *C. difficile* identified and toxino-typed by PCR. Strains were used to perform E-test and adhesion assays on human HT-29 cells. Clinical data were collected to correlate CDI to antibiotics use, disease activity, disease extent and location, type of therapy and hospitalization.

**Results:** In healthy controls CDI prevalence was 10%, however all strains were non toxigenic. In IBD patients in remission, *C. difficile* was identified in 9.1% with 55% of strains toxigenic (10% of UC with 40% toxigenic strains, 8.2% in CD with 63% toxigenic strains) whereas in patients with active disease CDI incidence was 11.1% with all strains toxigenic. *C. difficile* strains isolated from IBD patients with active disease were 75% toxin A and toxin B positive (A+B+) and 25% A+B-, whereas among patients in remission 78% were A+B+, 11% A-B+ and 11% A+B+binary-toxin-. All *C. difficile* strains from IBD patients were resistant to ciprofloxacin (MIC > 32 mg/L), but susceptible to metronidazole (MIC < 0.19–0.75 mg/L) and vancomycin (MIC < 0.5 mg/L). *C. difficile* strains isolated from patients with

pseudomembranous colitis were 3.3-fold more effective to adhere to HT-29 cells than strains isolated from IBD in remission. Multivariate analysis identified patients with ileal CD and patients treated with biologics as the groups at higher risk of CDI.

**Conclusions:** IBD patients are at higher risk of colonization by toxigenic *C. difficile* strains than controls and CDI is more frequent in patients with active disease. Patients with CD localized to the ileum and subjects treated with biologics are at higher risk of colonization.

**P2240** The 027/ST1 strains have greatest impact on white blood count and C-reactive protein during *Clostridium difficile* infection

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**Background:** White blood count (WBC) and C-reactive protein (CRP) are recognised biomarkers of *C. difficile* infection (CDI) severity. How these biomarkers vary across *C. difficile* strains is unknown.

**Methods:** *C. difficile* toxin enzyme immunoassay positive faecal samples from community and hospitalised patients in Oxfordshire from October 2006 to March 2011 were cultured and multi-locus sequence typed. The closest WBC, CRP, creatinine and albumin measurements within (-3,+1) days were obtained from the Infections in Oxfordshire Research Database. Repeat positives within 14 days were excluded. Mean biomarkers at CDI diagnosis were estimated using normal linear regression.

**Results:** WBCs were available for 1324 genotyped isolates, from 745 (56%) women, 1151 (87%) inpatients, with median age 78 (IQR 67–85) years. Four hundred and six (31%) isolates were the hypervirulent NAP1/027/ST1 strain. Mean WBC (x10<sup>9</sup>/L) at CDI diagnosis was 14.7 in ST1 vs. 12.3 in non-ST1 strains ( $p < 0.0001$ ). Mean WBC also varied significantly across non-ST1 strains; being 12.0 in the other major phylogenetic group ( $n = 806$ , clade 1), 15.3 in ST11 (078,  $n = 38$ ), and 15.6 ( $n = 50$ ) and 10.9 ( $n = 24$ ) in two smaller clades, 3 and 4 ( $p < 0.0001$ ;  $p < 0.0001$  ST1 vs. clade 1). Differences between ST groups were similar adjusting for age, sex and inpatient/outpatient at CDI, and were also similar for neutrophils. Smaller differences were observed for CRP (mg/L) at CDI diagnosis (1242 cases), with mean 114.7, 100.6, 96.5, 117.6 and 81.4 for ST1, clade 1, ST11, clade 3 and 4, respectively (adjusted  $p = 0.06$  across 5 groups;  $p = 0.04$  ST1 vs. clade 1). By contrast, serum creatinine concentrations were less variable (1326 cases: mean 107.7, 100.8, 98.7, 95.1, 91.2  $\mu$ M, respectively;  $p = 0.66$ ), as was albumin (1162 cases: mean 33.1, 33.5, 33.3, 34.8, 32.5 g/dL, respectively,  $p = 0.21$ ). In all 1859 sequence typed cases (with or without biomarker values), crude 14 day mortality following CDI was 19.8%, 11.6%, 26.7%, 6.8% and 13.7% respectively ( $p < 0.0001$ ).

**Conclusions:** 027/ST1 *C. difficile* strains have a significantly greater impact on WBC and CRP compared to other strains, consistent with their higher mortality risks. Heterogeneity in WBC between non-ST1 strains shows other virulent strains exist and may expand in the future; ongoing surveillance is essential.

**P2241** *Clostridium difficile* infection in the community – a cause for concern?

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**Background:** New cases of *Clostridium difficile* infection (CDI) are notifiable in Ireland since May 2008. A national voluntary enhanced surveillance scheme capturing information on case type, origin and onset of disease commenced in August 2009. This is currently undertaken by 36 acute hospitals.

**Methods:** Hospitals submit details on all CDI cases using standardised European definitions on a quarterly basis. Limited typing data is collected as Ireland does not have a *C. difficile* reference laboratory.

**Results:** Between the 1 August 2009 and 30 June 2011, enhanced data were submitted on 2466 cases (2238 new cases, 91%; and 220 recurrent

cases, 9%) of CDI. There has been a decrease in reporting of recurrent cases from 114% of all cases in 2009 to 8% in 2010 and 7.5% for the first half of 2011. The majority (74%) of cases were healthcare-onset (range, 73–76%) with 26% community-onset (range, 23–27%). Where onset of CDI is healthcare-onset, there has been an increase in the proportion occurring in nursing homes from 9% in 2009 to 10% in 2010 and to 13% in 2011. Seventy-seven percent of cases were healthcare-associated and 19% community-associated. The proportion of CDI cases originating in healthcare settings has decreased from 82% in 2009 to 75% in 2011, however, the proportion originating in the community has increased from 13% to 21% over the same period. Amongst healthcare-associated cases, the proportion that originated in a nursing home increased from 9.5% in 2009 to 12.5% in 2011. The proportion originating within the hospital has remained level at approximately 78–80% over the 3 years.

**Conclusion:** The decrease in recurrent CDI may represent an improvement in infection prevention and control strategies and patient management. However, it may also reflect changes in laboratory testing protocols. The increase in CDI outside acute hospitals indicates that CDI is not confined to hospitals and is increasingly common in community and nursing home settings. The addition of typing data to our surveillance scheme may help explain some of these trends. It is essential that CDI is considered in the differential diagnosis of all patients presenting with diarrhoea and that specimens are sent in a timely fashion for laboratory diagnosis.

**P2242** Hypervirulent *Clostridium difficile* PCR-Ribotype 027 subverts human intestinal epithelial cell response

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**Objectives:** In recent years a striking increase in *Clostridium difficile* nosocomial infections worldwide has been recorded, mainly due to the emergence of isolates that possess extended virulence properties, such as the *C. difficile* PCR-ribotype 027. The reasons for the emergence of these strains remain largely speculative, and there is widespread interest in understanding the factors contributing to the hypervirulence. The mucosal immune system needs to fulfill two conflicting functions, activate the immune response against invading pathogens, and prevent the induction of an excessive and detrimental inflammatory response. In this regard, a key function is played by intestinal epithelial cells, the initial sites of host response to mucosal infections. To further explore this role, we investigated the interactions of hypervirulent *C. difficile* with human intestinal epithelial cells and the induction of pro-inflammatory signals.

**Methods:** Adhesion assays were performed by infecting Caco-2 monolayers with the hypervirulent R20291 isolate (PCR-ribotype 027) and the control strain 630Derm (PCR-ribotype 012). Two mutant strains (Prof. N Minton, University of Nottingham) 630DeltaermDeltacwp84 and R20291Deltacwp84 deleted of cwp84, a protein responsible for the activation of the S-layer precursor protein, were also used. Expression levels of IL-8, RANTES and ICAM1 were assessed by Real Time RT-PCR.

**Results:** The results demonstrated that the hypervirulent PCR-ribotype 027 R20291 adhered to Caco-2 cells more strongly than the 630Deltaerm strain, which did not show differences with its mutant 630DeltaermDeltacwp84. On the contrary, a significant decrease in the adherence was observed in R20291Deltacwp84 compared to the parental strain. Further experiments performed on the same cell line have shown that both hypervirulent or control *C. difficile* strains were not able to induce upregulation of ICAM-1. Remarkably, when Caco-2 cells were infected with the hypervirulent strain the expression of pro-inflammatory chemokines IL-8 and RANTES was inhibited.

**Conclusions:** These studies highlight two strategies that might contribute to increased virulence of *C. difficile* PCR-ribotype 027: (i) the high levels of adhesion to intestinal epithelial cells, mainly mediated by S-layer proteins, improve the efficiency of the infection; (ii) the ability to inhibit chemokine expression acts as an escape mechanism and limits the induction of early immune responses.

**P2243** **Recombinational switching and glycosylation of the *Clostridium difficile* S-layer revealed by whole genome sequencing**

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**Objectives:** During the past 30 years temporal changes and geographic variation in incidence of clinically important *Clostridium difficile* genotypes have been observed, the causes of which are unknown. The outermost surface of *C. difficile* comprises multiple copies of a single protein which self-assemble to form a paracrystalline array or S-layer. The S-layer is an immunodominant antigen which determines serotype, facilitates binding to cells and causes an inflammatory response. It is encoded by the slpA gene located centrally in a 36.6 kb cell wall protein (cwp) gene cluster, along with other putative virulence determinants. Our aim was to investigate the evolutionary history of the cwp cluster and flanking genes, in the context of the population structure.

**Methods:** A total of 820 *C. difficile* clinical isolates obtained in Oxford and Leeds, UK, between September 2006 and August 2010 underwent whole genome sequencing using the Illumina HiSeq platform. Genomes were assembled de novo using Velvet. The assemblies were uploaded into a Bacterial Isolate Genome Sequence Database (BIGSdb) to allow simultaneous BLAST searching and retrieval of defined loci from multiple isolates. Sequences were investigated using Neighbour joining trees (MEGA version 4), BioEdit, and the Artemis Comparison Tool.

**Results:** *C. difficile* undergoes S-layer switching by homologous recombination involving DNA fragments between 12 and 36 kb. Switching events are size-constrained to maintain linkage of the genetically diverse and functionally inter-related slpA, cwp66 adhesin, and secA2 translocase genes, which together form a 10 kb ‘‘S-layer cassette’’. We also identified a 24 kb putative S-layer glycosylation gene cluster inserted within the S-layer cassette of 10–30% of recent clinical isolates, dependent on UK region. This gene cluster contained 19 identically orientated ORFs encoding all components needed for the synthesis, export and covalent attachment of a carbohydrate chain to a substrate.

**Conclusion:** This is the first report of S-layer switching, the genetic replacement of one S-layer variant for another by homologous recombination. This process appears analogous to capsular switching observed in other species. It is also the first time an S-layer associated glycosylation gene cluster has been identified in *C. difficile* or any other Gram positive human pathogen. S-layer switching may help to explain temporal changes and geographic differences in clinically important *C. difficile* genotypes.

**P2244** **Curtains as a source of *C. difficile*: the importance of sampling methods**

E.L. Best\*, C.E. Bosomworth, F. Pinker, P. Parnell, M.H. Wilcox (Leeds, UK)

**Objectives:** *C. difficile* spores may contaminate hospital curtains, which are handled frequently by patients and staff. Variable recovery of *C. difficile* from the environment can be method dependent. We aimed to identify the extent of *C. difficile* contamination of curtains in the vicinity of *C. difficile* infection (CDI) symptomatic patients using an intensive sampling method.

**Methods:** Curtains at windows of rooms of four CDI patients and four control (no CDI) hospitalised patients were intensively sampled. Sponge wipes (Polywipes™) were used to remove particulate matter from the entire surfaces of curtains. To ensure complete coverage of each curtain, the total surface area was split into nine segments on the front and nine on the back, with each segment (approximately

1600 cm<sup>2</sup>) sampled separately. This yielded 18 samples per curtain, and a total of 144 samples (72 from CDI cases and 72 from control curtain segments). Samples were enriched in Robertson’s cooked meat broth for 48 hours, and then subcultured onto Braziers CCEYL plates and incubated anaerobically for 48 hours.

**Results:** *C. difficile* were isolated from every curtain sampled in rooms housing known CDI cases (4/4 curtains tested), either from the front or back in three and from both sides of one curtain tested. According to the 18 segments per curtain, the proportion of each curtain total surface area associated with CDI cases that was *C. difficile* contaminated averaged 19% (range 11–50%). When comparing the *C. difficile* positive areas of each curtain we found no clear similarities. The areas of the curtain assumed to be handled most frequently and therefore the most contaminated were not always positive. *C. difficile* was not recovered from any of the 72 control curtain segments.

**Conclusions:** Our results emphasize the importance of sampling the entire curtain surface to detect *C. difficile*. Randomly sampling of a single curtain surface area of 1600 cm<sup>2</sup> had an approximate 80% chance of missing true *C. difficile* contamination. Curtains in the vicinity of CDI cases appear to be very frequently contaminated by *C. difficile*, likely reflecting the aerosolisation of spores, and may act as a reservoir for transmission.

**P2245** **Clinical significance of antigen-positive/toxin-negative results for *Clostridium difficile***

A. Ravindran\*, L. Ragunathan, M.M. Raza (Milton Keynes, UK)

**Objectives:** Testing for *C. difficile* associated diarrhoea (CDAD) remains problematic. This study was done to assess the clinical significance of Antigen positive/Toxin negative results by EIA.

**Methods:** Twenty-one patients with antigen positive/toxin negative stool samples by C. DIFF QUIK CHEK COMPLETE® (Techlab) between July and December 2010 were included in the study. Clinical presentation and management of current episode along with follow up data were then analysed to assess clinical significance of each result.

**Results:** All the patients in the study were symptomatic with diarrhoeal stools at the time of sampling. Eight of these patients were more than 80 years of age and one was a child aged 9. Seventeen of these patients had a history of admission to hospital within the previous year. Ten patients had been on proton pump inhibitors and eight had previous antibiotics. Four patients went on to become *C. difficile* toxin positive, three of which were within 2 weeks of toxin negative result and the other within 2 months. All four patients were treated for CDAD but two of them died but one had *Staphylococcus aureus* septicaemia and in the other the cause of death was not clear. Of the other 17 patients who did not develop toxin positive disease only four were treated and one had features suggestive of CDAD and died within 2 weeks. There were five other deaths in this group two on the same admission and the others on separate admission but none were linked to *C. difficile*. Six of these toxin negative patients have not been re-admitted but five others have been and two were challenged with co-amoxiclav but none have developed toxin positive disease in a year of follow up.

**Conclusion:** Our data suggest that patients with antigen positive/toxin negative stool samples, should not always be treated as colonisation but clinically evaluated. Those with clinical features of CDAD should be treated, as the toxin result can be false negative, as is likely the case with three of the four patients who developed toxin positive results within 2 weeks. However the majority of these do represent colonisation but even these patients should be monitored closely and should be isolated, while having diarrhoea, to limit environmental contamination. In our cohort although some of the 11 surviving patients have been challenged with hospitalisation and antibiotics none of them have gone on to develop CDAD. Therefore the factors associated with progression to disease and the significance of colonisation is not entirely clear.



**P2246** The unintended consequences of *Clostridium difficile* reduction programmes

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**Objective:** In 2008, a National Operating Framework was introduced by the Department of Health in England and laterally by Welsh Government in Wales to reduce *Clostridium difficile* infections. In response, Health Boards in Wales have introduced measures to reduce *C. difficile* including implementing restrictive antimicrobial policy guidelines. This paper looks at the unintended consequences of those restrictive guidelines on antimicrobial usage and antimicrobial resistance in Wales.

**Method:** Antimicrobial ward stock data for the acute hospitals in Wales was coded and measured using the ATC/DDD system. Antimicrobial susceptibility data was extracted from LIMS via regional DataStore systems. Duplicates were removed from the data sets prior to analysis.

**Results: Antimicrobial usage:** Since 2008, tetracycline usage has increased 121%; beta-lactam/beta-lactamase inhibitor combinations usage has increased 69%; carbapenem usage has increased by 79%; aminoglycoside usage has increased 42%; cephalosporin usage has decreased 44% and fluoroquinolone usage has decreased 24%. See Figure 1.

**Antimicrobial resistance:** Since 2008, co-amoxiclav resistance in *E. coli* from blood culture has increased by 10.9% to an All-Wales average of 41.9% ( $p < 0.05$ ) and gentamicin resistance has increased by 4.1% to an All-Wales average of 9.3% ( $p < 0.05$ ). The impact caused by the changes in antibacterial prescribing is not all negative; as cephalosporin and fluoroquinolone usage has decreased so resistance rates have decreased or stabilised e.g. fluoroquinolone resistance in *Klebsiella* spp. has decreased by 7.3% to an All-Wales average of 8.0% ( $p < 0.05$ ), and in *Enterobacter* spp. has decreased by 6.3% to an All-Wales average of 4.4%.

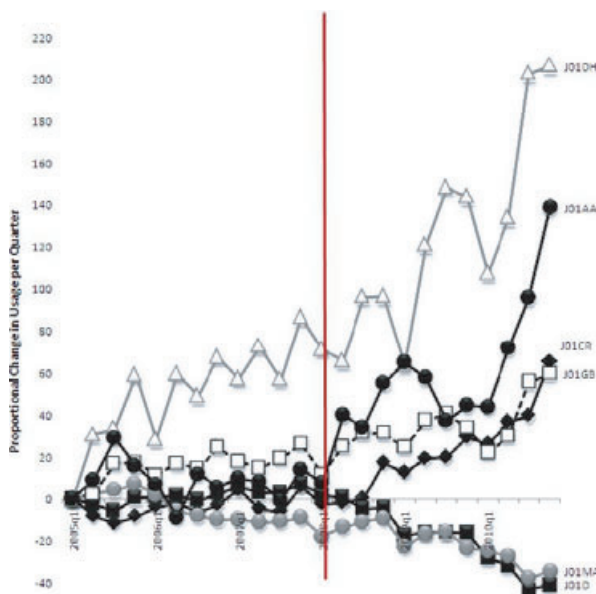


Figure 1: All-Wales trends in Antibacterial Usage in Secondary Care 2005-2010

Key for Figure 1: The red line shows the introduction of the AOF to reduce *C. difficile*  
 J01AA - tetracyclines; J01CR - beta-lactam/beta-lactamase inhibitor combinations; J01D - first, second and third generation cephalosporins; J01DH - carbapenems; J01GB - aminoglycosides; J01MA - fluoroquinolones.

**Conclusions:** The consequence of the imposed restrictions in cephalosporins and fluoroquinolone use has been an increase in usage of other antibacterial groups. In some instances it is too early to detect if the pressure from this increase will impact on resistance rates but in other instances the effect is measurable, with a significant increase in co-amoxiclav and gentamicin resistance rates in *E. coli* bacteraemias. Due to successful antimicrobial stewardship initiatives the last 3 years

has witnessed a marked change in antibacterial prescribing practices; continued surveillance is required to monitor the impact of these changes over time, and to detect future changes that may impact on resistance, and our ability to combat it.

**P2247** An antimicrobial stewardship economic analysis of *Clostridium difficile* infections

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**Objectives:** *Clostridium difficile* infection (CDI) is the most common infectious cause of nosocomial diarrhoea and is associated with extended lengths of stay (LOS) and around 20% require re-admission for recurrence. Economic costs of CDI and future lack of reimbursement for re-admissions within 30 days is staggering; yet, newer more costly antibiotics also challenge Antimicrobial Stewardship Programs. Our purpose is to define hospital costs of initial and recurrent readmissions for CDIs and to assess cost variance by age.

**Methods:** Adult patients with a positive stool assay for *C. difficile* hospitalized from 18 January 2010 to 31 July 2011 with hospital onset (HO) or community onset (CO) CDI were identified from the Epidemiology database. HO was defined as a positive test on or at hospital day 4 and CO was defined as a positive test within 3 days of admission or in an outpatient. Recurrence was 2-8 weeks after initial case. Hospital costs, length of stay, ICU admission and mortality were obtained from the information warehouse. All p-values determined by ranksum test or exact test, as appropriate.

**Results:** Hospitalization costs did not differ significantly by age groups or between initial and recurrent admissions ( $p > 0.05$ ). Overall recurrence rate was 12% (77); seven recurrent cases were seen in outpatient clinics and costs could not be captured. Readmission for recurrent CDI was 11%. Nineteen cases (3%) readmitted within 30 days represent a potential reimbursement loss of \$335 443 USD. CDI onset was: HO 40% of which 29% were in the ICU, CO 46% of which 13% were in the ICU and 15% unknown. Overall mortality for initial and recurrent CDI was 5.5% and 5.6%, respectively.

Age Group	Initial Cases	Cost USD	LOS days	Mortality	Recurrent Admitted Cases	Cost USD	LOS days	Mortality
<40	81	24,223 (6,966-56,695)	10 (4-25)	2	11	14,708 (8,752-100,589)	6 (2-32)	0
40-64	278	21,708 (8,646-59,326)	9 (5-21)	14	36	16,826 (7,298-32,049)	7 (5-13)	2
65-84	193	21,250 (12,074-50,247)	10 (7-20)	15	19	17,271 (11,057-32,598)	11 (7-13)	1
>84	30	17,020 (10,777-30,737)	8 (6-14)	1	4	25,092 (12,663-30,508)	9.5 (4.5-16.5)	1

**Conclusions:** Analysis of CDI costs extend beyond the cost of a patient's initial CDI hospitalization. Stewardship programs should consider this when CDI treatment guidelines are developed.

**P2248** Comparison of vancomycin and metronidazole for the treatment of *Clostridium difficile* associated disease

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**Purpose:** To compare the outcome of patients with *Clostridium Difficile* Associated Disease (CDAD) after first treatment with Vancomycin, Metronidazole or both.

**Material and method:** A retrospective study based on the records of all patients admitted to the Clinic of Infectious Diseases between January 2011 and August 2011 with the diagnosis of CDAD or who developed diarrhoea after admission. A clinical diagnosis was made and toxin A and B detection was carried out using Enzyme Linked Fluorescent Assay technology (Vidas bioMerieux). We performed a statistical analysis using Kaplan-Meier survival curves, ROC curves and univariate analysis taking into consideration age, gender,

comorbidities, laboratory results, history of antibiotic treatment and hospital admission.

**Results:** One hundred and seventeen patients with 148 admissions, 74 women, median age 71 years (min 15 years, max 90 years, average 67 years). Eighty-three patients (71%) had prior hospitalization and 98 (84%) had received prior antibiotic treatment. Comorbidities such as chronic renal failure and diabetes mellitus were rare conditions in our patients. Treatment was performed with Vancomycin in 34 (29%) patients, Metronidazole in 46 (39%) patients and with both in 37 (32%) patients. The average duration of treatment was 9.7 days with min 1 day and max 21 days. Twenty-five patients (21%) developed one or more recurrences after 5–87 days and six patients died (5%). Patients with recurrences did not differ significantly from those who were cured as far as age, gender, comorbidities, history of hospitalization or antibiotic treatment were concerned. Metronidazole and vancomycin or both produced similar rates of clinical cure and relapses.

**Conclusions:** (i) In our study, CDAD was mainly non-severe with a low mortality rate; (ii) the rate of recurrences was high; (iii) the outcome did not differ significantly regardless of antibiotic treatment, which had a median duration of 9–10 days.

**P2249** **Cadazolid and fidaxomicin are active against strains isolated from primary and recurrent *Clostridium difficile* infections**

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**Objectives:** *Clostridium difficile* is the major cause of nosocomial diarrhoeal diseases in elderly patients after treatment with antimicrobial agents. About 20% of the patients develop a recurrent infection after the primary episode. The aim of this study was to investigate the antimicrobial sensitivity of isolates from primary and from recurrent *C. difficile* infection (CDI) as well as the microbial factors that may contribute to the recurrences of CDI.

**Methods:** Fifty-five patients with a primary CDI were enrolled in this study. Faecal samples were, when possible, collected at 1, 2, 4, 6 and 12 months after the primary infection and analysed for the presence of *C. difficile* and toxin B. All isolates were investigated by antimicrobial susceptibility tests and ribotyping.

**Results:** The mean age of the 55 patients was 74 years and 29 were females and 26 males. Ten of the patients died during the follow-up period due to underlying diseases not directly attributed to CDI. Twenty-seven patients were positive for *C. difficile* during the follow-up period. Nineteen were colonised with the same ribotype as the primary CDI and eight switched ribotype. The most common ribotype was 020 followed by 078. In eight of the patients a new *C. difficile* ribotype was isolated after a period of negative samples. In one patient, four different ribotypes were isolated during the 1-year period. Another patient changed the ribotype twice during the follow-up period. No PCR-ribotype 027 was found in any of the samples. Sixty-seven isolates were analysed for antimicrobial susceptibility. All isolates were sensitive to cadazolid (MIC range) (0.125–0.25 mg/L), fidaxomicin (0.016–0.125 mg/L), metronidazole (0.125–1 mg/L), vancomycin (0.125–1 mg/L), tigecycline (0.008–0.125 mg/L), fusidic acid (0.064–0.5 mg/L) and linezolid (0.5–8 mg/L). Five isolates were resistant to moxifloxacin (0.064–32 mg/L) and tetracycline (0.064–32 mg/L) and three to rifampicin (0.002–64 mg/L). Forty-one of the *C. difficile* isolates were resistant to clindamycin (0.25 to  $\geq 128$  mg/L).

**Conclusion:** Among the 55 patients enrolled in this study, 27 were positive for *C. difficile* after the initial episode. During the study period, 8/55 (14%) changed the PCR-ribotype. The 078 PCR-ribotype was isolated from five patients. No PCR-ribotype 027 was detected in any samples. All tested strains were sensitive to cadazolid, fidaxomicin, metronidazole, vancomycin, tigecycline, fusidic acid and linezolid. Forty-one strains (61%) were resistant to clindamycin.

**P2250** **Enteric microbiome profiles during a phase 2 clinical trial of CB-183 315 or vancomycin for treatment of *Clostridium difficile* infection**

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**Objective:** The interplay of killing of *C. difficile* and suppression of the normal microbiota during treatment of *Clostridium difficile* infection (CDI) are major determinants for clinical response and recurrence. The objective of this analysis was to analyze by real-time quantitative PCR the major components of the enteric microbiome from fecal samples obtained from a subset of patients enrolled in the Phase 2 LCD-09-03 trial.

**Methods:** Fecal samples from 26 patients from a single center (CB/125 mg: n = 9; CB/250 mg: n = 9, vancomycin: n = 8) were collected on days 0, 4, 10, 14, 21, 28 and 38 for quantitative cultures of *C. difficile*, cytotoxin B fecal filtrate concentrations and for microbiome profile analysis. DNA from 0.25 g fecal samples obtained by Qiagen QIAamp extraction/NanoDrop quantification were probed (Biorad iQ5 and CFX96) with primers targeting *Bacteroides*, *Bacteroides/Prevotella*, *C. coccoides*, *C. leptum*, *Veillonella*, *Desulfovibrio*, *Lactobacillus*, *Bifidobacteria*, *Enterobacteriaceae* and *Enterococcus* species.

**Results:** Clinical cure was achieved in 25/26 patients (failure in one CB/125 mg patient). Recurrence of CDI was observed in 4/8 CB/125 mg, 2/9 CB/250 mg and 1/8 VAN subjects. Treatment with CB did not reduce *Bacteroides* counts and reduction of Firmicutes appeared to be less notable as compared to VAN (days 0, 10, 14 and 21 shown). Compared to VAN, CB appeared to be more also sparing of *Prevotella* and *Bifidobacteria*. No differences were observed in *Desulfovibrio*, *Lactobacillus*, or *Veillonella* by treatment group. Enterococcal counts were reduced by all treatments and Enterobacteriaceae counts were 2–3 logs higher than those found historically in normal control stools. CB/250 mg and VAN reduced *C. difficile* counts to the lower limit of detection, whereas CB/125 mg was less effective. Regrowth of *C. difficile* to counts similar to study entry with toxin re-expression was observed in all subjects with recurrence.

Genera/spp	Treatment Group	mean Log <sub>10</sub> CFU/g ± SD			
		Day 0	Day 10	Day 14	Day 21
<i>Bacteroides</i>	CB/125mg	7.7 ± 1.8	8.5 ± 1.6	8.9 ± 1.7	8.1 ± 1.6
	CB/250mg	8.2 ± 1.2	8.4 ± 1.2	8.3 ± 1.7	8.6 ± 1.0
	VAN	8.2 ± 2.0	5.8 ± 1.5*	6.3 ± 1.6*	7.2 ± 1.4
<i>Bacteroides/Prevotella</i>	CB/125mg	7.8 ± 1.6	8.5 ± 1.6	8.8 ± 1.7	7.9 ± 2.0
	CB/250mg	8.2 ± 1.4	8.1 ± 1.8	8.7 ± 1.1	8.8 ± 0.9
	VAN	7.9 ± 2.1	4.8 ± 1.8*	6.0 ± 1.5*	7.2 ± 1.6
<i>C. coccoides</i>	CB/125mg	7.8 ± 1.5	6.6 ± 0.9	7.7 ± 1.1	8.5 ± 0.7
	CB/250mg	7.5 ± 1.2	5.9 ± 0.6**	6.5 ± 1.1	8.4 ± 0.6
	VAN	8.1 ± 1.4	5.6 ± 0.6*	6.4 ± 1.9	8.6 ± 0.5
<i>C. leptum</i>	CB/125mg	6.4 ± 1.5	5.7 ± 1.2*	5.9 ± 1.6	6.0 ± 1.0
	CB/250mg	6.1 ± 1.3	4.3 ± 1.3**	4.3 ± 1.4*	5.4 ± 1.4
	VAN	6.0 ± 1.5	3.2 ± 0.7*	3.4 ± 1.3*	6.0 ± 0.9
<i>Clostridium difficile</i> total counts	CB/125mg	5.4 ± 2.1	3.1 ± 1.9*	4.3 ± 2.3	2.9 ± 1.5
	CB/250mg	6.4 ± 0.9	2.0 ± 0.6†	2.0 ± 0.1†	3.6 ± 2.3
	VAN	5.8 ± 2.0	2.0 ± 0.0†	2.0 ± 0.1†	1.8 ± 0.4

\* p < 0.05

\*\* p < 0.01

† p < 0.001

**Conclusions:** CB-183 315 250 mg BID reduced recoverable *C. difficile* as effectively as vancomycin in this subset of CDI patients. Compared to vancomycin, CB-183, 315 is more sparing of gut microbes, primarily *Bacteroides* and *Prevotella* spp. and may reduce the risk of recurrent CDI. The 250 mg dose of CB-183 315 BID appears to be the optimal dose for further clinical studies

**P2251** **Risk factors for CDI recurrence: comparison of CB-183 315 and oral vancomycin**

S. Rege\*, H. Patino, P. Bernardo, C. Lee, K. Mullane, S. Veticaden, I. Friedland (Lexington, US; Hamilton, CA; Chicago, US)

**Objectives:** A recent Phase 2 trial showed reduced recurrences for CB-183 315 compared to vancomycin in patients with CDI. The objective

of this post-hoc analysis from the Phase 2 trial was to evaluate the risk factors associated with CDI recurrences.

**Methods:** A Phase 2, randomized, controlled, double-blind, trial compared two dosing regimens of the oral lipopeptide, CB-183 315 (125 or 250 mg BID) to oral vancomycin 125 mg QID in 209 patients with CDI (randomized in 1:1:1 ratio). The effect of the following risk factors on recurrences were evaluated with univariate analyses: age, CDI severity, concomitant antibiotic exposure during treatment and during follow-up only, use of proton pump inhibitors, isolation of NAP1/BI/027 strain, region (US vs. Canada), albumin <2.5 g/L, WBC count >15 000/ $\mu$ L and prior CDI episode. The variables with the greatest impact on recurrence were identified using a stepwise effect-selection method for the logistic regression model. Variables with a p-value <0.1 were retained in the stepwise effect-selection process. The selected variables were included in a final logistic regression model in addition to treatment group and other previously described risk factors (age, WBC count >15 000/ $\mu$ L and presence of NAP1/BI/027 strain).

**Results:** CDI recurred in 48/209 patients during the study duration. All variables examined, with the exception of CDI severity, had a p-value <0.1 based on the univariate analyses and were included in the stepwise effect-selection process. This method identified prior CDI episode and concomitant antibiotic exposure during follow-up only as the variables with the greatest impact on recurrence. The final logistic regression model included these two variables in addition to treatment group and known risk factors. Treatment with 250 mg of CB-183 315 was associated with a 20% odds reduction in CDI recurrence when compared with oral vancomycin, after adjusting for all variables in the model.

**Conclusion:** Concomitant antibiotic exposure during follow-up and the number of prior CDI episodes were significant predictors of CDI recurrences. Treatment with 250 mg of CB-183 315 was associated with odds reduction of CDI recurrence by 20% when compared to oral vancomycin, after adjusting for all variables in the model.

**P2252** **Narrow spectrum penicillins and exposure to beef as risk factors for *C. difficile* infection in community. A case-control study among patients attending general practice in Denmark**

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**Objectives:** The aim of this study was to identify risk factors for *Clostridium difficile* infection (CDI) and to describe the clinical symptoms in patients who attended general practice because of gastrointestinal complaints.

**Methods:** Stool samples submitted from general practice on suspicion of gastroenteritis were analysed for bacterial, viral and parasitic gastrointestinal pathogens including *C. difficile*. A matched case-control study was conducted to reveal risk factors for CDI. Covariates investigated were primarily antibiotics, other drugs, admission to hospital, various food items, contact to animals and children <2 years. Furthermore clinical symptoms and severity of disease was evaluated. A multivariate main effects model was fitted using conditional logistic regression.

**Results:** Three hundred and fifty-five cases (*C. difficile* culture positive) and 455 controls (*C. difficile* culture negative) were included in the study. Age ranged from 0.25 to 94 years of age. Fifty percent of cases were <2 years of age. In patients  $\geq$ 2 years of age, hospitalization and beef consumption were significantly more often reported by cases compared to controls (OR 8.4; 95% confidence interval (CI) 3.1–22.8) and (OR 5.5; 95% CI 2–15.1), respectively. Phenoxymethylpenicillin, Dicloxacillin and penicillins with extended spectrum were all significantly associated to CDI (OR 14.8; 95% CI 2.7–81.7) and (OR 27.4; 95% CI 3.6–211) and (OR 9.2; 95% CI 1.9–45.4), respectively. Proton pump inhibitors were not associated to CDI. In patients  $\geq$ 2 years of age weight loss and stool frequency  $\geq$ 10 times a

day were reported significantly more often in cases compared to controls in univariate analysis (OR 2.8; 95% CI 1.5–5.1) and (OR 3.1; 95% CI 1.7–5.9), respectively.

In patients <2 years of age neither hospitalization nor antibiotics were associated to CDI. Apart from stomach ache no differences in clinical symptoms were found between cases and controls in patients <2 years of age.

**Conclusions:** This study of CDI in a community setting suggests intake of beef as a possible risk factor and reveals narrow-spectrum penicillins to be significantly associated to CDI. Analysis of clinical symptoms indicates CDI to be of clinical importance with symptoms at least as severe as gastroenteritis caused by other gastrointestinal pathogens in patients  $\geq$ 2 years of age. Data displayed a discrepancy in risk factor- and symptom profile between children <2 years of age and all other patients  $\geq$ 2 years of age.

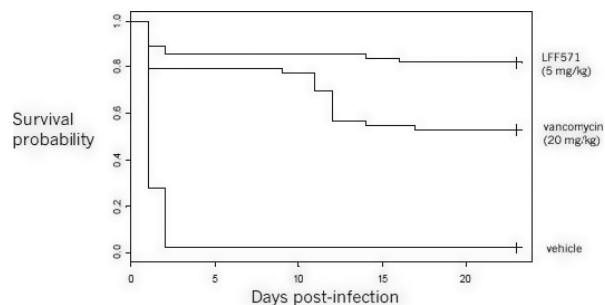
**P2253** **Efficacy of LFF571, a novel semi-synthetic thiopeptide, in a hamster model of *C. difficile* infection**

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**Objective:** LFF571 is a novel semi-synthetic thiopeptide with potent activity against a variety of Gram-positive pathogens. In vivo efficacy of LFF571 was evaluated against vancomycin in a hamster model of *Clostridium difficile* infection.

**Methods:** Infection was induced in Golden Syrian hamsters using a toxigenic strain of *C. difficile* (ATCC 43255). Treatment started 24 hours post-infection, and consisted of saline, vancomycin (20 mg/kg, PO QD), or LFF571 (5 mg/kg, PO QD). Cox regression was used to analyse survival and relapse data. Survival was right censored; animals were not observed beyond day 21. At death or end of study, cecal contents were tested for toxins A/B using the Wampole *C. difficile* TOX A/B IITM kit.

**Results:** Compilation of seven separate studies showed that LFF571 decreased the risk of death by 96% and 69% compared with saline and vancomycin, respectively ( $p < 0.001$ ). Further analysis of the pooled data indicated that the survival benefit of LFF571 treatment compared to vancomycin was due primarily to a decrease in the risk of relapse after day 7. Overall, LFF571 reduced relapse rate to 3.6% compared with 32.1% for vancomycin. Animals successfully treated with LFF571 or vancomycin had no detectable *C. difficile* toxin.



**Conclusions:** LFF571 was more efficacious, with fewer relapses, when compared with vancomycin in the hamster model of *C. difficile* infection. LFF571 is being assessed in humans for the treatment of *C. difficile* infections.

**P2254** **Epidemiological investigation of *Clostridium difficile* infection mandatory surveillance reports in patients with established renal failure in England**

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**Objectives:** Recent studies allude to *Clostridium difficile* infection (CDI) being an increasing problem in patients on Renal replacement

therapy (RRT). We review patient characteristics and trends of CDI in RRT patients, as reported to the English mandatory surveillance scheme since 2007.

**Methods:** Data including sex, age, and whether the patient had established renal failure were extracted from the CDI surveillance database.

Renal population data were obtained from the renal registry annual report 2010 (<http://renalreg.com/Reports/2010.html>) for use as a renal RRT population baseline.

Examination of patient characteristics between national CDI reports and the RRT population were made using Stata 11.

**Results:** More than 140 000 CDI cases were reported, only 1% were in patients undergoing RRT. The percentage of renal cases reported with CDI has remained constant over time, and represents ~7% of the RRT population (range 5.6–7.8%). RRT, once thought to be a driver for MRSA bacteraemia infection, now only account for 4.8% MRSA bacteraemia cases having reduced over time since 2007, however the number of RRT patients reported with CDI is 2.5 times higher (range 2.4–4.2/year) than those reported with MRSA.

Males accounted for 54% of the cases (range 58% in 2007 to 51% in 2009), this is lower than the 62% males reported for 2009 by the Renal Registry. Comparatively 41% male was seen in all CDI reports. Most renal CDI cases were in the 75–79 years age group (both sexes), consistent with the RRT population but contrary to the national CDI data, where most cases occur in the >85 years group.

A higher percentage of RRT CDI cases were repeat or relapse episodes (13.5%) compared with the national CDI data (7.9%), where completed.

**Conclusions:** Incidence of RRT and the occurrence of CDI in renal patients remained stable in England over time, with a higher number of patients affected when compared to MRSA data; however fluctuations in patient characteristics have been noted in renal CDI cases. We cannot substantiate the reports of increasing CDI in renal patients however cases have not reduced over time. With the higher repeat/relapse results investigations will be undertaken to identify whether a resistant strain or particular serotype is affecting this patient group.

Additional understanding of differences between national and renal CDI cases may be beneficial to these units, further work is planned to review patient risk factors affecting infection and look more closely at RRT population rates.

## Clostridium difficile – diagnosis, pathogenesis and epidemiology

### P2255 Diagnosis of Clostridium difficile infection – getting any easier?

G. Morgan, S. Hasnie, D.J. Brown, J.E. Coia\* (Glasgow, UK)

**Objective:** Recent work has highlighted issues with toxin enzyme immunoassays (EIA) for CDI diagnosis. We examined a range of methods to evaluate the Scottish diagnostic algorithm (SDA).

**Methods:** Two hundred and fifty-two routine unformed faecal diagnostic samples from inpatients over 65 years were tested. Assays were: Cepheid® GeneXpert PCR, Techlab® C.DIFF CHEK-60 EIA glutamate dehydrogenase (GDH), Techlab® C. difficile TOX A/B 11 EIA (toxin A and B) and vero cell cytotoxin test. All samples were cultured on Braziers' medium following alcohol shock and isolates tested for toxin production (vero cell cytotoxicity and toxin EIA). Results were used to predict diagnostic outcomes with the SDA. Retrospective casenote review was undertaken on samples positive by any assay.

**Results:** Two hundred and nineteen samples (87%) were negative; 33 samples (13%) were positive in at least one assay. Toxin was detected in the same 10 samples by EIA and cytotoxin tests. PCR yielded 27 positives, GDH EIA 26 positives. Twenty-five samples were culture positive; 21 isolates were toxigenic. Case notes were available for 26/33 positives. CDI was suspected clinically in eight cases. Four of the eight were positive by all methods; a further three were positive by PCR, two by GDH EIA, and two by culture (all toxin producers). In this limited study GDH screening followed by PCR, and GDH screening followed by toxin EIA with toxigenic culture of samples with discrepant results,

performed equally well compared with toxigenic culture of all samples (19/21 positives detected).

**Conclusions:** PCR targets DNA encoding toxin and not toxin itself therefore a positive result may reflect carriage of organisms not expressing toxin. Five PCR positives were negative in all other tests, including culture. One was clinically suspicious of CDI. GDH EIA was a good initial screen, but required a confirmatory test. Toxin EIA is not recommended as a standalone test in the SDA. However cytotoxin assay, which can be used standalone, fared no better in this limited study than toxin EIA.

The current SDA includes options to reflect local circumstances of individual laboratories. Our study generally supported use of the SDA. However sensitivity of the cytotoxin test was lower than expected. This may reflect degradation of toxin due to transit from off-site hospitals. If confirmed, this has implications for standalone use of this assay. PCR was sensitive but correlated better with toxigenic culture if an initial GDH screen was performed, as is an option in the SDA.

### P2256 Evaluation of the analytical sensitivity (limit of detection) of the BD MAX™ Cdiff assay, a new fully automated molecular assay

N. Paquette\*, C. Lehouillier, C. Roger-Dalbert (Quebec, CA)

**Objective:** The BD MAX™ Cdiff Assay performed on the BD MAX™ System is an automated in vitro diagnostic test for the direct, qualitative detection of the Clostridium difficile toxin B gene (tcdB) in human liquid or soft stool specimens from patients suspected of having a Clostridium difficile infection (CDI). The BD MAX™ Cdiff Assay is intended to aid in the diagnosis of CDI. The objective of this study is to evaluate the analytical sensitivity of the BD MAX™ Cdiff Assay.

**Methods:** The analytical sensitivity also referred as the Limit of Detection or LoD of the BD MAX™ Cdiff Assay was determined with one strain of Toxinotype 0 Clostridium difficile carrying the tcdB gene (ATCC 43255). In addition, LoD was confirmed with a second Toxinotype 0 (ATCC 9689) and with Toxinotypes IIIa (SE844) and VIII (ATCC 43598). C. difficile strains, quantified by culture, diluted into negative stool matrix were tested in 24 replicates per concentration by two different operators using three different production lots of the BD MAX™ Cdiff Assay reagents and nine different BD MAX™ systems. The LoD95% value was determined using a method that models the positive response (expressed in percentage) as a function of Log (CFU) per swab. The logistic model equation of the fitted curve allows for the computation of the LoD95% by inverse prediction using the parameter estimates and its 95% confidence interval.

**Results:** The BD MAX™ Cdiff Assay LoD95% was determined as 26 485, 15 581, 20 483 and 12 431 CFU/mL of stool for C. difficile ATCC 43255, ATCC 9689 (Tox 0), Toxinotype IIIa and VIII respectively.

**Conclusion:** The BD MAX™ Cdiff Assay LoD ranged between 12 000 and 27 000 CFU/mL of stool. This new automated molecular assay demonstrated consistent LoD95% values between all different toxinotypes.

\*The BD MAX™ Cdiff Assay is not available for sale or use in the U.S.

### P2257 Evaluation of potential inhibitory effects of biological and chemical materials on the BD MAX™ Cdiff assay, a new fully automated molecular assay

N. Paquette\*, S. Matte, R. Therrien, C. Roger-Dalbert (Quebec, CA)

**Objective:** The BD MAX™ Cdiff Assay performed on the BD MAX™ System is an automated in vitro diagnostic test for the direct, qualitative detection of the Clostridium difficile toxin B gene (tcdB) in human liquid or soft stool specimens from patients suspected of having a Clostridium difficile infection (CDI). The BD MAX™ Cdiff Assay is intended to aid in the diagnosis of CDI. The objective of this study was to evaluate the potential inhibitory effects of biological (biological

liquids and organisms) and chemical materials, which may be present in stool specimens, on the BD MAX<sup>TM</sup> Cdiff Assay.

**Methods:** Twenty-five (25) chemical or biological substances occasionally found in stool specimens were evaluated for interference with the BD MAX<sup>TM</sup> Cdiff Assay. In the first part of the study, two *Clostridium difficile* strains ATCC 43255 (Toxinotype 0) and ATCC 43598 (Tox VIII) spiked at  $2-3 \times \text{LoD}95\%$  in negative stool matrix were tested with the highest amount of each compound likely to be found in stool specimens. Negative samples were also tested. In the second part of the study microbial interference was assessed. *C. difficile* strains at  $2-3 \times \text{LoD}95\%$  were tested in presence of non toxigenic *Clostridium difficile* strain ATCC 700057 and *Escherichia coli* strain ATCC 25922 at  $\geq 1 \times 10^8$  CFU/mL (as potentially competing or interfering organisms) with the BD MAX<sup>TM</sup> Cdiff Assay. For all the different tested specimens, a 10  $\mu\text{L}$  loop was dipped into the bacterial suspension and was then placed in a BD MAX<sup>TM</sup> Cdiff Sample Buffer Tube containing the evaluated chemical material and negative stool matrix.

**Results:** Out of 25 substances tested, only hydrocortisone cream led to a slight inhibition of PCR. Some samples gave an unresolved status and needed to be repeated. This substance does not give any false-positive or false-negative results. The remaining substances tested showed no detectable interference with the BD MAX<sup>TM</sup> Cdiff Assay. Moreover no interference with high microbial load of bacterial species was observed.

**Conclusion:** Only one chemical substance gave non conforming results and required a repeat test from the same sample buffer tube. These data demonstrate the high robustness of this new automated molecular assay for the detection of *C. difficile* Toxin B gene.

\*The BD MAX<sup>TM</sup> Cdiff Assay is not available for sale or use in the U.S.

**P2258 Evaluation of the analytical reactivity (inclusivity) and analytical specificity (cross-reactivity) of the BD MAX<sup>TM</sup> Cdiff assay a new fully automated molecular assay**

N. Paquette\*, I. Paradis, C. Lehoullier, R. Therrien, C. Roger-Dalbert (Quebec, CA)

**Objectives:** The BD MAX<sup>TM</sup> Cdiff Assay performed on the BD MAX<sup>TM</sup> System is an automated in vitro diagnostic test for the direct, qualitative detection of the *Clostridium difficile* toxin B gene (tcdB) in human liquid or soft stool specimens from patients suspected of having a *C. difficile* infection (CDI). The BD MAX<sup>TM</sup> Cdiff Assay is intended to aid in the diagnosis of CDI. The first objective of this study was to evaluate the analytical specificity (cross reactivity) of the BD MAX<sup>TM</sup> Cdiff Assay towards organisms found in stools. The second objective was to challenge the BD MAX<sup>TM</sup> Cdiff Assay with a large variety of toxigenic *C. difficile* strains in the analytical reactivity study (inclusivity).

**Methods:** The analytical specificity testing has been performed with four non toxigenic *C. difficile* strains, two *C. difficile* strains of Toxinotype XI lacking tcdB gene and 30 other-*Clostridium* strains (including four strains of *Clostridium sordellii*), along with 99 closely related organisms and other pathogenic and commensal flora found in the gut and stools (representing globally 90 species). All strains were tested at a concentration of  $\geq 1 \times 10^8$  CFU/mL. Moreover, cross reactivity testing has been performed with seven viruses potentially present in stools. All viruses were tested at a concentration of  $\geq 1 \times 10^5$  PFU/mL. The analytical reactivity testing has been performed on 59 toxigenic *C. difficile* strains (including 17 Toxinotypes), present in over 21 countries, from well-characterized clinical isolates or public collections. Some hypervirulent strains (NAP1) were also tested. *C. difficile* strains were tested at a concentration corresponding to three times the LoD95% of the assay.

**Results:** None of the bacterial species or viruses used in the analytical specificity study tested positive with the BD MAX<sup>TM</sup> Cdiff Assay. The assay correctly identified all 59 *C. difficile* strains carrying the tcdB gene in the analytical sensitivity study.

**Conclusion:** No cross reactivity has been observed with the BD MAX<sup>TM</sup> Cdiff Assay even with *C. sordellii*, which Lethal Toxin is genetically similar to *C. difficile* Toxin B. All toxigenic *C. difficile* strains tested from different geographical areas were successfully detected with the BD MAX<sup>TM</sup> Cdiff Assay. This new molecular assay for the detection of *C. difficile* tcdB gene demonstrated a high level of sensitivity and specificity.

\*The BD MAX<sup>TM</sup> Cdiff Assay is not available for sale or use in the U.S.

**P2259 Evaluation of a loop-mediated isothermal amplification technique for detection of toxigenic *Clostridium difficile* strains in diarrhoeal stools**

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**Background:** The illumigene<sup>TM</sup> *C. difficile* (Meridian Bioscience) is a molecular assay, based on loop-mediated isothermal amplification (LAMP) targeting a conserved sequence of the toxin A gene. We evaluated the performances of illumigene<sup>TM</sup> for diagnosing *C. difficile* infection on diarrheal stools as a standalone test and also in algorithms where three immunoassays, the *C. diff* Quik Chek Complete<sup>TM</sup> (toxins A and B and GDH) (Techlab®), the Premier *C. difficile* glutamate dehydrogenase (GDH) and the Immunocard toxin A and B (Meridian Bioscience®), were compared as a screening method. Toxigenic culture was used as Gold Standard. Algorithms were as follows: in case of negative results for both GDH and toxin, stool was considered negative. In case of positive GDH and or positive toxin, the stool was considered positive. In case of discordant results, the illumigene<sup>TM</sup> result was used.

**Materials and methods:** Stools were from adult inpatients of the University Hospital St-Luc-UCL suffering from diarrhoea. Cultures were performed on CCFA and on CCFA with added bile salts. In case of positive culture, strains were tested for toxin production using cell cytotoxicity assay. The illumigene<sup>TM</sup> and all immunoassays were performed according to the manufacturer's instructions.

**Results:** Between October 2010 and January 2011, 296 stools were tested and 22 samples were shown to contain toxigenic *C. difficile* by toxigenic culture (prevalence: 7.4%). The sensitivity (SE), specificity (SP), PPV and NPV of illumigene<sup>TM</sup> were: 85.7%, 99.6%, 94.7% and 98.9%. Unresolved result was recorded in one instance and the sample was excluded from our calculation. Screening with *C. diff* Quik Chek Complete<sup>TM</sup> or with Premier *C. difficile* GDH combined with Immunocard and followed by illumigene<sup>TM</sup> on GDH positive/toxins A and B negative samples gave SE, SP, PPV and NPV of respectively : 81%, 99.6%, 99.4%, 98.6% and 81%, 98.9%, 85%, 98.5%.

**Conclusion:** The illumigene<sup>TM</sup> assay is a highly performant molecular test compared to existing immunoassays. The combination of a GDH assay with a very high NPV and the illumigene<sup>TM</sup> assay reduces cost while maintaining a very good sensitivity and specificity and allowing results in <2 hours.

**P2260 Evaluation of CHROMagar<sup>TM</sup> *C. difficile* (CHROMagar), a new chromogenic medium for rapid detection and direct identification (24 hours) of *Clostridium difficile* in comparison to CLO medium (bioMérieux) and Xpert *C. difficile* PCR test (Cepheid)**

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*Clostridium difficile* (CD) is the leading cause of hospital-acquired antibiotic-associated diarrhea (AAD) and colitis. Both toxins A and B of CD are responsible for around 25% of AAD and in most cases of pseudomembranous colitis.

**Purpose:** To evaluate CHROMagar<sup>TM</sup> *C. difficile* (CHROMagar), a novel chromogenic medium for rapid detection ( $\leq 24$  hours) and direct identification of *C. difficile* in comparison to Xpert *C. difficile* PCR test (GeneXpert, Cepheid) and existing selective medium (48 hours) CLO (bioMérieux).

**Methods:** CHROMagar<sup>TM</sup> *C. difficile* was firstly evaluated with 16 pure strains of CD inoculated directly onto the medium and then

incubated in anaerobic conditions. After 24 hours the plates were examined (characteristic morphology, fluorescence under UV). All the 16 strains display typical colony morphology with fluorescence production at 24 and 48 hours.

A total of 98 AAD fecal specimens collected (March–June 2011) for routine *C. difficile* analysis. On each fecal sample, toxin research was performed with Xpert *C. difficile* PCR test and culture was performed on CLO as gold standard, and on CHROMagar *C. difficile*. Plate reading was made at 48 hours for CLO medium, and at 24 and 48 hours for CHROMagar *C. difficile*.

**Results:** PCR tests were positive on 25/96 (26%) of the samples. Cultures were found positive on 24/25 of the PCR-positive samples, giving all (24/24) characteristic morphology and fluorescent colony in 24 hours with CHROMagar *C. difficile*, and typical colony aspect in 48 hours with CLO. No positive culture was recovered from any PCR-negative samples. The PCR-positive sample showing a negative culture response on medium was considered as a true positive regarding to the clinical case description (patient with colitis treated for 4 days). Sensitivity and specificity of CHROMagar *C. difficile* were 96% and 100% vs. PCR, respectively. Performances of CHROMagar *C. difficile* at 24 hours were equivalent to CLO results at 48 hours.

**Conclusion:** CHROMagar *C. difficile* is an accurate and easy to use chromogenic medium for the detection of CD. The high specificity and sensitivity of this medium, after only 24 hours of incubation, makes it an efficient tool for the direct detection and identification of *C. difficile* in fecal specimens. In parallel to the PCR solution (rapid toxin detection in 1 hour), culture remains less expensive, useful for epidemiological and antibiotic resistance survey and faster (24 hours) with a new generation of culture medium.

#### **P2261** Evaluation of two real-time PCR, two antigen tests and culture for the detection of toxigenic *Clostridium difficile*

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**Objectives:** To define the performance of four commercial tests for the detection of *C. difficile* (CD) in the stool

**Methods:** During the period of September 2010 to February 2011 stools from patients with suspicion of CD infection were collected (n = 117). We meant to retrieve an similar number of positive or negative stools. The rapid immunochromatographic tests C.DIFF Quik Chek COMPLETE® detecting toxin A and B plus the presence of CD (Techlab) and RIDA® QUICK Toxin A/B (r-biopharm) were used for antigenic testing. Two multiplex PCR Xpert® *C. difficile* (Cepheid) and RIDA® GENE CD +ToxB (r-biopharm) after stool extraction on EasyMag (bioMérieux), were used for genomic testing on the same stools. Culture using selected plates after alcohol choc on the stool were used to recover *C. difficile* but no toxigenic test on cell culture was done on stools or isolated strains. A positive stool was considered when at least three tests or the two PCR were positive.

**Results:** Fifty stools (42.7%) were determined to be positive. PCR tests showed sensitivities of 98% and 100% and specificities of 92.5% and 98.5% with Xpert and RIDA GENE, respectively. Antigenic detection of toxin A and B tests showed sensitivities of 62% and 70% and specificities of 94% and 97% with QUIK CHEK and RIDA QUICK, respectively. We also compared the utility of tests detecting only the presence of *C. difficile* in stools. Sensitivities of 100%, 90% and 90% and specificities of 89.5%, 85.6% and 85.1% were obtained for RIDA GENE CD, QUIK CHEK antigen and CD culture, respectively.

Both genomic test are highly sensitive and adequate to detect toxigenic strains in stools. Xpert CD showed, however, a slightly poorer specificity. Antigenic tests have low sensitivities missing up to 38% positive stools, with good specificities. The use of a combined test with CD antigenic detection to enhance sensitivity does not improve the overall detection as no further toxigenic strains were detected. Such a strategy lowers specificity (85.6%) leading to unnecessary treatment.

**Conclusion:** Genomic amplification tests are more adequate to diagnose CD infections. The high sensitivity enables to give a quick and reliable response in a few hours without need for repeat samples.

#### **P2262** Utility of Copan SL-solution for liquefaction of mucus in stool samples for molecular detection of *Clostridium difficile* toxin gene

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**Background:** *Clostridium difficile* infection (CDI) is the major cause of antibiotic associated diarrhea and colitis with symptoms ranging from mild, self-limiting diarrhea to pseudomembranous colitis. CDI requires a rapid and reliable testing method for optimal patient care and infection control management. Molecular amplification method is most sensitive in detection of *C. difficile* toxin(s) or the gene. Presence of excess mucous in the stool, which is often the case in setting of CDI may plug the pipette tips as well as prevent nucleic acid (NA) extraction for the molecular amplification. The Copan SL-Solution (SLS) (Copan: Brescia, Italy) liquefies mucoid specimens.

**Objectives:** To evaluate the ability of SL-Solution to liquefy mucous in stools, release and preserve *C. difficile* toxin (NA) for molecular detection.

**Methods:** Three hundred and twenty-five adult stool samples submitted for the detection of *C. difficile* toxin gene were processed as per standard laboratory operating procedure (untreated) and also pre-treated in a 1:5 ratio with SLS. Both untreated and pre-treated specimens were extracted by EasyMAG™ (bioMérieux), amplified by qualitative PCR, and measured for DNA yield by spectrophotometer.

**Results:** Of the 325 stool samples, pretreatment with SLS resulted in detection of additional 10 positives which were deemed negative without the SLS treatment. Pretreatment with SLS also prevented repeating the NA extraction with EasyMAG™ due to plugging of the pipette tips.

**Conclusion:** The Copan SL-Solution effectively liquefies muous in stools and improves NA extraction with EasyMAG™ and results in higher detection of NA. It is easy to use and eliminates the need to re-extract due to plugging of the pipettes.

#### **P2263** Detection of *Clostridium difficile* in stool by the search for glutamate dehydrogenase, evaluation of a new immunoassay test unit

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**Background:** As part of *Clostridium difficile* infection (CDI), the European guidelines recommend to make a bacteriological diagnosis within 24 hours. The diagnostic algorithms are available in two or three stages: the first step is to use a rapid test with an excellent negative predictive value and the second step is to determine the power of toxigenic bacteria. The search for the glutamate dehydrogenase (GDH) can be used in the first stage. A new unit test immunoassay Immunocard GDH *C. diff*® (Icard GDH), (Meridian France), marketed in France since June 2011 is to evaluate in this context.

**Methods:** Comparative evaluation of the two GDH tests (Icard *C. DIFF* and Quik Chek [QC GDH] [Alere, France]) and toxigenic culture as reference method. These tests were performed according to manufacturers' recommendations, the same day by two different operators and interpreted blindly. In parallel, each stool was cultured on TCCA selective medium (taurocholate, cycloserine, cefoxitin). The toxin-producing CD was investigated by cytotoxicity test done from a supernatant of a broth culture of the strain. Discordant results were checked by performing a second enzyme immunoassay and/or an enrichment culture.

**Results:** A total of 395 stools were analyzed and for 64 stools (16.4%), a strain of CD was found in culture. The prevalence of toxigenic strains was 12.4%. Compared to culture, the performance of the test Icard

GDH were for sensitivity, specificity, negative and positive predictive value, 92.3%, respectively, 97%, 98.5% and 85.7%. To test the GDH QC, they were 89.2% 99.4% 97.9% and 96.7%. The correlation of the two tests with culture was 96.2% for Icard GDH and 97.7% for QC GDH. Among 10 samples negative for culture but Icard GDH positive, two were positive with QC GDH. Enrichment culture performed on eight of them allowed to find five CD more.

**Conclusion:** In this study, the Immunocard GDH test allowed to obtain very good negative and positive predictive value for use in the new strategy for the diagnosis of CDI. This new test has also sensitivity comparable to immunoassay QC GDH test.

**P2264 Evaluation of a new rapid and simple enzyme immunoassay for detection of *Clostridium difficile* common antigen, glutamate dehydrogenase, in stool**

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**Objectives:** The objective of the study was to evaluate the performance of a new rapid test, the ImmunoCard *C. difficile* GDH (ICD GDH; Meridian Bioscience, Inc. OH), for the detection of glutamate dehydrogenase (GDH) in stool samples. The test is not intended to discriminate between toxigenic and non-toxigenic strains of *C. difficile*, but can be used in selecting those samples in need of further toxin testing.

**Methods:** The study was conducted at two sites of a large general hospital in Antwerp, Belgium (Ziekenhuis Netwerk Antwerpen, ZNA), between May and July 2011, using stool specimens from patients suspected of having a *Clostridium difficile*-associated disease. All samples were analyzed with the new rapid test, in parallel with the routinely used C. DIFF QUIK CHEK COMPLETE (QCC; TechLab), which detects both GDH and the toxins A and B.

Samples showing discrepant results between GDH and toxins with the Quik Chek Complete test and/or between both GDH analyses were cultured anaerobically on a selective *Clostridium difficile* agar (BioMérieux) for at least 48 hours. These samples were also analyzed by a commercial PCR assay (BD GeneOhm Cdiff Assay) for the presence of toxigenic *C. difficile*.

**Results:** In total, we analyzed 295 stool specimens, the results of which are summarized in Table 1.

Keeping in mind that culture results are only available for discrepant results, we may estimate the performance of the GDH assay by assuming positive cultures when both ICD GDH and QCC (GDH and toxins) are positive, and assuming negative cultures when all of the tests are negative. The sensitivity and the specificity of the ICD GDH would then be 100% and 98% respectively (as compared to 100% and 96% for the QCC GDH); the negative and positive predictive values would be 100% and 85% respectively (as compared to 100% and 78% for the QCC GDH).

ICD GDH	QCC GDH	QCC Toxins	Culture	Number of samples
NEG	NEG	NEG	ND**	250
NEG	POS	NEG	NEG	4
POS	POS	NEG	NEG	6
POS	POS	NEG	POS	18*
POS	POS	POS	ND**	17

Table 1. Test results

\*9/18 were positive with the PCR assay

\*\*ND: not determined

**Conclusion:** Our study suggests that the new ImmunoCard *C. difficile* GDH test is an accurate assay, enabling a cost-effective "first step" approach in order to separate the bulky negative samples from those that are in need of further toxin testing.

**P2265 Detection of toxigenic *Clostridium difficile*: comparison of the cell culture neutralisation, Xpert<sup>®</sup> *C. difficile*, Xpert *C. difficile*/Epi and the Illumigene<sup>™</sup> *C. difficile* assays**

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**Objectives:** Several laboratory techniques are available to detect *Clostridium difficile* (CD) in fecal specimens. Because questions have been raised about the reliability and turn-around time of the cytotoxin (Toxin B) in the cell culture neutralization assay (CCNA), a study was performed comparing the performance of CCNA with molecular assays. The detection of the toxin B gene by real time PCR (Xpert<sup>®</sup> *C. difficile*; Cepheid), and the detection of toxin A gene by loop-mediated isothermal amplification (Illumigene<sup>™</sup>; Meridian Bioscience) were evaluated. The isolation of CD in culture followed by CCNA (toxigenic culture-TC) was used as the gold standard for discrepant testing. Furthermore, we evaluated the Cepheid Xpert *C. difficile*/Epi (Xpert CD/Epi) for the detection of the epidemic NAP-1 (027/NAP/BI).

**Methods:** Two hundred prospectively collected diarrheal stool specimens were tested simultaneously by the CCNA, Xpert<sup>®</sup> CD, Xpert CD/Epi and Illumigene CD assays. Additionally, 50 retrospective stool specimens (previously positive by CCNA) were included in the study. Discrepant specimens were tested by the TC. The NAP-1 positive result was confirmed by pulsed-field gel electrophoresis and/or by sequencing of the *tdcC*. A sample was considered discrepant if even one assay was not in agreement with the other assay results. In the latter case, TC was utilized as gold standard.

**Results:** Of the 200 prospective stools tested, 10.5% (n = 23) were positive by CCNA, 17.5% (n = 35) by Illumigene CD, and 21.5% (n = 43) by both the Xpert CD and Xpert CD/Epi. Of the 50 retrospective stools, previously positive by CCNA, 94% (n = 47) were positive by Illumigene CD and 100% (n = 50) by both the Xpert CD and Xpert CD/Epi. Of the 11 discrepant specimens (negative by Illumigene<sup>™</sup> but positive by Xpert), 10 tested positive by TC and for one specimen, we were unable to isolate *C. difficile* from the stool. The Xpert CD/Epi detected a frequency of 21% NAP-1 positive CD.

**Conclusion:** The Xpert CD and Illumigene CD offer greater sensitivity and quicker turnaround time as compared to the CCNA test. The Xpert CD and the Xpert CD/Epi assays detected a higher number of toxigenic CD as compared to the Illumigene assay. 11/250 Xpert CD toxB positive and toxigenic culture positive specimens were not detected by the Illumigene assay. Some of these specimens were also CCNA and/or NAP-1 positive. The Xpert CD/Epi is useful for epidemiologic surveillance and infection control.

**P2266 Comparison of VIDAS<sup>®</sup> GDH automated immunoassay with Cepheid GeneXpert<sup>®</sup> *C. difficile* PCR assay and an in-house PCR assay for GluD, for the detection of *C. difficile* in faecal samples**

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**Objectives:** To compare a new automated immunoassay, VIDAS<sup>®</sup> GDH, with the Cepheid GeneXpert<sup>®</sup> *Clostridium difficile* toxin Polymerase Chain Reaction (PCR) assay and an in-house PCR assay for the GluD gene Glutamate dehydrogenase (GDH) of *C. difficile*, for the laboratory diagnosis of *C. difficile* infection (CDI).

**Methods:** Three hundred diarrhoeal samples routinely submitted for *C. difficile* testing were collected (100 at each of three test sites in Leeds, Berlin and St Etienne). The commercial assays (VIDAS<sup>®</sup> and GeneXpert<sup>®</sup>) were performed, as per manufacturer's instructions, at the respective sites, except for GeneXpert<sup>®</sup> in Berlin, which was performed in Leeds. All samples were shipped at -20°C to Leeds for testing with an in-house GluD PCR. Samples that were positive by the VIDAS<sup>®</sup> assay but negative by GluD PCR were re-tested using the GluD PCR, cultured onto ChromID<sup>®</sup> *C. difficile* agar (bioMérieux, France) and Brazier's agar (Oxoid, UK) and tested using a CE marked GDH assay (Alere). If samples were positive by repeat GluD PCR

testing, or negative on repeat GluD PCR testing but positive by the Alere assay and/or culture, the sample status was determined to be GDH positive. Results were analysed both before and after repeat testing.

**Results:** All 300 samples gave a result with the VIDAS<sup>®</sup> GDH assay, 296 gave a result with the GeneXpert assay (two invalid samples), and 280 gave results using the in-house GluD PCR assay (20 insufficient samples). The VIDAS<sup>®</sup> GDH assay was 98.5% (95% CI 94.2–99.7) sensitive and 88.1% (95% CI 81.8–92.5) specific compared with the GeneXpert<sup>®</sup> PCR assay, and 92.6% (95% CI 86.5–96.2) sensitive and 91% (95% CI 84.8–94.9) specific compared with GluD PCR. The Pearson's correlations were 0.86 and 0.84 for GeneXpert<sup>®</sup> PCR and GluD PCR, respectively. After repeat testing of 11 discrepant samples as above (total n = 278), the VIDAS<sup>®</sup> GDH assay remained 92.6% (95% CI 86.5–96.2) sensitive but specificity increased to 95.7% (95% CI 90.6–98.3), with a Pearson's correlation of 0.89.

**Conclusions:** The VIDAS<sup>®</sup> GDH assay has comparable accuracy to the GeneXpert<sup>®</sup> *C. difficile* PCR assay and our in-house GluD PCR assay. The VIDAS<sup>®</sup> GDH assay could be an option as a first line test in a *C. difficile* testing algorithm. The optimal combination of tests depends on the clinical question to be answered.

#### **P2267** One-year of loop-mediated isothermal amplification and toxigenic culture for *Clostridium difficile* diagnosis

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**Objectives:** We have evaluated our 1-year experience of toxigenic culture (TC) and loop-mediated isothermal amplification (LAMP) for routine diagnosis of *Clostridium difficile* infection (CDI).

**Methods:** Loop-mediated isothermal amplification (LAMP, Illumigene; Meridian Bioscience Inc., Cincinnati, USA), targeting a highly conserved 204 bp sequence within the *tcdA* region of the *C. difficile* pathogenicity locus (PaLoc), is a six primer nucleic acid amplification technique (NAAT), with amplification at 63°C. This 1-hour test was introduced in clinical routine in October 2010 for CDI diagnosis. PCR ribotyping according to Stubbs et al. was used to investigate any variation in performance characteristics due to genotype. Recurrent CDI was defined as positive test result >10 days past initial positive test.

**Result:** From 1 October 2010 to 30 September 2011 a total of 2242 clinical specimens were analyzed by LAMP and TC. After removing 131 duplicates (positives 21 and negatives 110), 346 specimens turned positive in LAMP. This presented a positivity rate of 18%. Thirty-one (8%) isolates were tested non-toxicogenic from positive culture and none of these were positive in LAMP. Recurrent episodes were 21%. Two samples were invalid and negative in TC and an additional four bloody specimen were initially invalid but positive in rerun after dilution. In total LAMP had a sensitivity of 0.96 (346/362); CI 0.93–0.97 and a specificity of 0.98 (1853/1880); CI 0.97–0.99. Positive predictive value (PPV) was 0.93 and negative predictive value 0.99. Of 167 PCR ribotyped isolates, 15 LAMP negative were evenly distributed among nine different ribotypes. Four isolates belonged to toxin A negative ribotype 017 but were positive in LAMP.

**Conclusion:** LAMP is a rapid and easy-to-use test system that does not require PCR laboratory facilities. Our 1-year evaluation showed high sensitivity (96%) and specificity (98%) and considering a positivity rate of 18% the PPV of 93% is up to this point reassuring. Presenting the clinician the test result within a few hours can effectively guide therapy.

#### **P2268** A CE-marked DNA molecular assay on the Abbott m2000 for the detection of toxigenic and hypervirulent strains of *C. difficile*

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**Objectives:** *Clostridium difficile* (*C. difficile*) is a prevalent health care-associated infection and the major cause of hospital-associated diarrhea.

Because it is easily spread by contact, early detection is critical to reducing hospital stays and costs. We have developed a cost-effective, rapid and sensitive automated molecular diagnostic test, the CE-marked IMDx *C. difficile* for Abbott m2000, to address the growing unmet need for early *C. difficile* detection. This assay is the first fully automated assay for detection of toxigenic *tcdA*, *tcdB*, and *tcdB*-variant genes in the NAP1/B1/027 and 1470 strains of *C. difficile*.

**Methods:** IMDx has developed NGENix<sup>™</sup>, a proprietary bioinformatics platform for designing multiplexed, real time PCR-based assays. NGENix<sup>™</sup> identifies unique regions within sequenced genomes, iteratively designs sets of primers and probes, assesses the thermodynamics of the DNA multiplex, and determines potential cross-reactivity to other genomes. We used NGENix<sup>™</sup> to design oligonucleotide solutions to the toxigenic *tcdA*, *tcdB*, and *tcdB*-variant genes in the NAP1/B1/027 and 1470 strains of *C. difficile* and then assessed analytical performance on the Abbott m2000 system.

**Results:** Direct analysis of swabs from 251 stool samples demonstrated 95.7% sensitivity and 99.5% specificity. No cross-reactivity was observed in a panel of 58 common enteric viruses (tested at  $\geq 1 \times 10^6$  TCID50/mL) and microorganisms (tested at  $\geq 1 \times 10^5$  CFU/mL). No interference was seen in the presence of 22 endogenous and exogenous substances, evidenced by non-significant changes in *C<sub>i</sub>* and *MR* values. The assay is highly specific for *C. difficile*;  $\geq 95\%$  of *tcdA*, *tcdB*, and *tcdB*-v sequences tested using synthetic constructs that represent different genotypes and possible primer and probe mismatches were identified. Precision studies demonstrated >98% concordance across all variables and inter-assay %CV range for *C<sub>t</sub>* values fell between 1.7% and 4.3%. Additional performance characteristics are shown in Table 1.

**Table 1.** IMDx *C. difficile* for Abbott m2000 Performance Characteristics

<b>Limit of Detection</b>	33.87 CFU/mL for <i>tcdA</i> /NAP1 (95% CI: 33.84 - 33.90)
	35.74 CFU/mL for <i>tcdB</i> /NAP1 (95% CI: 35.71 - 35.77)
	9.5 CFU/mL for <i>tcdA</i> /1470 (95% CI: 9.48 - 9.53)
	9.3 CFU/mL for <i>tcdB</i> /1470 (95% CI: 9.26 - 9.35)
<b>Assay Dynamic Range</b>	10 <sup>2</sup> – 10 <sup>7</sup> CFU/mL (6 logs) for NAP1 strain 10 <sup>1</sup> – 10 <sup>7</sup> CFU/mL (7 logs) for 1470 strain

**Conclusion:** The IMDx *C. difficile* for Abbott m2000 assay can analyse 94 patient samples in under 4 hours providing rapid, accurate results that can enable physicians to make more informed therapeutic decisions. The assay has been CE-marked.

#### **P2269** Development of a simple novel sample preparation for the rapid detection of *Clostridium difficile* in stool with LAMP-BART

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**Background and objective:** *Clostridium difficile* (Cd) is a Gram positive anaerobic, spore-forming bacillus that causes *C. difficile* associated disease through the expression of toxin genes *tcdA* and *tcdB*. Molecular methods are proving to be more effective and faster in detecting Cd than cytotoxic cell culture or immunoassays.

Current molecular tests rely upon either a complex manual/robotic extraction or a multi-step protocol involving a 200–700-fold dilution of the sample to prepare DNA for detection. Convenient extraction methods, such as those provided by fully integrated platforms, are often too expensive for many clinical settings; hence there is a need for a less expensive approach that offers similar ease of use. Here we describe a rapid (10 minutes), very low cost, two step sample preparation method with minimal hands on time requiring no specialist instruments, which produces eluate with only a 70-fold dilution factor that can be used to directly reconstitute lyophilised molecular amplification reagents.



**Methods and results:** A swab taken from stool sample is sealed inside a low cost device containing amplification buffer and other proprietary components that is then incubated for 10 minutes. Eluate from the device is then pipetted onto lyophilised molecular amplification reagents, in this case the method known as LAMP-BART was employed. A preliminary study performed on 80 stool samples previously determined by PCR to be positive (47) or negative for Cd (33). The method showed 100% correlation with the PCR status.

**Conclusion:** The novel sample preparation provides a rapid and sensitive detection method for Cd from stool with minimal hands-on requirements, but without the cost and complexity of fully integrated molecular devices or robotic systems. This system is ideal for near patient testing in particular.

**P2270 Evaluation of RIDA<sup>®</sup>GENE *Clostridium difficile* and Toxin A/B (R-Biopharm) real-time PCR for diagnosing *Clostridium difficile* infections**

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**Objective:** Quick and reliable diagnosis of *Clostridium difficile* infections is essential for optimal treatment of infected patients. The RIDA<sup>®</sup>GENE *Clostridium difficile* and Toxin A/B (R-Biopharm) test is a real time PCR test that detects fragments of specific *C. difficile* genes (RIDA<sup>®</sup>GENE *Clostridium difficile*) and its toxins A and B (RIDA<sup>®</sup>GENE toxin A/B) in stools. The objective of this study was to evaluate these two tests.

**Methods:** The study was carried out on 462 consecutive, non-repetitive diarrheic stool samples. DNA extraction was done with the Maxwell<sup>®</sup> 16 system (Promega). The RIDA<sup>®</sup>GENE *Clostridium difficile* test was compared to culture on selective medium (TCCA: brain heart infusion agar supplemented with defibrinated horse blood, taurocholate, cycloserine and cefoxitin) and the RIDA<sup>®</sup>GENE toxin A/B test to a cytotoxicity test (CTA) and toxigenic culture (CT). CTA was carried out by inoculating a stool filtrate on MRC-5 cells. If a culture was positive, the toxigenicity of the strain (CT) was directly examined from a 5-days supernatant broth culture using the cytotoxicity test. For discrepant results, samples were controlled with an enriched culture (brain-heart infusion broth, taurocholate, cycloserine and cefoxitin) or a new amplification of a new extract.

**Results:** Culture was positive for 57 samples (12.3%), CTA and CT were positive for 22 (4.8%) and 43 (9.3%) samples, respectively. Real-time PCR for detecting *C. difficile* (RIDA<sup>®</sup>GENE *Clostridium difficile* test) and toxin genes (RIDA<sup>®</sup>GENE toxin A/B test) gave invalid results in 51 (11%) and 53 (11.5%) cases, respectively. Invalid results dropped to 1.1% and 1.3% after 1/10 dilution according to the manufacturer's instruction. After resolving discrepancies, the sensitivity and specificity of RIDA<sup>®</sup>GENE *Clostridium difficile* were 96.6% (CI95% 87–99.4) and 95% (CI95% 92.2–96.8) using culture as the gold standard and that of RIDA<sup>®</sup>GENE toxin A/B was 85.7% (CI95% 62.6–96.2) and 93.8 (CI95% 91–95.8) compared to CTA and 81.4% (CI95% 66.1–91.1) and 97.6% (CI95% 95.4–98.8) compared to CT.

**Conclusion:** The RIDA<sup>®</sup>GENE *Clostridium difficile* and Toxin A/B test is a quick and sensitive test for detecting toxigenic strains of *C. difficile* in stool samples.

**P2271 Evaluation of real-time PCR in a two-step diagnostic algorithm for diagnosis of *C. difficile* associated diarrhoea**

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**Objectives:** The aim of this study was to evaluate the effective utilization of PCR assay in toxin A/B-negative patients.

**Methods:** We performed EIA for *C. difficile* toxin A/B, and real-time PCR assay for the *tcdB*-gene was subsequently performed in toxin-negative patients suspected as CDAD between May 2011 and September 2011.

**Results:** During the study period, 1331 stool specimens were examined for toxigenic *C. difficile* infection by using toxin assay and

131 (9.8% of 1331) of them were positive. One hundred and fifty-five toxin-negative specimens of patients suspected as CDAD were subsequently performed by real-time PCR assay and 49 (31.6% of 155) were positive. A total of 180 specimens (13.5% of 1331) contained toxigenic *C. difficile*, and 27.1% (49/180) was detected by real-time PCR assay. Except asymptomatic carriers, 117 patients were diagnosed as CDAD. The median age of these patients was 66.5 ± 14.7 years and 55.6% were males. One hundred and sixteen (99.1% of 117) had healthcare-associated CDAD and one (0.9% of 117) had community-associated CDAD. Ninety (76.9% of 117) were toxin-positive CDAD and 27 (23.1% of 117) were toxin-negative CDAD. Comparing between toxin-positive CDAD and toxin-negative CDAD, there were no significant differences in underlying conditions, medication history and severity of CDAD. The rates of clinical failure and recurrence in toxin-negative CDAD were higher than that in toxin-positive CDAD, but there were no significant differences (Table 1).

Table 1. Demographic and clinical characteristics of CDAD according to results of toxin A/B

Characteristic	Toxin-positive CDAD (n=90)	Toxin-negative CDAD (n=27)	P-value
Male (%)	51 (56.7)	15 (55.6)	0.545
Age in years, mean (SD)	66.8 ± 14.3	65.7 ± 16	
Hospitalization (%)			0.346
On admission	41 (45.6)	21 (77.8)	
Length of hospital stay (Day)	22.8 ± 22.2	20.1 ± 15.2	
Underlying diseases or comorbid conditions (%)			
Diabetes mellitus	21 (23.3)	6 (22.2)	0.586
Cardiovascular disease	21 (23.3)	7 (25.9)	0.511
Chronic kidney disease	4 (4.4)	2 (7.4)	0.591
Malignancy	29 (32.2)	7 (25.9)	0.356
Recent operation	24 (26.7)	8 (29.6)	0.579
Nasogastric tube	11 (12.2)	3 (11.1)	0.488
Previous CDAD	1 (1.1)	1 (3.7)	0.339
Concurrent medication history (%)			
Received antibiotics therapy within 14 days prior to onset of CDAD	81 (90.0)	24 (88.9)	0.270
Use of Pepsin pump inhibitors	19 (21.1)	5 (18.5)	0.594
Use of chemotherapy	14 (15.6)	5 (18.5)	0.478
Positive in stool exam for RBC or WBC	36 (40)	13 (48.1)	0.456
CDAD (%)			
Severity			0.883
Mild to moderate	61 (67.8)	15 (55.6)	
Severe	29 (32.2)	12 (44.4)	
Clinical response			0.381
Clinical failure	9 (10)	3 (11.1)	
Recurrence	18 (20)	6 (22.2)	0.326

**Conclusions:** In this study, subsequent performance of PCR assay improved the rate of diagnosis for CDAD in toxin-negative patients. And clinical states in CDAD were similar regardless of result of toxin assay for *C. difficile*.

**P2272 Diagnosis of *Clostridium difficile*: real-time PCR detection of toxin genes in faecal samples is more sensitive compared to toxigenic culture**

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**Objective:** The aim of this study was to evaluate four DNA-amplification methods detecting toxigenic *Clostridium difficile* (CD), including CD with PCR ribotype 027 (CD027), directly from faecal samples. Thereby to establish a rapid primary diagnostic test of CD based on the evaluation.

**Methods:** A total of 300 faecal samples from 284 Danish hospitalized patients with diarrhoea were included consecutively from mid February to the beginning of April 2011. CD was detected routinely by anaerobic culture on cycloserine cefoxitin fructose agar for 48 hours. Genotypic toxinprofiling by PCR and PCR ribotyping were performed on CD culture positive samples. In parallel, the samples were analysed for toxigenic CD by (i) an "in-house" multiplex Real-time (RT) PCR two-step algorithm and three commercial DNA-amplification methods, (ii) Illumigene (R) *C. difficile* [Meridian Bioscience], (iii) PCRFast (R) *C. difficile* [Simoco Diagnostics] and (iv) Xpert (R) *C. difficile* [Cepheid]. (i) and (iv) detects presumptive CD027; (ii) and (iii) detects *tcdA/B* only.

**Results:** Assay (i) and (iv) were significantly more sensitive compared to toxigenic culture. The concordance between (i) and (iv) was 97.3% (292/300). Re-culture and/or prolonged incubation time (3–7 days) of

the culture negative, but a) and (ivd) CD positive samples, increased the culture positive rate by 29% (from 38 to 49/300). Compared to initial culture as the reference standard, the re-culture data resulted in an increased positive predictive value (>80%) and specificity (>95%) for all assays, although a reduced negative predictive value (NPV) (>90%) and sensitivity (63–95%) of all but (iv) which had a NPV and sensitivity of 100%. RT PCR inhibition was <1% (0 and 2/300) in a) and d) respectively that is, in Bovine Serum Albumin (BSA) optimized assays. In contrast, PCR inhibition was 13% (38/300) in (iii) not containing BSA. Preliminary results from PCR ribotyping showed 94% and 97% concordance of (i) and (iv) respectively for the detection of CD027.

**Conclusion:** Assay (i) and (iv) are sensitive diagnostic methods and their high concordance suggests that (i) and (iv) CD positives, but culture negative faecal samples are true positives. Both methods are usable as rapid primary diagnostic tests for toxigenic CD and for presumptive identification of CD027. The current toxigenic culture method is sub-optimal for primary diagnostics of CD. BSA efficiently reduces PCR inhibition and could be considered included in PCR protocols for detection of CD genes in faecal samples.

**P2273** Evaluation of the DNA amplification assay Illumigene™ system for the diagnosis of *Clostridium difficile* infection

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**Objective:** *Clostridium difficile* variant strains in the genetic region Pathogenicity Locus (PaLoc) have been involved in *C. difficile* - associated disease (CDI) and outbreaks in hospital settings. The increased incidence and severity of CDI in the last decade, particularly in North America and Europe, have brought renewed focus on the most appropriate method to detect *C. difficile* and/or its toxins in stool.

The aim of this prospective study was to evaluate the diagnostic performance of the gene amplification system Illumigene™ *C. difficile* (Meridian Bioscience, USA), targeting a fragment of the toxin *C. difficile* A gene (*tcdA*), in routinely investigation as compared to toxigenic culture and Immuno-Chromatographic assay (IC) for toxin A/B and glutamate dehydrogenase (GDH).

**Methods:** Analytic sensitivity was determined by using experimentally seeded samples with known amounts of reference *C. difficile* strains VPI10463 (ToxA+ ToxB+), ATCC 70057 (ToxA- ToxB-). Analytic specificity was tested by using the DNA extracted from faecal samples containing *Salmonella* spp., *Helicobacter pylori*, *Staphylococcus aureus*, *Giardia intestinalis*, *Entamoeba coli*, *Entamoeba dispar*, *Blastocystis hominis*, as the template.

A total of 201 faecal samples from 189 hospitalized patients with suspected CDI, collected over a 3 months period, were investigated by Illumigene, toxigenic culture and IC (C.DIFF QUIK CHEK Complete: TechLab, USA) for the presence of toxin A/B and GDH.

Faecal samples with discordant results were analyzed by a duplex PCR for the detection of *tcdA/tcdB* genes.

**Results:** For reference strains a detection limit of 5 and 1000 CFU/g for Illumigene and for IC, respectively, was observed. DNA from the enteropathogenic bacteria other than *C. difficile* and from parasites was not detected by Illumigene. Among 201 analyzed samples, 70 were positive and 131 negative, based on the combination of the results of the different assays. Of the 131 negative samples, 127 were negative with all methods (concordance 96.9%) and four were positive by the GDH assay alone. Forty-three samples were positive for all assays with a 61% concordance. Results of discordant samples showed a 100% agreement with Illumigene.

**Conclusion:** The Illumigene system showed sensitivity and specificity comparable to those of the toxigenic culture and IC. It is currently advantageously applied in our laboratory: results are available within 1 hour and a specific antibiotic therapy can be promptly administered to the patients.

**P2274** Impact on patient length of stay and cost-effectiveness of rapid molecular testing for *Clostridium difficile*

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**Objectives:** Xpert *C. difficile* PCR is a rapid molecular assay for the detection of *C. difficile*. Previous studies have shown it to be reliable and accurate as compared to CCNA and clinical assessment in the diagnosis of CDI. In this study we aim to assess the cost-effectiveness and impact of its routine use for testing for *C. difficile* infection (CDI) on patient length of stay in comparison to a conventional diagnostic method (CCNA) in an acute hospital setting.

**Methods:** In March-September 2011, Xpert *C. difficile* PCR was introduced as the routine test used on all patients with suspicion of CDI in Singleton and Morriston Hospitals, Swansea. TAT from sample collection to result and length of stay (LOS) were compared for 168 (83 negatives, 85 positives) prospective patients tested by PCR and 161 (82 negatives, 79 positives) control patients tested by cytotoxin neutralisation assay (CCNA). Test results were systematically reported to the respective wards as soon as they became available. Both tests were costed using a micro-costing bottom-up approach including costs for test materials, staff time, capital, overhead cost and waste from repeat samples due to false negative results. A cost-effectiveness analysis was undertaken with regards to LOS.

**Results:** No significant differences were found in the patient demographics of the four groups. Average time to result for PCR samples was 1.6 hours compared to 46.54 hours for CCNA negatives and 22.45 hours for CCNA positives. The delay in the availability of CCNA results was reflected in an on average 2.6 days longer hospital stay for positive CCNA patients in comparison to positive PCR patients (6.9 days for negative patients). Based on the micro-costing, CCNA as a test costs £9.45, PCR costs £35.99 per reportable result. The incremental cost of PCR per bed day saved is £6. Considering the cost of a hospital bed day, the reduction in LOS would lead to a cost saving of up to £904 for every patient with suspected CDI in Swansea hospitals if samples were to be tested with PCR instead of CCNA in the future.

**Conclusion:** The routine use of a rapid molecular test for *Clostridium difficile* in an acute hospital setting produced quick results that were well received by clinicians and nurses and led to a decrease in LOS compared to CCNA control patients. This could result in considerable savings through reduced excess inpatient days despite the fact that costs of PCR testing per sample are higher than costs of CCNA.

**P2275** Comparison of Illumigene® *C. difficile* and Geneohm™ Cdiff assays on glutamate dehydrogenase positive faecal samples

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**Objectives:** To compare the new Illumigene® *C. difficile* assay molecular assay using loop-mediated amplification (LAMP) technology which targets a conserved 204 bp sequence of the *tcdA* gene of *Clostridium difficile* with the BD GeneOhm Cdiff assay which targets the *tcdB* gene.

**Methods:** Over a 2 months period spanning late February–late April 2011 we collected faecal samples that were positive with the C. Diff Chek™-60 kit (GDH). The tests were done every afternoon and positive samples were then frozen until the next morning. Friday samples were frozen over the weekend. The GeneOhm and Illumigene assays were carried out according to manufacturer's instructions. A further 52 GDH negative samples were collected and tested with the Illumigene assay.

**Results:** Total number of GDH positive faecal samples tested by Illumigene and GeneOhm was 111. The "gold standard" was a positive result from all three tests. Sixty five samples (58.55%) were positive by all three methods. Forty four (39.6%) were negative by both Illumigene and GeneOhm, and one was positive by GDH and GeneOhm, and another by GDH and Illumigene. The sample that was GeneOhm positive but Illumigene negative was cultured and grew *C. difficile*. The

*C. difficile* underwent PCR for toxin profiling and was negative for the *tcdA*, *tcdB* and *CDT* genes. This result was classed as a false positive for the GeneOhm assay as the cultured *C. difficile* was non-toxinogenic. The faeces of the second discordant result was not cultured but it was also classed as a false positive as it was not positive by both PCR assays. There was 98.2% correlation between the two assays which had exactly the same sensitivity of 100% and specificity of 98%. The PPV and NPV for both were 98.48% (91–99%) and 100% (92–100%), respectively.

Of the other 52 GDH negative samples that were collected and tested with the Illumigene assay, 49 tested negative and three were invalid. The three invalid samples all contained blood.

**Conclusion:** In summary, the Illumigene® *C. difficile* and the GeneOhm™ Cdiff were shown to be comparable PCR assays for the presence of *C. difficile* in faecal specimens. The Illumigene machine had a very small footprint and the only extra equipment necessary for the assay were a vortex mixer and a heating block. The sample preparation for both assays were similar, however the Illumigene had a simplified protocol and shorter assay time which would make it more adaptable and easier to implement into a routine laboratory setting.

**P2276** An alternative protocol for toxigenic *Clostridium difficile* detection: cheap, sensitive, labour friendly and timely enough?

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**Objectives:** Current diagnostic algorithms in detecting toxigenic *Clostridium difficile* (CDIF) tend to be costly and labor intensive. We explored the performance and potential role of innovative culture based and molecular techniques in a low prevalence setting.

**Methods:** During September and October 2011, all stool samples for CDIF detection were included. Standard protocol (protocol 1) for toxigenic CDIF uses direct GDH and toxin A/B detection (Techlab C. diff Quik Chek Complete) (TL) followed by selective bloodagar (Biomérieux CLO) (BA) inoculation and maximum 72 hours of anaerobic incubation. Growth of suspect colonies from direct toxin A/B negative samples initiates a repeated toxin test on these colonies. An alternative protocol (protocol 2) without direct toxin detection but with inoculation on a chromagar (Biomérieux ChromID CDIF) (CA) with anaerobic incubation for maximum 48 hours was set up. Only when growth of suspect (black) colonies on CA is observed, a direct real time molecular toxin detection (Cepheid Xpert *C. difficile*) (GX) is performed on the feces sample detecting toxin B, binary toxin and *tcdC* deletion within 1 hour. Cost per test for TL, BA, CA and GX is respectively 14, 1, 1.5 and 40; estimated hands on time per test respectively 5, 1, 1 and 3 minutes.

**Results:** Toxigenic CDIF was detected in 12/192 (6.3%) samples. Sensitivity was 67% (8/12) for protocol 1, 83% (10/12) for protocol 2. Using protocol 1 5/8 toxigenic CDIF were detected within day, 2/8 after 2 days and 1/8 after 3 days. Using protocol 2 8/10 toxigenic CDIF were detected after 1 day, 2/10 after 2 days. Calculated cost/sample was 16 for protocol 1, 4 for protocol 2. Estimated hands on time/sample was 6.3 minutes for protocol 1, 1.2 minutes for protocol 2.

**Conclusion:** Using a chromogenic agar with molecular toxin confirmation is a reliable, cost and labor efficient algorithm for detection of toxigenic CDIF especially when CDIF prevalence is low. Although a minimum time to detection of 1 day has to be taken into account the sensitivity gain is substantial.

**P2277** Evaluation of the GenoType® Cdiff for detection of *Clostridium difficile*-DNA and the multiplexed identification of toxins and different ribotypes from stool specimens

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**Objectives:** The purpose of this study was to evaluate the new PCR based *Clostridium difficile* (CD) assay GenoType® Cdiff (Hain

Lifescience, Nehren, Germany). This assay is able to identify *Clostridium difficile*, toxins A and B, the binary toxin *cdtA/B*, and the highly pathogen and virulent ribotypes 078, 126 and 027. The detection is done in a line probe format (DNA-strip).

**Methods:** DNA isolation from stool was performed with an automated nucleic acid purification instrument (GenoXtract) and the GXT Stool Extraction Kit (Hain Lifescience). The GenoType® Cdiff assay was performed according to manufacturer's instructions.

**Results:** One hundred and seventy-five stool samples positive in the Glutamate dehydrogenase (GDH) antigen test (C.diff Check™-60-EIA; Techlab, Blacksburg, VA, USA) were compared to results from an EIA for the detection of toxin A and B (Premier™ TOXINS, A&B, Meridian, Saco, ME, USA) performed on cultured CD colonies and direct from stool. EIA based toxin detection direct from stool had a sensitivity of 73% and a NPV of 29%.

In 167 GDH positive and culture positive stool specimens *C. difficile* was confirmed in 161 cases by PCR (sensitivity 96%). Eight GDH positive stool specimens remained negative when cultured.

One hundred and fifty-two samples were congruent positive with PCR and tox EIA (culture + direct testing), 17 were congruent negative and four only positive in the EIA. Two samples were excluded (sensitivity toxin 97%, specificity 100%, PPV 100%, NPV 81%).

The GenoType® Cdiff assay was able to identify ribotype 027 in nine specimens and in two specimens 078/126.

**Conclusions:** The GenoType® Cdiff assay for the direct detection of *Clostridium difficile* and major ribotypes from stool shows rapid, sensitive and specific results. The DNA isolation, amplification and detection is automated. The turnaround time (including hands on time) is ~4–5 hours. The assay provides more information (ribotypes, toxins, binary toxins and Moxifloxacin resistance) as any presently available commercial CD test.

**P2278** Improved *C. difficile* detection in a community hospital in Israel by implementing glutamate dehydrogenase and molecular technology-based algorithm

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**Objectives:** *Clostridium difficile* is a Gram positive spore forming rod that grows anaerobically. Strains of *C. difficile* that produce the toxins A (*tcdA*) and B (*tcdB*) are known to be the causative agents of *C. difficile*-associated diarrhea (CDAD), antibiotic associated diarrhea and pseudo membranous colitis (PMC).

Accurate and rapid diagnosis of *C. difficile* is essential for patient management particularly in an older population, and infection control. New *C. difficile* guidelines were recommended by ESCMID (December 2009), SHEA-IDS (May 2010) and ASM (September 2010). In our laboratory we adopted the new guidelines in May 2011 (the ASM two step algorithm: glutamate dehydrogenase [GDH] + toxin followed by molecular confirmation). The aim of this study is to have a look back on the utility and benefit of this new algorithm.

**Methods:** In 2009 and 2010 diarrheal stool samples were collected from symptomatic patients as part of routine clinical care at the E. Wolfson Medical Center in Israel. Samples were tested by ELISA Premier™ Toxins A&B (Meridian Bioscience). As of May 2011 the diarrheal stool samples were screened by C. diff Quik-check completeR (TechlabR). Positive results for either one of the tests – GDH or TOX- were followed by Illumigene (Meridian Bioscience), a molecular method that served as a confirmatory test (LAMP).

**Results:** In 2009 a total of 655 patients (59 pediatric, 596 adult) were tested, of which 47 cases were positive (7%). In 2010 a total of 608 patients (38 pediatric, 570 adult) were tested, of which 51 cases were positive (8%). In 2011 a total of 658 (39 pediatric, 619 adult) patients were tested, of which 86 cases were positive (13%). The mean age of patients over 2009–2011 was similar.

**Conclusions:** While the number of tests performed to look for *C. difficile* over 2009–2011 did not increase, suggesting a steady incidence of *C. difficile*, there was a significant rise in the rate of positive tests in 2011 compared to 2009 (OR = 1.94, p-value <0.001) and 2010

(OR = 1.64, p-value = 0.004). No significant rise was found between 2009 and 2010. These findings indicate that the new adopted algorithm (using the Illumigene molecular assay for confirmation), albeit costly, is more sensitive than the former one for the detection of *C. difficile*. It leads to a rapid and accurate diagnosis of *C. difficile* resulting in better patient-care and infection control. It would probably be found in the near future to be cost-effective.

#### **P2279** Impact of a rapid diagnosis on the management of patients suspected of *Clostridium difficile* infection

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**Objectives:** *Clostridium difficile* (CD) is a major agent responsible for healthcare-associated diarrhea. Rapid diagnosis is essential for patient's management and implementation of infection control measures. Our objective was to assess the changes in patient's management after implementing a rapid diagnosis of *C. difficile* infection (CDI) by PCR.

**Methods:** A prospective time-series study comparing two 3-month periods was performed in a 750-bed university-affiliated hospital. During P1 CD diagnosis was based on both the cytotoxicity assay and the toxigenic culture and during P2 the diagnosis was performed by real time PCR (Xpert *C. difficile*, Cepheid). During these two periods, information on isolation days and empiric treatment were collected among patients suspected of CDI. CD lab results were reviewed daily, ward rounds were made to determine isolation days, and charts reviewed for diarrheal symptoms and treatment. The following criteria were used to assess quality of patients' management:

1 Time for result restitution and frequency of repeat testing within 7 days,

2 For patients with CDI: time elapse between stool collection and beginning of treatment, mortality at D10 and D30,

3 For patients without CDI: frequency and length of preemptive (empiric) treatment for *C. difficile*.

**Results :** Seven hundred and thirty-three stool samples (P1 n = 359 and P2 n = 374) were studied: 36 (10.0%) were positive during P1 and 47 (12.6%) during P2. Time for result restitution was 75 ± 62 and 15 ± 15 hours for P1 and P2, respectively (p < 0.001).

Frequency of redundant stool samples within 7 days was lower in P2 compared to P1 (7.4% vs. 15.8%, p = 0.02).

Patients with CDI were more frequently treated by vancomycin or metronidazole during P2 (93.3% vs. 80.8%, p = 0.08) and treatment was started earlier (0.49 ± 0.5 day vs. 2.0 ± 1.7 day, p < 0.001) as compared to patients during P1. Crude mortality at D10 and D30 was not significantly different during the two periods but length of hospital stay following the diagnosis of CDI was longer in P1 as compared to P2 (median : 10.5 days vs. 8 days, p = 0.05).

Empiric therapy among patients without CDI decreased from 15.8% during P1 to 7.4% during P2 (p = 0.0007). Number of unnecessary treatment-days was 228 and 65 for P1 and P2, respectively.

**Conclusion:** A rapid CDI diagnosis based on PCR impacts positively on patient care.

#### **P2280** Molecular characterisation of *Clostridium difficile* toxin genes of Bulgarian strains

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**Objectives:** The aim of the study is the application of Real time PCR methods for detection of *tcdA*, *tcdB* and *cdtA/cdtB* genes in *C. difficile* clinical strains from Bulgarian hospitals.

**Methods:** During the period of November 2008 to March 2010, 65 stool samples from patients of nine hospitals with mild to severe enterocolitis and history of previous antibiotic treatment were analyzed. The patients were selected according to the ECDC accepted criteria.

Isolation and identification of *C. difficile* was performed by standard microbiological techniques and latex-agglutination test (Cultured, BD, USA). Toxin A and B production was analyzed by Immuno Card Toxins A&B-EIA (Meridian, Bioscience, USA). Bacterial DNA was isolated by QIAampR DNA Mini Kit (Qiagen). Detection of toxin genes *tcdA*, *tcdB*, *cdtA* and *cdtB* was performed by Eva Green Real-time PCR and confirmed by melting curve analysis. Discrimination of A+/B+ and A-/B+ *C. difficile* isolates was performed by PCR with primers NKV011/NK9 targeting a specific deletion in *tcdA* gene (714 bp). Presence of this deletion leads to production of inactive toxin A. All amplification fragments were separated either by QIAxcel capillary electrophoresis system (Qiagen) for determinate right amplicon size (bp) according molecular DNA marker (Fermentas). For positive control in performed analysis were used reference ECDC *C. difficile* collection.

**Results:** The overall *C. difficile* culture isolation rate was 23% (15/65). Production of Toxin A and B was detected in 21.5% (14/15) stool samples by EIA. The *tcdA* gene was detected by Real-time PCR with primers Tox-A-s/Tox-A-as in 86.7% (13/15 isolates) whereas 13.3% were negative. Deletion in *tcdA* gene (714 bp) was detected in 40% (6/15) of the strains and they were toxin A-negative. The intact *tcdA* gene (2535 bp) was amplified in 46.7% of the strains, which were considered as toxinA-positive. The *tcdB* gene was detected in 93.3% (14/15) *C. difficile* strains and only a single strain was negative with primers NK104/NK105. Three toxigenic variants have been distinguished by EvaGreen Real time PCR: 46.67% (7/15) toxin A+B+; 46.67% (6/15) A-B+ and 6.67% (2/15) A-B-.

**Conclusions:** In our investigation predominant toxigenic variants were toxin A+B+ and A-B+. The binary-toxin genes *cdtA* and *cdtB* was PCR detected in one of the A+B+ strains.

#### **P2281** Hospital-endemic *Clostridium difficile* strains are one of the multidrug-resistant organisms in our hospital

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**Background:** To investigate the difference of antimicrobial resistance rates according to PCR-ribotyping of *Clostridium difficile*, antimicrobial susceptibility test for clinical isolates from healthcare-associated *C. difficile* infection (HA-CDI) was performed.

**Methods:** From Sept 2008 through Jan 2010, all the isolates from HA-CDI were included in our study. With cultured *C. difficile* organisms, PCR-ribotyping was performed Antimicrobial susceptibility test to clindamycin (CM), moxifloxacin (MX), metronidazole (MT), or vancomycin (VC) was performed using E-test, and to rifaximin (RF) by agar dilution test. Distinct PCR-ribotype composed with ≥10 strains and four to nine strains were regarded as major and minor PCR-ribotypes, respectively.

**Results:** During study period, 143 *C. difficile* showed 42 distinct PCR-ribotypes with three major and five minor PCR-ribotypes. There was no resistant strain against MT or VC. Most common PCR-ribotype (AB17) including 37 strains (25.9%) showed resistance rates of 91.7% to CM, 91.9% to MX, and 5.4% to RF. Second most common PCR-ribotype (aB, ribotype 017) comprising 15.4% (22 isolates) of total isolates was also very resistant to antibiotics; CM (100%), MX (95.5%), and RF (95.0%). However, among minor PCR-ribotype strains, only two PCR-ribotype strains [AB2 (R112), AB8] revealed high resistant rate to CM (100%, 75%) and MX (80%, 75%). Overall resistance rates of five minor PCR-ribotype strains were 45.8% to clindamycin, 37.5% to moxifloxacin, and 0% to rifaximin. The other strains which did not belong to major or minor ribotypes revealed lower resistance rate to CM (35.9%), MX (28.9%) and RF (10.3%). Comparing resistance rate between three groups of isolates, resistance rates were higher in order; major, minor, and the other PCR-ribotype strains (p for trend <0.001, <0.001, respectively).

**Conclusion:** Antimicrobial resistance rate of *C. difficile* isolates was closely correlated with frequency of isolation in hospital. The effect of decreasing antibiotic selection pressure to epidemiology of CDI in the hospital is necessary.

**P2282** *Clostridium difficile*: antimicrobial resistance depending on binary toxin production

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**Objectives:** Some studies indicate that the production of binary toxin correlates with the severity of *Clostridium difficile* infection (CDI), rendering the strains with binary toxin more virulent, irrespective of PCR ribotype. The aims of this study were (i) to study the proportion of direct toxin A/B from stool samples, and (ii) to determine the antimicrobial resistant patterns of binary toxin producing and non-producing *C. difficile*.

**Methods:** Between February and September 2011, 1084 stool samples were studied for *C. difficile* detection. The presence of GDH and toxin A/B were analyzed in a single immunochromatographic device (ICD) Techlab *C. diff* Quik Chek Complete (Inverness Medical). All GDH positive samples were analyzed for detection of Toxin B and binary toxin genes by GenXpert System (IZASA). All samples with positive Toxin B gene were cultured on CLO selective medium (BioMerieux) and incubated in anaerobic chamber for 48–72 hours at 37°C. Antimicrobial susceptibility was performed by E-test AB Biodisk (Suecia) for metronidazole (Mz), vancomycin (Va), tetracycline (Te), clindamycin (Cd), moxifloxacin (Mo) and erythromycin (E).

**Results:** One hundred and twenty-five (11.5%) stool samples were GDH (+), 38 (3.5%) of them were Toxin A/B (+) by ICD and 89 (8.2%) had the Toxin B (+) gene by GenXpert System. The percentage of samples with binary toxin producing *C. difficile* (BTPCD) was 34.8% (31/89). The percentage of positive samples by direct Toxin A/B among samples BTPCD was 48.4% (15/31) and among non-producing 39.6% (23/58) ( $p = 0.211$  ns). The percentages of resistance to antibiotics tested were: Mz: 0%, Va: 0%, Mo: 52.6%, Te: 45.5%, E: 43.6% and Cd: 51.3%. 25.3% of *C. difficile* were sensitive to all antibiotics tested, 20% were resistant to one antibiotic and 54.7% were resistant to  $\geq 2$  antibiotics. The percentage of resistance of BTPCD and non-producing were: Mo: 78.6–42% ( $p < 0.01$ ); Te: 96–23.1% ( $p < 0.01$ ); E: 70.4–23.5% ( $p < 0.01$ ) and Cd: 59.2–45.1% ( $p = 0.11$  ns).

**Conclusions:** 34.8% of *C. difficile* were binary toxin producing. The production of binary toxin does not increase the detection of direct Toxin A/B from stool samples. The strains binary toxin producing *C. difficile* were significant more resistant than non-producing. It was not detected resistance to metronidazole or vancomycin.

**P2283** Susceptibility of *Clostridium difficile* from the UK to alternative agents

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**Objectives:** *Clostridium difficile* is a leading cause of nosocomial diarrhoea worldwide and is associated with high rates of morbidity and mortality. A 35-fold increase in the reported incidence of *C. difficile* Infection (CDI) in the UK over the last decade has been associated in part with the emergence of the highly virulent clone PCR-ribotype 027. Recommended empiric therapy for CDI is currently metronidazole or oral vancomycin but as rates of relapse and re-infection increase, new pharmacological approaches are needed. This study aimed to investigate the antimicrobial susceptibilities of *C. difficile* strains to some less commonly used agents.

**Methods:** Two hundred and seventy-six *C. difficile* isolates from 38 different ribotypes (of known susceptibility to vancomycin and metronidazole) submitted to the UK Anaerobe Reference Unit between 2001 and 2011 were tested. Susceptibility was assessed by agar dilution MIC by the CLSI method for rifaximin, rifampicin, fusidic acid and teicoplanin.

**Results:** Summary MIC data presented by ribotype are shown in the Table. The ‘Other’ category consists of 110 isolates from 34 less common ribotypes. The great majority of isolates were susceptible to all of the agents tested, irrespective of ribotype (or indeed year of isolation). The one isolate resistant to vancomycin (MIC 4 mg/L) remained sensitive to teicoplanin (MIC 0.25 mg/L). Of note, two

isolates were resistant to both rifampicin and rifaximin, while four isolates were resistant to rifampicin but remained sensitive to rifaximin.

Table: MICs (mg/L) for different ribotypes

Ribotype	Rifampicin		Rifaximin		TEI		FUS	
	Range	MIC90	Range	MIC90	Range	MIC90	Range	MIC90
001	<0.002 – 32	<0.002	<0.002 – >32	0.008	0.125-0.5	0.25	0.06-16	2
027	<0.002 – >32	0.004	<0.002 – >32	0.008	0.125-0.5	0.25	0.06-16	2
078	<0.002 – 0.004	<0.002	<0.002 – 0.008	0.008	0.125-1	0.25	0.06-2	1
106	<0.002 – >32	<0.002	<0.002 – 0.008	0.008	0.125-1	0.5	0.03-2	2
Other	<0.002 – >32	<0.002	<0.002 – >32	0.008	0.06-1	0.25	0.03->128	2

**Conclusions:** Recent isolates of *C. difficile* from across the UK appear sensitive to rifampicin, rifaximin, teicoplanin, and fusidic acid. This suggests that they may be effective alternative agents in the treatment of severe CDI.

**P2284** The role of human intestinal microbiota in the development of *Clostridium-difficile* associated infection

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**Objectives:** *Clostridium difficile* is the leading infectious cause of nosocomial diarrhea in developed countries. Host factors appear to determine the differences in clinical presentation. The human intestinal microbiota acts as a barrier, preventing colonization and inhibiting overgrowth of pathogens such as *C. difficile*. However, the effects of bacterial community structure in the intestines on *C. difficile* infection are not well documented. Our objective was to explore the difference in the composition of intestinal microbiota between patients with *C. difficile* associated disease, asymptomatic carriers and normal hosts.

**Methods:** Patients with a stool assay for *C. difficile* toxin were identified via the microbiology laboratory in our institute. Using Real Time PCR, bacterial populations were quantified from stool samples of four groups of patients: *C. difficile*-associated diarrhea patients (group 1); *C. difficile* asymptomatic carriers (group 2); antibiotic associated diarrhea patients without *C. difficile* (group 3); and normal controls (group 4). Stool was examined for three genes – *C. difficile* toxin A gene, 16S rRNA gene from *Clostridium thermocellum* and 16S rRNA gene from bacteroides fragilis.

**Results:** Fifty-six patients underwent analysis of the stool (group 1 – 12, group 2 – 14, group 3 – 15, group 4 – 15). Patients in groups 1 and 2 were older than the other groups, and more exposed to previous antibiotic treatment. There was no significant difference in demographic parameters such as gender, functional status and charlson score. *C. difficile* isolates from group-1 and 2 underwent ribotyping and were tested for resistance to quinolone antibiotics. The NAP-I/O27 ribotype was identified in 8%. Thirty percent of isolates were resistant to moxifloxacin and 45% were resistant to ciprofloxacin. As expected, *C. difficile* toxin A gene was significantly higher in groups 1 and 2. Significantly higher concentrations of 16S rRNA gene from *Clostridium thermocellum* were found in group 4 compared with groups 1 and 2, while concentrations of 16S rRNA genes from bacteroides fragilis were significantly higher in group 4 compared with group 2, but not group 1.

**Conclusions:** Data concerning the role of the intestinal microbiota in *C. difficile* infection is gradually being assembled. We have shown significant differences in the composition of the microbiota between *C. difficile* patients and carriers and normal hosts. Larger study samples and more specific PCR analyses are required for future trials.

**P2285** High frequency of partial tcdC deletion in *Clostridium difficile* isolated in Sao Paulo

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**Introduction:** *Clostridium difficile* is the major etiologic agent of diarrhea associated with antimicrobial use. It can produce enterotoxin A

and/or cytotoxin B, both encoded by genes located in a pathogenicity locus. Toxin A causes fluid accumulation and damage to the intestinal mucosa, while cytotoxin B is a potent cytotoxic agent. Some strains also produce binary toxin CDT. All may be present in the same strain, but some express only the cytotoxin B. Clinical manifestations can range from a self-limited diarrhea to pseudomembranous colitis, which in its most severe form can be fatal. TCDC protein plays a key role, because the hyper virulent strains usually have a partial deletion of the *tcdC* gene and are associated with more severe forms of disease.

**Objectives:** To determine the frequency of the genes *tcdA*, *tcdB*, *cdtB* and *cdtA*, and deletions in *tcdC*, in a collection of 61 strains of *C. difficile* isolated from feces of patients treated in private hospitals in São Paulo, Brazil, during the years of 2009 and 2010. In all cases the test for detection of toxins in the stool was positive by ELISA.

**Material and methods:** Frozen cultures were thawed and grown on Brucella agar with horse blood for 24 hours under anaerobic conditions. The bacterial suspensions were prepared in reagent grade water and then were boiled for 10 minutes. *C. difficile* species identification was confirmed using PCR amplification of a species-specific region of the *tpi* gene. The presence of genes *tcdA*, *tcdB*, *tcdC*, *cdtA* and *cdtB* was detected by multiplex PCR as described by Antikainen et al., except that Platinum Taq DNA polymerase was used. *tcdC* gene sequences were obtained as previously described by Spigaglia and Mastrantonio.

**Results:** All samples analyzed harbored the genes *tcdA* and *tcdB*. *tcdC* partial deletion was found in 13 samples (21%), *cdtA* gene was detected in 26 samples (42%). When sequences obtained were compared to those available at GenBank an 18 bp deletion was detected in most of the isolates. A 36 bp deletion was detected in a single isolate.

**Discussion and conclusions:** This is the first Brazilian study indicating the high frequency of samples with partial deletion of *tcdC*. Our findings (21%) contrast to the previous reports in the literature which indicate a frequency of *tcdC* deletion around 6%. Our findings indicate a high frequency of strains of *C. difficile* with partial *tcdC* deletion in Sao Paulo, Brazil.

## Clostridium difficile – diagnostics and therapy

### P2286 Enhancing laboratory capacity for Clostridium difficile detection in Europe

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**Objectives:** *Clostridium difficile* infections (CDI) is an important healthcare problem across Europe. To improve recognition and awareness of CDI and to enable surveillance at a European level, the European Centre of Disease Prevention and Control (ECDC) funded a project to enhance laboratory capacity for CDI detection and surveillance in Europe (2010–2014): ECDIS-net (www.ecdisnet.eu).

**Methods:** A questionnaire on national laboratory capacity for CDI was sent to the national coordinators of 32 European countries. Furthermore, each national coordinator was asked to randomly select 10% of the laboratories, with a minimum of 3, for more detailed information.

**Results:** Thirty-one of 32 countries responded to the national questionnaire, in 10 of which (32%) a national recommendation for a test-algorithm for CDI diagnostics is present. In 12 (38%) countries, national guidelines or recommendations with selection criteria for routine laboratory testing of CDI are used. Estimations of the percentage of CDI cases that are laboratory diagnosed ranged from 1% to 100% per country and the estimated total number of patients diagnosed with CDI ranged from 11 to over 24 000, with a median of 2439. Five (16%) countries indicated the existence of limitations for healthcare providers to request primary CDI diagnostics in their country, including financial, technical and organizational. In 22 (71%) countries at least one laboratory performed typing of *C. difficile*. None of nine countries that answered no had made arrangements with other countries for typing. In 14 (of 27 answering, 52%), typing was performed at a national reference laboratory and in 14 (of 20) the typing

was nationally/officially funded. In 20 labs typing was done by PCR ribotyping.

Fifteen (48%) indicated that there was a need for training in culture of *C. difficile* in their country and 18 (58%) a need for training in PCR-ribotyping.

For more detailed information, 205 hospital laboratories from the 32 countries were approached to participate in a follow-up questionnaire on local CDI diagnostic practices and capacity.

**Conclusion:** The questionnaire among the national representatives show that a wide variety exists across Europe in practices and volume of CDI diagnostics. In the majority of countries, the capacity to perform typing for outbreaks and/or surveillance was present, but some restrictions existed in several countries and about half the countries indicated a need for training in culturing and typing of *C. difficile*.

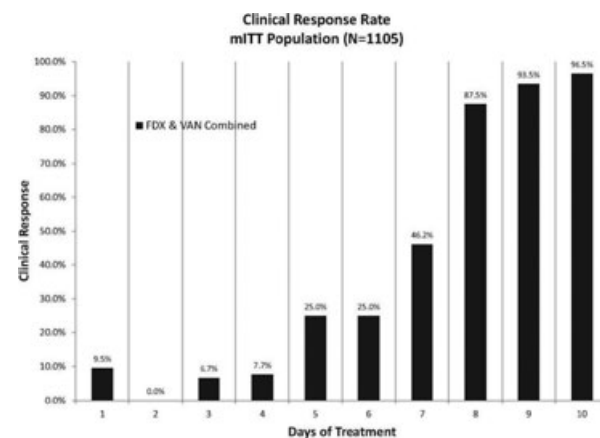
### P2287 Predictors for treatment failure with fidaxomicin and vancomycin in Clostridium difficile infection

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**Objectives:** Treatment failure, defined as greater than three unformed bowel movements per day, was observed in 13% of 1105 CDI patients in Phase 3 randomized clinical trials equally in both FDX and VAN arms. A multivariate analysis of risk factors and clinical observations was conducted to discern associations with failure of therapy.

**Methods:** Patients were randomized to receive FDX 200 mg twice daily (539 patients) or VAN 125 mg four times daily (566) for 10 days. Number of bowel movements was recorded each day. The modified intent-to-treat (mITT) population consisted of 1105 patients with CDI who were enrolled in the 003 trial (596 patients) or 004 (509 patients) trials. For this mITT population analysis all durations of treatment were examined. Univariate analyses were performed on categorical and continuous variables. All predictors that were statistically significant in univariate analysis were used in the multivariate analysis to identify independent predictors of clinical failure using stepwise logistic regression backwards selection method (exit criteria  $p = 0.05$ ). These variables were age (in decades), number of unformed bowel movements, white blood count, neutrophils, lymphocytes, haemoglobin, sodium, creatinine, blood urea nitrogen (BUN), albumin, BI group, baseline severity, concomitant antibiotics, cancer, renal impairment, country, treatment duration, and study drug treatment.

**Results:** The final model of multivariate analysis showed that shorter (every decrease of 1 day) treatment significantly increased the risk of not responding to treatment by about twofold (OR = 2.33, 95% CI 1.97–2.75;  $p = 0.0001$ ). Treatment 9–10 days seems optimal. Lower serum albumin (every decrease in 1 g/L) significantly increased the risk of failure by 12% (OR = 1.12, 95% CI 1.01–1.19,  $p = 0.0002$ ). Among CDI patients with cancer, VAN treatment, but not FDX treatment, was



associated with increased risk of failure (OR = 4.02, 95%CI 1.47–10.99,  $p = 0.0068$ ). Excluding treatment duration as a covariate, for all patients, BI strain (OR = 1.73, 95%CI 1.04–2.88,  $p = 0.04$ ), serum albumin (OR = 0.91, 95% CI 0.87–0.95,  $p = 0.0001$ ), Leukocytosis (OR 1.05, 95%CI 1.01–1.08,  $p = 0.005$ ) and renal insufficiency (OR = 2.54, 95% CI = 1.53–4.20,  $p = 0.0003$ ) were predictors of failure.

**Conclusion:** A shortened treatment course appears to be associated with lower cure rates; the analysis supports current treatment guidelines. Reduced serum albumin as a marker of CDI severity, is also related to failure.

#### **P2288** Recurrences of *Clostridium difficile* infections in Stockholm relapse or re-infection?

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**Objectives:** *Clostridium difficile* is a Gram-positive, spore-forming anaerobic rod which cause a wide range of diseases in humans, from mild diarrhea to severe life-threatening pseudomembranous colitis. Approximately 20% of the patients treated for a first episode of *C. difficile* infection (CDI) suffer from a recurrent CDI within 8–10 weeks. A recurrence can correspond to a relapse, that is, infection with the same strain, or a re-infection, that is, infection with a different strain. The purpose of the study was to evaluate if a recurrence of CDI is a relapse or a re-infection.

**Methods:** A total of 149 toxigenic *C. difficile* strains collected between 2008 and 2011 from 67 patients were analysed by PCR-ribotyping. All patients had at least one *C. difficile* positive sample at a minimum of 8 weeks after the first sample indicating a recurrent CDI. The isolates were identified by characteristic colony morphology, typical smell and Gram staining. PCR ribotyping was used to analyse the isolated strains. The PCR products were separated on 5% polyacrylamide gels by electrophoresis. The gels were scanned and analysed by Bionumerics software version 6.5. The banding patterns were compared to a database including *C. difficile* reference strains.

**Results:** Fifty-five (82%) patients had one episode of recurrence. In 32 (58%) of the patients, the isolate from the recurrent CDI was of the same ribotype as the one isolated from the primary infection, indicating a relapse. In 23 (42%) of the patients the recurrent CDI was due to a different ribotype, indicating a re-infection. Twelve of the patients suffered from more than one recurrent CDI. Four were due to relapse and eight were due to both relapse and re-infection. A total of 43 different ribotypes were identified. The dominating ribotypes were 020 (56%), 001 (33%), SE21 (23%), 023 (23%), 231 (19%). No 027 ribotypes were found.

**Conclusion:** A recurrence of CDI could be due to the same or due to a different *C. difficile* strain. Here it is shown that 58% of the patients, the recurrent CDI was due to relapse and in 42% due to a re-infection. In addition, eight of 12 patients with more than one recurrent CDI, both relapse and re-infection were present.

#### **P2289** Clinical outcomes for cancer patients with *Clostridium difficile* infection

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**Objectives:** Cancer patients are at high risk for developing CDI but it is unclear if they have poorer clinical outcomes. In Phase 3 trials of *Clostridium difficile* infection (CDI) treated with fidaxomicin (FDX) or vancomycin (VAN) we observed 183 (16.6%) patients with a current diagnosis of cancer among 1105 total modified-intent-to-treat (mITT) patients. Their responses to therapy were subjected to post-hoc analysis.

**Methods:** The patients in this analysis had a current diagnosis of cancer at the time of CDI diagnosis and were receiving various forms of treatment. Patients with CDI were given FDX 200 mg twice daily (539

patients) or VAN 125 mg four times daily (566) for 10 days. Clinical endpoints included cure after  $\geq 8$  days of treatment (in 153 patients), recurrence after 28 days of follow-up and sustained clinical response (cure with no recurrences). Number of bowel movements was recorded daily to determine Time To Resolution of Diarrhoea (TTROD).

**Results:** Compared to 922 CDI patients without cancer, those with cancer had a lower clinical cure rate (88.6% without cancer vs. 79.2% with cancer,  $p = 0.0006$ ) but no difference in recurrence rates (21.4% and 20.0%, respectively;  $p = 0.69$ ). TTROD was prolonged in *Clostridium difficile* infection (CDI) cancer patients (55 hours without cancer vs. 100 hours with cancer,  $p = 0.0004$ ). Among the 153 cancer patients who took  $\geq 8$  days of treatment, all clinical endpoints showed significant superiority with FDX compared to VAN (clinical response [97.3% vs. 87.5.0%], recurrence [14.1% vs. 30.0%], and sustained response [83.6% vs. 61.3%]). The odds ratios demonstrate that FDX is five times more likely than VAN to produce a clinical response (OR 5.07, 95%CI 1.07–23.98,  $p = 0.025$ ) and three times more likely to lead to a sustained response (OR 3.22, 95%CI 1.50–6.91,  $p = 0.002$ ). VAN showed a 2.6-fold greater odds of recurrence (OR 0.38, 95%CI 0.16 = 0.89,  $p = 0.023$ ) than FDX.

**Conclusions:** *Clostridium difficile* infection (CDI) represents a serious illness for cancer patients, with more clinical failures and prolonged diarrhoea than in patients without cancer. VAN treatment is associated with worse outcomes for clinical cure, recurrence and sustained clinical response than FDX treatment.

#### **P2290** Development of resistance in *C. difficile* with fidaxomicin, vancomycin, and rifaximin

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**Introduction:** Fidaxomicin (FDX) is a novel antibiotic that was recently approved in the United States for the treatment of *C. difficile* associated diarrhea. It is an inhibitor of RNA polymerase as are the rifamycins. Although it lacks cross-resistance to other classes of antibiotics, the question arises regarding the relative frequency of spontaneous resistance.

**Methods:** The rate of spontaneous resistance development to fidaxomicin (FDX) in *C. difficile* was evaluated in comparison to that of rifaximin (RFX) and vancomycin (VAN) by plating dense suspensions of four *C. difficile* strains (ATCC 9689 [ORG 74] and ATCC 700057 [ORG 830] plus two clinical strains, ORG911 and ORG916) on Brucella agar plates containing 4 $\times$  and 8 $\times$  the MIC of the respective antibiotic. The number of colonies observed after 48 hours was divided by the number of colonies inoculated (determined by serial dilution and plating of the inoculum), to give the value of the frequency of spontaneous resistance (FSR) to each drug.

**Results:** Mutation frequencies to FDX and VAN were very low for all strains, with FSR values ranging from  $<1.4 \times 10^{-9}$  to  $12.8 \times 10^{-9}$  for fidaxomicin and  $<1.4 \times 10^{-9}$  for VAN. While no resistant colonies were generated at 8 $\times$  MIC concentrations of either drugs, at 4 $\times$  MIC of FDX one or two resistant colonies (per agar plate) were seen in some experiments in the ATCC strains ORG 74 and ORG 830, with MIC values at 4  $\mu\text{g}/\text{mL}$ . Rifaximin, on the other hand, selected for the highest number of spontaneously arisen resistant mutants at both drug concentrations in all strains (with FSR values between  $6.67 \times 10^{-9}$  to  $1.73 \times 10^{-7}$  and a 32 $\times$  to  $>64\times$  rise in MIC values). No cross resistance between RFX, VAN and FDX was observed in any of the resistant strains.

**Conclusion:** The present study demonstrates lower mutation frequencies in *C. difficile* with FDX and VAN than with RFX, both in the order of  $10^{-9}$ . RFX had multiple spontaneous mutants in all strains at  $10^{-9}$  to  $10^{-7}$ . This study confirms the lack of cross-resistance between these antibiotics.

## Hepatitis

### **P2291** The Influence of inflammation on liver stiffness – A study in acute hepatitis

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**Background and objectives:** Liver stiffness measurement by transient elastography (Fibroscan®) is currently used to quantify the degree of liver fibrosis. The elasticity of the liver can also be influenced by other factors, such as inflammation, edema, necrosis and steatosis, and if these factors exist, their contribution in the result of the investigation is hard to establish. This study aims to compare the characteristics of the same liver in different conditions: during extensive inflammation (acute hepatitis) and resolution period.

**Methods:** Twenty-six patients with acute hepatitis (10 with A hepatitis and 16 with B hepatitis) were included. The patients were monitored until ALT levels became  $<2 \times \text{ULN}$ . Fibroscan® was performed at three time points: at presentation, 14 days later and when ALT was  $<2 \times \text{ULN}$ . At the same points, ALT, AST and bilirubin were tested. None of the patients had any previously known liver disease.

**Results:** The mean age of the patients was 28.5 ( $\pm 6.73$ ) years. At presentation, 61% of the patients had liver stiffness values of over 7.2 kPa (usually consistent with significant fibrosis), while during resolution only 11% had this high values. 5/26 patients at presentation had liver stiffness of over 15 kPa, values usually considered significant for cirrhosis; only 2/26 patients had normal values (both with VHA). At presentation, liver stiffness was only influenced by etiology, not by ALT, AST or bilirubin level, being significantly higher in acute B hepatitis than in A hepatitis ( $p = 0.001$ ,  $r = 0.6$ ). The stiffness values decreased in parallel with the decrease of ALT during the monitoring period, and they became correlated with ALT at the last two evaluations ( $p = 0.001$ ,  $r = 0.61$  and respectively  $r = 0.751$ ). Liver stiffness at peak increase of ALT may be a good indicator of the disease progression as it is statistically significant correlated with ALT and AST values at 14 days after admission ( $p = 0.004$ ,  $r = 0.543$  and  $p = 0.001$ ,  $r = 0.623$ ).

No differences regarding ALT, AST and bilirubin levels, were found between the two etiologies at admission.

**Conclusions:** Liver stiffness is influenced by inflammation, but not always correlated with ALT or AST levels. In acute hepatitis B, at presentation, liver stiffness was significantly higher than in A hepatitis, regardless of ALT levels.

Liver stiffness at admission may be a predictor of the evolution of the hepatitis as its values are correlated with ALT levels found 14 days after.

### **P2292** Occult hepatitis B among healthy blood donors

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**Objectives:** HBsAg detection is routine for screening blood donors in Iran. An HBsAg negative donation is generally considered safe. In this study we aimed to determine the presence of anti-hepatitis B core (anti-HBc) and HBV genome among Iranian healthy blood donors which have occult hepatitis B (OBI).

**Methods:** Three hundred serum samples negative for both HBsAg and anti-HCV collected from healthy blood donors were tested for the presence of anti HBc antibody. All samples positive for anti-HBc antibody then were investigated for determination of anti-HBc titre by enzyme immunoassay (EIA). Each sample which tested positive for anti-HBc was also examined for the presence of HBV-DNA by PCR.

**Results:** Of the 300 samples tested, 25 (8%) blood samples were found to be positive for anti-HBc. HBV DNA was not detected among all of anti-HBc positive specimens.

**Conclusion:** The prevalence of OBI among HBsAg negative blood donors is variable according to the level of HBV endemicity, and to the assays employed in routine serological or NAT screening.

Some investigations have shown OBI among Middle Eastern healthy blood donors. On the other hand, special HBV mutations can render HBsAg undetectable by conventional Eliza technique so further studies with more sensitive techniques such as nested PCR or Real time PCR is suggested to ensure about existence of OBI among healthy blood donors.

### **P2293** Public awareness of familial transmission of Hepatitis B in Turkey

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**Objectives:** Turkey has a moderate endemicity level of HBV infection. Detection of perinatal and intrafamilial transmission of the virus is essential for the prevention of chronic liver diseases. In this study we investigated how the patients were aware of their HbsAg positivity.

**Methods:** The records of HbsAg positive patients between April 2011 and November 2011 were examined retrospectively.

**Results:** A total of 200 HbsAg positive patients (51.8% female, 48.5% male) were included to study. The mean age was  $37.2 \pm 11$  (14–60 years). Of this patients only five cases has a history of acute hepatitis B. Forty-three percent of patients had HbsAg positivity in their family members. HbsAg positivity was most commonly discovered by laboratory tests during the marriage procedures ( $n = 35$ , 17.5%). Others were diagnosed by the test during pregnancy (17%), preoperative evaluation (15%), blood donation (15%), acceptance prior to military service (4%), family history of hepatitis B (4%), in the presence of acute hepatitis B (2.5%), private reasons (include job applications, school surveys, sports) (9%) and work up of the other diseases (16%), respectively.

**Conclusion:** Although about half of the cases had HbsAg positive family members, family screening tests had been performed less than expected. Hence most cases were diagnosed at third-fourth decades even. This study indicated that there is a need for more public awareness of familial transmission of hepatitis B.

### **P2294** A sero-epidemiological study on hepatitis B virus infection among pregnant women in Beijing

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**Objectives:** Maternal–neonatal transmission or contact with infected mothers in early childhood accounted for half of all chronic HBV infections in China. Thus, the critical strategy to prevent HBV infection is to immunize newborns against hepatitis B. We examined the prevalence of HBV serological markers among pregnant women in Beijing.

**Methods:** A total of 3550 pregnant women who received prenatal examination during March 2008 and December 2010 were recruited in this study. Serological markers of hepatitis B virus were measured by the chemiluminescence microparticle immunoassay. The local ethics committee approved the study.

**Results:** In our cohort, 3.8% of these pregnant women were positive for HBsAg, indicating an ongoing infection. Among the 134 women positive for HBsAg, the vast majority (99.25%) were also positive for anti-HBc, the other one was positive for HBsAg and HBeAg. Overall, 20.1% of HBsAg (+) pregnant women were also HBeAg (+), and these rates were lower than rates estimated for China in 1979 and then in 2006. About 24% of these pregnant women exhibited anti-HBc seropositivity. Moreover, serological markers of past HBV infection with spontaneous recovery (anti-HBc (+) and anti-HBs (+)) were observed in 18.6% of participants whereas 44.2% presented isolated anti-HBs (+), which indicated vaccination-induced protection.

As shown in Table 1, when the study population was grouped by age by 5-year increments, there were significant differences in positive rate



of HBsAg between groups ( $p < 0.001$ ). However, there were no significant differences in positive rate of HBeAg among HBsAg (+) women ( $p = 0.227$ ). Contrary to positive rate of HBsAg, the prevalence rate of isolated anti-HBs (+) was highest in the 25–29 years group (53.0%) and lowest in the  $\geq 35$  years group (24.3%). The prevalence of previous HBV exposure marker (anti-HBc (+)) was significantly higher in  $\geq 35$  years group than any of the other age groups ( $p < 0.001$ ).

Table 1 The prevalence rate of HBV serological markers among pregnant women in Beijing

Group	N	HBsAg (+)	P value	HBeAg (+) among HBsAg (+)	P value	anti-HBc (+)	P value	anti-HBc (+) only	P value	anti-HBc (+) and anti-HBs (+)	P value
Age, y											
<25	245	4.5	0.000	36.4	0.227	23.7	0.000	35.1	0	17.6	0.000
25-29	1525	3		22.2		19.5		53		15.5	
30-34	1224	3.1		10.5		25.6		44		21	
$\geq 35$	556	7.2		22.5		32.7		24.3		22.3	
total	3550	3.8		20.1		24		44.2		18.6	

HBsAg: hepatitis B surface antigen, HBeAg: hepatitis B e-antigen, anti-HBc: antibody to hepatitis B core antigen, anti-HBe: antibody to hepatitis B surface antigen.

**Conclusion:** In China, the neonatal vaccination program that was implemented in 1992 has reduced hepatitis B infection. However, the positive rate of HBeAg among HBsAg (+) pregnant women was still as high as 20.1% in our cohort. Because HBsAg and HBeAg positive pregnant women had a 90% chance of infecting their newborns within half to 1 year of birth, it was necessary to improve HBV infection screening in pregnant women and further strengthen infant immunization programs.

#### P2295 Different genotype on codon 173 of HBV polymerase gene

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**Objective:** In most cases, treatment with any single Nucleos(t)ide Reverse Transcriptase Inhibitor generate rapid suppression of HBV replication, but the effect does not keep on due to emerge of drug resistant HBV strains, which is the single most important factor in treatment failure. PyroStar HBV Resistance test is designed to allow detection of mutation on codon 169, 173, 180, 181, 184, 194, 202, 204, 236, and 250 of the HBV polymerase (pol) gene which all confer drug resistance. These mutations are detected with six pyrosequencing reactions which are generated in reverse (antisense) direction. Therefore the sequencing results correspond to the antisense strand of HBV genome. To determine the mutation in pol gene, we use the kit and system mentioned above in our Molecular Microbiology Lab. We detected a different result on codon 173. This difference is about single nucleotide variation not resulting in phenotypic changing. We have not obtained any document about it in scientific references and manuals of kit and system presented by manufacturer. We wanted to submit and share this data especially with system users because we thought that it may be important as well as interesting.

**Methods:** The serum sample was obtained from a patient with chronic hepatitis B, who was treated in the Ataturk University Research Hospital. After completing DNA extraction and PCR amplifications that are targeted to pol gene for quantitative rtPCR and pyrosequencing, the PCR products were sequenced with pyrosequencing primers to allow detection of mutation on 10 codons mentioned above. For position of codon 173 antisense sequence of wild type was CAC indicating valine in phenotype and for mutant type it was CAG indicating leucine in phenotype.

**Results:** In the serum sample, we obtained the antisense sequence as CAA on codon of 173. This sequence also indicating leucine when translated.

**Conclusion:** The mutation on codon 173 of pol gene has been detected 9% of cases of lamivudine resistance cases. The sequence presented was not assigned as mutant type by manufacturer of the kit and/or the system previously. Although this changing has not depicted different amino aside in phenotype, it is very important in genotyping. Because pyrosequencing method capable to detect minor population of nucleotides, and is more sensitive and suitable for short sequences at a specific position, the scientist who interested in pyrosequencing method for HBV pol gene should be informed that kind of mutation.

#### P2296 Cytokine response of peripheral blood lymphocytes from patients with persistent HBV DNA despite seroconversion to anti-HBs antibodies

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**Objectives:** Chronic hepatitis B virus (HBV) infection is among the most common persistent infections in humans. Long-term complications of chronic HBV infection include liver cirrhosis, or hepatocellular carcinoma, especially in hyperendemic areas. Our previous study showed that HBV DNA may persist for a long time after therapy despite the appearance of anti-HBs antibodies. The mechanisms that induce immunosuppression and lead to viral persistence are not clear. However, inactivation of antiviral T cells may be a key obstacle preventing the clearance of persistent infections by a wide range of viruses, including HBV.

In this study we analysed the profile of Th1 and Th2 cytokines released by peripheral blood lymphocytes from HBV DNA-positive and HBV DNA-negative patients in whom seroconversion to anti-HBs antibodies was confirmed.

**Methods:** Blood samples were obtained from 20 patients at a mean of 9 years (range: 8–10 years) after the last dose of rIFN- $\alpha$ . All patients were HBsAg, HBeAg-negative, but anti-HBs, anti-HBc and/or anti-HBe-positive. Despite of anti-HBs presence, 10 patients were HBV DNA-positive and 10 were HBV DNA-negative. The following parameters were determined: (i) lymphocyte proliferation; (ii) absolute number of B, T and T cell subsets; (iii) concentration of IFN- $\gamma$ , IL-2 (Th1) and IL-4, IL-10 (Th2) cytokines in cell culture supernatants stimulated with mitogens or rHBsAg. In addition, cytokine levels were compared with distribution of specific anti-HBs antibodies among IgG subclasses.

**Results:** In both groups no significant differences between lymphocyte proliferation and absolute number of B and T cells were observed and the secretion of cytokines stimulated by non-specific mitogens was at the similar level. Statistically significant differences with respect to concentration of particular cytokines stimulated by rHBsAg were found when HBV DNA status was considered. In all patients anti-HBs/HBV DNA+, lower concentration of IFN- $\gamma$  (300 vs. 673 pg/mL;  $p \leq 0.01$ ) and higher of IL-4 (160 vs. 88 pg/mL;  $p \leq 0.03$ ) and IL-10 (798.4 vs. 130 pg/mL;  $p \leq 0.001$ ) were found. High IL-10 correlated with detectable HBV DNA and increased contribution in the response of IgG4 anti-HBs.

**Conclusion:** The results of the present study confirm, in part, recent observations on animal models that persistent viral infection increases production of IL-10, which, in turn, may be responsible for suppression of cellular immune response, and prevents viral clearance.

#### P2297 Improved HBV HBsAg mutant detection with ADVIA Centaur®

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**Introduction and objective:** In the recent years diagnostic companies have worried about the possibility of false negative serology results achieved with HBsAg assays due to the emergence of HBsAg mutants. Once new HBsAg tests are developed or improved, those tests should be evaluated not only with samples of recombinant origin but also with natural patient samples. With that purpose, 65 patient serum samples (1:40 diluted) of chronic VHB carriers genotypes A, D, E and F with known HBsAg mutations (distributed along HBsAg 1–4 regions) were chosen from the National Centre of Microbiology serum bank to be tested.

**Methods:** The HBsAg (former test) and HBsAgII (new test), were evaluated with the ADVIA Centaur® XP, (Siemens Healthcare Diagnostics, Deerfield, IL, US) according to the manufacture guidelines.

**Results:** Both assays were able to detect 83.4% (35/42) of samples with isolated mutations in regions 1–3, while HBsAgII reached a



0.16–15.97 IU/mL for the HBsAg I and II kit, respectively. Comparison with an existing quantitative HBsAg assay in 20 samples showed a good correlation between the results ( $R^2 = 0.975$ ). Based on these results, the quantitative HBsAg assay for the Advia Centaur® XP has been implemented in the laboratory. For each quantitative assay, at least two sample dilutions and a calibrator are tested. The HBsAg load in the undiluted sample is calculated in IU/mL based on measurement of at least two dilutions.

**Conclusion:** The quantitative HBsAg assay for the Advia Centaur® XP has been successfully developed and validated and can be used as a tool for the monitoring of chronic HBV patients.

**P2301 IL28B genotype study in a cohort of patients infected with hepatitis C virus genotype 1**

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**Objective:** Our main objective was determining the genotypes of IL-28B in patients-cohort and the predictive potential of virological response to therapy.

**Methods:** We carried out an retrospective analysis in a patient-cohort with chronic Hepatitis C genotype 1, either mono-infected or HIV co-infected, to identify genotypes of IL28B and the probability of sustained virological response to pegylated interferon alfa and ribavirin therapy.

**Results:** Of the 111 patients studied, a total of 61 were co-infected, within this group 29 had completed treatment with INF + RBV, 62% of the group were genotype TC, 30% genotype CC, 8% TT. Seventy-five percent of the patients with sustained virologic response presented with genotype CC.

Of the 50 patients mono-infected 31 had completed treatment, 39% were genotype TC. Seventy-six percent of patients with SVR presented genotype CC.

**Conclusions:** The most frequent genotype of IL28B is the non-responsive TC. We consider that, given the high negative prediction value of the TC genotype to reach an SVR, the genotype of each patient should be determined prior to the start of treatment.

We propose delaying treatment of patients who do not have an advanced of fibrosis and who have an unfavourable IL-28B genotype until such time as new medicines can be used.

**P2302 Comparative study of virological and clinicopathological features of anti-hepatitis C virus – reactive Egyptian patients with normal and elevated liver enzymes**

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**Objectives:** The study investigated the clinicopathologic and virologic features of 30 Egyptian patients chronically infected with hepatitis C virus (HCV).

**Methods:** Patients were stratified into two groups; group 1: 15 patients with normal alanine aminotransferase (ALT) activity, group 2: 15 patients with raised ALT activity. All patients underwent physical examination, ultrasound liver evaluation, routine liver function tests and serum HCV RNA quantitative detection. Percutaneous ultrasound-guided liver biopsy was followed by routine histological evaluation according to a well established grading and scoring system. Total RNA was isolated from liver tissue and HCV RNA was detected by reverse-transcription polymerase chain reaction (RT-PCR). Liver sections were immunostained for detection of HCV antigen.

**Results:** All patients had hepatitis C viraemia. The mean titer of hepatitis C virus RNA (logarithmic transformed copy number per milliliter of serum) was significantly higher in patients with elevated enzymes ( $5.0 \pm 0.26$ ) than in those with normal enzymes ( $4.72 \pm 0.60$ ),  $p = 0.04$ . There was a trend towards a higher HCV RNA titer with progression of histological changes in the liver. Histological evidence of chronic hepatitis was documented in all cases and the severity of liver disease was significantly correlated with increase in age. The mean histological activity index score was significantly higher in patients

with elevated enzymes ( $5.53 \pm 2.36$  vs.  $2.93 \pm 2.20$ ,  $p = 0.005$ ). Tissue HCV RNA was detected in 19 cases (63.33%) (nine in group 1, 10 in group 2) and was related to a higher amount of serum HCV RNA and to a higher degree of inflammatory activity. Positive staining for HCV antigen was found in 16 specimens (53.33%), (seven in group 1, nine in group 2). Staining was exclusively cytoplasmic, with a high percentage of stained cells observed in relation to increased grade of necro-inflammatory changes.

**Conclusion:** The results of the presented work show that in patients with chronic HCV infection, serum and intrahepatic HCV RNA may be detected despite the presence of normal serum ALT levels. This can be accompanied by histologically and clinically progressive disease. Liver biopsy could be considered useful in patients with normal ALT levels, not only to reassure those with minimal liver disease but also to define patients with more significant histologic findings who may benefit most from antiviral therapy.

**P2303 Comparison of a newly developed automated and quantitative hepatitis C virus core antigen test with the hepatitis C virus RNA assay for the clinical usefulness of confirming anti-hepatitis C virus results**

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**Objectives:** HCV is a global healthcare problem. Diagnosis of hepatitis C virus (HCV) infection is mainly based on the detection of anti-HCV antibodies as a screening test on sera samples. Recombinant immunoblot assays are used as supplemental tests and in the final detection and quantification of HCV RNA in confirmatory tests. In this study, we aimed to compare the HCV core antigen test with the HCV RNA assay for confirming anti-HCV results to determine whether the HCV core antigen test may be used as an alternative confirmatory test to the HCV RNA test and to assess the diagnostic values of the total HCV core antigen test by determining the diagnostic specificity and sensitivity rates compared with the HCV RNA test.

**Methods:** A total of 212 treatment-naive patients provided serum were analysed for anti-HCV and HCV core antigen assay, both with Abbott Architect, and the molecular HCV RNA assay is a confirmatory test by using a reverse transcription-polymerase chain reaction method.

**Results:** The diagnostic sensitivity, specificity and the positive and negative predictive values of the HCV core antigen assay compared to the HCV RNA test were 96.3%, 100%, 100%, and 89.7%, respectively. The levels of HCV core antigen showed a good correlation with those from the HCV RNA quantification ( $r = 0.907$ ).

**Conclusion:** In conclusion, the Architect HCV Ag assay is highly specific, sensitive, reliable, easy to perform, reproducible, cost-effective and applicable as a screening, supplemental and pre-confirming test for anti-HCV assays in the laboratory procedures used for the diagnosis of hepatitis C virus infection.

**P2304 HCV antigen testing in a busy diagnostic laboratory**

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**Objectives:** Studies have indicated that the ARCHITECT® HCV Ag assay can be used as an alternative to HCV RNA testing. Our aim was to determine if the HCV antigen assay could be used as a replacement for HCV antibody (anti-HCV) and/or HCV PCR testing for hepatitis screening. The clinical utility of HCV antigen (Ag) test in two different clinical situations: hepatitis screening and follow up of patients in a renal dialysis unit was assessed.

**Methods:** The Abbott ARCHITECT® HCV Ag assay was used following the manufacturers protocol. The Abbott ARCHITECT® Anti-HCV assay and an in-house real-time HCV PCR test were compared to the HCV antigen levels in the above mentioned sample groups.

**Results:** There were 4768 samples sent for routine hepatitis screening (anti-HCV) which were also tested for HCV Ag (Table). Fourteen samples were HCV Ag positive anti-HCV negative, only five were PCR positive, indicating acute infection. These five samples were taken from three patients. The remaining nine samples were false positives. Analysis of samples with Ag levels <35 fM indicated that 37% of these low positive values were actually false positives. There were two patients who contracted HCV infection in a renal dialysis unit. In accordance with Department of Health guidelines follow up of all renal patients exposed was required. Over a period of 6 months there were 1438 samples from 435 patients taken from four different renal units tested. The two infected renal patients had a total of 27 PCR and HCV Ag tests done during this time period, all samples gave a Ag result while 33% of the samples had an invalid PCR result due to inhibitory factors within the positive samples.

	HCV PCR result					HCV antibody			Number tested
	PCR >1000IU/ml	PCR <1000IU/ml	PCR negative	PCR invalid	PCR not tested	Anti-HCV positive	Anti-HCV weak reactive	Anti-HCV negative	
HCV Ag Negative (<3 fmoIU)	0	7	261	2	4297	203	9	4355	4567
HCV Ag Positive (>=10 fmoIU)	155	2	7	2	7	159	0	14	173
HCV Ag Equivocal (3-10 fmoIU)	3	4	15	3	3	10	0	18	28

**Conclusion:** Using the ARCHITECT® HCV Ag assay as a 1st line screening test we were able to detect three acute infections in patients that would otherwise have been missed by routine screening of anti-HCV. The test was of value during the renal unit outbreak, where four renal units were involved and where invalid PCR results frequently occur within renal dialysis patient samples. We found that low positive antigen values <35fM should be retested. It is important to determine if a patient has an active HCV infection, rather than patients who have had and cleared an HCV infection in the past (anti-HCV positive only). We propose to use only the ARCHITECT® HCV Ag assay as our front line screening test.

#### **P2305** Efficacy of early diagnosis and treatment of patients with acute hepatitis C with interferon-alpha 2b monotherapy

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**Objectives:** (i) To investigate the effects of treatment with interferon alfa-2b on rapid virological response (RVR) and sustained virological response (SVR) and correlation between RVR and SVR. (ii) To investigate the effectiveness of different duration of therapy on different viral genotypes. (iii) To investigate the changes of biochemical parameters during and after therapy.

**Materials and methods:** (i) Patients are diagnosed with acute hepatitis C based on epidemiological anamnesis, symptoms, cyclic characteristics of disease, results of biochemistry test (aminotransferases, bilirubin, serum proteins), detection of HCV RNA and their genotype by PCR and HCV antibodies to the virus by ELISA. (ii) Patients with acute hepatitis C are initiated with interferon-alfa 2b therapy (Altevir®, 3 million units three times a week). Duration of therapy for patients with undetected viral genotype and viral genotype 1 is 24 weeks whereas for genotype 2 and 3 is 12 weeks. (iii) Levels of HCV RNA and biochemical parameters are monitored for the duration of 48 weeks.

**Results:** Fifteen patients are diagnosed with acute hepatitis C (average age = 31.87 ± 14.54, Male = 80%, Female = 20%). Rapid virological response (RVR) or undetectable plasma hepatitis C virus (HCV). RNA is achieved on fourth week in 93.3% of patients and on eighth week in 6.67% (a patient with viral genotype 1). Sustained virologic response (SVR), defined as undetectable HCV RNA at least 6 months after cessation of therapy is achieved in all patients. 33.33% of patients have normalization of aminotransferases on 12th week after starting of therapy, 16.67% patients on 24th week, 16.67% on 36th week and

33.3% on 48th week. Although RVR, SVR and normalization of biochemical parameters have been detected, observation of all patients will be continued for once every 6 months for another 2 years.

**Conclusion:** RVR is achieved in most patients during the 4th week of therapy and during 8th week in a patient. SVR is achieved in all patients. In this connection, RVR on 4th and 8th week can be a good indicator of which patients will go on to achieve SVR. Treatment for 12 weeks was effective in patients with viral genotype 3, whereas genotype 1 required 24 weeks of therapy. Normalization of biochemical parameters (aminotransferases) has been detected in all patients but at different time intervals. Interferon-alfa 2b monotherapy is cheap (socio-economic relevance), effective in treating acute hepatitis C infection and decreases the risk of progression to chronic disease.

#### **P2306** Outcome of interferon-based therapy on fibrosis stage at patients with chronic hepatitis C genotype 1b depending on IL28b genetic variants

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**Objectives:** Today a direct link between rs12979860 C/T polymorphism in the IL28B gene and sustained virology response (SVR) rate in patients with chronic hepatitis C (CHC) genotype 1 is established. The modern clinical observations allow us to suggest a similar relation between T allele of this gene and accelerated CHC progression towards cirrhosis and Hepatocellular Carcinoma (HCC). Our aim was to assess the differences in the dynamic of HCV-related fibrosis by non-invasive tests in the outcome of interferon-based therapy in patients with different genetic variants of IL28b.

**Methods:** In a confirmatory factor analysis (CFA) liver fibrosis and rs12979860 genotypes were analyzed in 48 patients with CHC 1b genotype. Fibrosis by METAVIR score was determined before and after standard interferon-based therapy using non-invasive method Fibrotest. IL28b genotypes were determined by melting curve analysis of hybridization probes using the LightCycler.

**Results:** SVR was achieved at 66.6% of treated patients, 33.3% were non-responders and relapsers. Among patients with «cc» genotype SVR was achieved in 90.5%, among «ct\*/t't» genotype IL28b – in 48%. Twenty-five percent of patients achieved significant decrease in fibrosis score (average 0.35 points by Fibrotest scale), 39.6% patients – moderate decrease (average 0.17 points); 27% – no decrease and 8.3% had increase in fibrosis score (average 0.24 points). The highest average degree of decrease in fibrosis of 0.19 points occurred in patients with genotype «cc» of IL28b vs. 0.1 points in patients with genotype «ct» and «t't» of IL28b (p < 0.05). We found no significant correlation between the degree of fibrosis decrease and its baseline value.

**Conclusions:** This study showed that T allele of the rs12979860 polymorphism of the IL28b gene may be used as negative predictor for fibrosis regression in patients with CHC 1b after interferon-based therapy. It may be recommended to continue observation for fibrosis or HCC progression in carriers of T allele of the rs12979860 polymorphism of the IL28b gene even in case of achieved SVR.

#### **P2307** Changing HCV seroepidemiology and low vaccination-induced protection rates against hepatitis A and B among active injecting drug users in Athens, Greece: a 10-year retrospective study of the Greek Organisation Against Drugs (OKANA)

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**Objectives:** Viral hepatitis represents a well known health problem among injecting drug users (IDUs). The aim of the study was to determine the prevalence of serological markers for viral hepatitis A, B and C among a large group of active IDUs in Athens metropolitan area.

**Methods:** Between 1997 and 2007 a total of 2.668 active IDUs who admitted to the Emergency Help and Support Unit of the Greek

Organization Against Drugs were serologically evaluated for viral hepatitis A (anti-HAV), B (HBsAg, antiHBc, antiHBs) and C (anti-HCV), using commercially available methods.

**Results:** The overall prevalence of anti-HAV, HBsAg, antiHBc, antiHBs and anti-HCV was 36.21% (966/2668), 3.52% (94/2668), 45.01% (1201/2668), 40.82% (1089/2668) and 72.23% (1927/2668), respectively. The majority of anti-HCV (1137/1927, 59%) and antiHBc (806/1201, 67.11%) positive cases reported injecting drug use beginning before 1991 ( $p < 0.0001$ ). There was a significant reduction in anti-HCV seroprevalence among users who reported injecting drug use beginning in '70s (653/762, 85.7%), '80s (734/970, 75.67%), '90s (404/716, 56.42%) and after 2000 (15/44, 34.09%),  $p < 0.001$  in all comparisons. Isolated antiHBs positivity was observed in 338 (23.04% of the antiHBc-negative) cases, mainly in those who reported injecting drug use beginning after 1991 (235/338, 69.53% of isolated antiHBs positive cases). There was also observed a significant age-related reduction in anti-HAV seroprevalence (15–24 years: 14.09%, 25–34 years: 22.24%, 35–44 years: 42.94%, 45–54 years: 77.47%, 55–64 years: 91.48%, >65 years: 100%,  $p < 0.001$  in all comparisons).

**Conclusion:** Although hepatitis C remains the major infectious diseases related health problem among active IDUs the anti-HCV seroepidemiology is changing during the last decades. Less than a fourth (23.04%) of the susceptible study population was effectively vaccinated against hepatitis B whereas the vast majority (>85%) of IDUs younger than 25 years were unexposed/unvaccinated and susceptible to hepatitis A.

#### **P2308** Role of the CD4+ count monitoring during pegylated interferon therapy in chronic HCV infected patients

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Even though hematological cytopenias are the most frequent adverse effects during interferon treatment, no data exist concerning the decrease of lymphocytes, especially CD4+.

**Objectives:** Describing the variation in lymphocyte and CD4 count during interferon treatment in order to identify the need for opportunistic infections prophylaxis.

**Methods:** Prospective cohort study of patients receiving pegylated interferon. Lymphocyte counts, CD4+, CD8+, CD4/CD8 ratio were evaluated at baseline, and after 3, 6, 9 and 12 months of interferon.

**Results:** One hundred and twenty-one patients with HCV hepatitis have met the inclusion criteria. The lymphocyte counts decreased during treatment; the mean decrease was  $1442.3/\text{mm}^3$ . The mean value of CD4+ at baseline was  $838.2/\text{mm}^3$  (SD:  $\pm 184.9$ , CI95%: 813.4; 898.9). The mean decrease of CD4 count vs. baseline was:  $342.32/\text{mm}^3$  at 3 months (SD:  $\pm 201.32$ ; CI95%: 292.58; 372.27),  $445.36/\text{mm}^3$  at 6 months (SD: 178.95; CI95%: 378.2; 484.4),  $456.26/\text{mm}^3$  at 9 months (SD: 194.5; CI95%: 411.27; 514.33),  $470.33/\text{mm}^3$  at 12 months (SD: 186.45; CI95%: 403.75; 538.19). No patient had at baseline a CD4+ count below  $200/\text{mm}^3$ . The number of patients with CD4+ below  $200/\text{mm}^3$  significantly increased between visits (4.5% at 3 months, 15.8% at 6 months, 21.9% at 9 months, 25.3% at 12 months) ( $p = 0.00004$ ). The decrease of CD4+ count was correlated with the decrease of lymphocytes. The CD4/CD8 ratio also decreased during interferon treatment: the CD4/CD8 ratio below 1.5 was recorded in 8.32% of patients at baseline and 46.96% of patients at end of treatment ( $p = 0.005$ ). We also reported a patient who developed severe pneumonia during interferon therapy. The diagnosis was *Pneumocystis jirovecii* pneumonia. The CD4 level was  $254/\text{mm}^3$  after 6 months and  $210/\text{mm}^3$  after 9 months of treatment. In the last week of treatment when pneumonia was diagnosed the CD4 count was  $98/\text{mm}^3$  and ratio CD4/CD8 was 0.8.

**Conclusions:** The CD4+ count may decrease in some patients receiving interferon to levels that increase the risk of opportunistic infections. More important seems to be the ratio CD4/CD8. The monitoring of CD4 count in patients who developed lymphocytopenia is very important.

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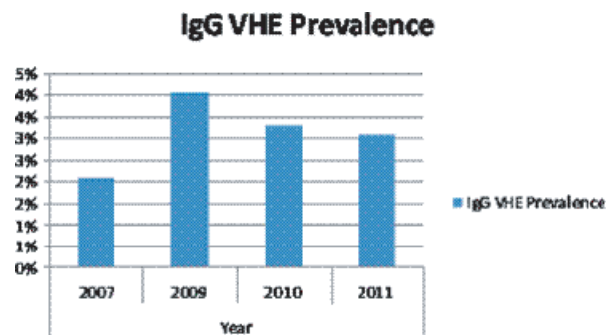
#### **P2309** Has the prevalence of hepatitis E infection changed among pregnant women in Spain?

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**Objective:** Hepatitis E virus (HEV) causes a high number of epidemic outbreaks in low-income countries, whereas only sporadic cases, especially in travelers, are described in developed countries. Although acute HEV infection usually causes mild and self-limited hepatitis, fulminant hepatic failure and high mortality frequently can occur in pregnant women during the third trimester. The aim of this work was to determine the seroprevalence of HEV antibodies in pregnant women and the clinical features related to its detection in blood.

**Method:** One thousand three hundred and sixty-four pregnant women (mean age 30 years, 15–45) attended in the Obstetric Department for routine screening, were included in this study. One hundred and eighty-one women were screened in 2007, 588 in 2009, 271 in 2010 and 324 in 2011. None of the patients presented clinical symptoms related with hepatitis at the time of blood sample collection. For the IgG anti-HEV antibodies detection in serum, a commercial immunoenzymatic method was employed and all positive samples were further studied for the presence of IgM anti-HEV antibodies (HEV Ab and HEV IgM; Dia.Pro Diagnostic Bioprobes, Milan, Italia). A result was considered positive when the sample's optical density/cut off optical density was superior to 2. Positive results by the immunoenzymatic method were confirmed by Western Blot analysis (RecomBlot HEV IgG/IgM; Mikrogen, Martinsried, Germany). In those patients presenting positive results, transaminases ALT/AST levels and clinical symptoms were assessed.

**Results:** the prevalence of anti-HEV IgG among the pregnant women population in Madrid was 2.1% (5/181) in 2007, 4.08% (24/588) in 2009, 3.3% (9/271) in 2010 and 3.09% (10/324) in 2011, with an overall prevalence of 3.51% (48/1364). 0.61% (2/324) tested positive for anti-HEV IgM antibodies which is used as an acute phase marker of HEV infection. None of the woman with a positive data for IgM or IgG presented symptoms.



**Conclusions:** The rate for IgG antibodies in serum among pregnant women in Madrid in 2011 was 3.09% which indicates that the seroprevalence has not changed in the last 5 years despite the increasing rate of immigration and travels to endemic areas.

**P2310** Genotypic, drug resistance and phylogenetic analysis of reverse transcriptase gene of hepatitis B virus from patients concurrent for HBsAg and anti-HBs tests in China

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**Objective:** Antibody to hepatitis B surface antigen (HBsAg) (anti-HBs) can exist in patients with chronic hepatitis B virus (HBV) infection. This present study was to analyze the genotypic profile, drug resistance pattern and the evolution pattern of HBVs from patients positive for HBsAg and anti-HBs tests.

**Methods:** HBV reverse transcriptase (RT) sequences were isolated and sequenced from 88 HBV carriers, including 21 cases concurrent with anti-HBs. The nucleotide RT sequences obtained were translated into amino acid sequences to analyze the drug resistance pattern, aligned and compared with HBV sequences of the same genotype found in the genotyping reference set available on the NCBI Web site. MEGA version 4.0 was used to reconstruct phylogenetic trees using the maximum likelihood method under best-fit model, which was estimated by jModeltest 0.1.1 in advance. HBV diversity was evaluated by three parameters: the mean genetic distance (d), the number of synonymous substitutions per synonymous site (dS) and the number of non-synonymous substitutions per non-synonymous site (dN).

**Results:** 0.74% patients with chronic HBV infection were positive for anti-HBs. HBV carriers with anti-HBs were easier to infect genotype C HBVs than patients without anti-HBs (61.9% vs. 43.3%) in present study. The resistant frequencies of HBV isolates to Lamivudine, Telbivudine, Adefovir and Entecavir are 9.5%, 4.8%, 13.6% and 0%, respectively. The YMDD mutations in Lamivudine resistant patients infected with genotype B HBVs were much more common than that in patients infected with genotype C HBVs ( $p < 0.05$ ). The genetic diversity of genotype C HBVs was higher than that in B genotype HBVs ( $p < 0.05$ ). And the nucleotide substitution type of genotype C HBV isolates from HBsAg and anti-HBs patients were main the transition ( $p < 0.05$ ).

**Conclusion:** Concurrent HBsAg and anti-HBs was still not a common serologic pattern in our district, suggesting anti-HBs did not indicate recovery and immunity from re-infection any longer. HBsAg carriers with anti-HBs tended to be infected with genotype C HBVs, the diversity of which mainly caused by transition instead of transversion, seemed more complicated and less Lamivudine resistant than that of genotype B HBVs.

**P2311** Performance evaluation of VIDAS® Anti-HCV, a new automated immunoassay test for the qualitative detection of antibodies anti-HCV in human serum and plasma samples

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**Objective:** The VIDAS® automated system (bioMérieux) using the Enzyme Linked Fluorescent Assay (ELFA) technique is suitable for routine, emergency, specific or complementary testing, notably in infectious diseases diagnosis. In view to complete its existing HIV-Hepatitis (HAV and HBV) panel, a VIDAS® Anti-HCV prototype is currently in development allowing qualitative detection of antibodies to hepatitis C virus (anti-HCV) in human serum or plasma. We performed an evaluation of this prototype in terms of sensitivity and specificity of VIDAS® Anti-HCV compared to four already CE-marked tests: Architect (Abbott), Elecsys (Roche), Centaur (Siemens) and Ortho HCV 3.0 ELISA (Ortho-Clinical diagnostics).

**Methods:** The VIDAS® Anti-HCV principle combines a two-step enzyme immunoassay indirect sandwich method with a final chemiluminescence detection. Anti-HCV antibodies present in the sample bind with antigens representing the HCV core, NS3 and NS4

proteins (solid phase) and with a monoclonal anti-human IgG antibodies conjugated to alkaline phosphatase (revelation step).

Sensitivity study was performed on 150 in-house collection of low and high level Anti-HCV positive samples, 252 positive specimens of different genotypes and 30 seroconversion panels.

Specificity study was performed on 5216 French blood donor samples, 203 negative hospitalized patients and 273 putative interference specimens. Negative and positive status of the specimens were provided by reference methods.

**Results:** The comparative study performed on 150 samples shows that VIDAS® Anti-HCV has a sensitivity equivalent to Architect, Centaur and Elecsys systems while Ortho seems less sensitive for low positive samples. Proper antibody detection of the VIDAS assay was demonstrated for the six HCV genotypes and analysis of the 30 seroconversion panels showed that VIDAS® Anti-HCV is among the best tests for early detection of anti-HCV.

Study on the blood bank donor specimens demonstrated a high specificity of 99.7%. In the comparative specificity study performed on 450 negative samples, VIDAS® Anti-HCV showed comparable performance to the four others competitors (>99.50%) as also observed on the 476 negative clinical samples (hospitalized and putative interference populations).

**Conclusion:** Evaluation of VIDAS® Anti-HCV sensitivity and specificity showed that this new prototype VIDAS assay is as performant as other already CE-marked tests, notably Architect and Elecsys assays.

**P2312** Evaluation of Liaison® XL Murex HCV AB assay under routine laboratory conditions

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**Introduction:** The LIAISON® XL MUREX HCV Ab assay is a new assay for the detection of hepatitis C (HCV) antibodies in human serum or plasma specimens. This assay uses chemiluminescence immunoassay (CLIA) technology for the qualitative determination of specific antibodies to hepatitis C virus (anti-HCV). This method is an indirect CLIA designed with two recombinant antigens (core and NS4) specific for HCV coating magnetic particles (solid phase), while a third HCV antigen (biotinylated NS3) is provided lyophilized, as a separate reagent.

**Objective:** To evaluate LIAISON® XL MUREX HCV Ab assay under routine laboratory conditions and to compare results with the method in use in our laboratory (Architect® HCV Ab assay, Abbott).

**Material and methods:** A total of 444 unselected serum samples received from April to May of 2011 in our laboratory for routine HCV-antibody screening were analysed by both assays. Additionally 71 samples from hemodialysis patients were tested. Discordant results were resolved by InnoLIA HCV assay. All assays were performed and interpreted according to the manufacturers' instructions.

**Results:** Of 515 tested samples, 487 were negative and 20 positive by both assays (concordance/agreement 98.4%). Eight discordant results were obtained, four positive results only by Liaison XL and four positive only by Architect; only one discordant result was from hemodialysis patients. All this samples showed negative results by InnoLIA. S/CO values of discordant samples were 1.1, 1.7, 2.1 and 2.6 by LiaisonXL and 1.6, 2.4, 3.0 and 7.7 for Architect. Mean S/CO values for true positives samples were 5.39 (SD: 2.34) by Liaison XL and 10.81 (SD: 4.54) by Architect. Mean S/CO for true negatives were 0.048 (SD: 0.091) by Liaison XL and 0.045 (SD: 0.047) by Architect. After implementation of the results of the InnoLIA, the calculated sensitivity of the Liaison XL assay was found to be 100% (CI 79.95–99.54%), the specificity 99.19% (CI 97.80–99.74%), PPV 83.33% (CI 61.81–94.52%) and NPV 100% (CI 99.03–99.98%).

**Conclusions:** We observed a good overall agreement between the two assays under routine laboratory routine conditions. Both assays showed equal false positives results.

**P2313** Source of outbreak of nosocomial hepatitis C virus infection resolved by genetic analysis of HCV RNA from a 4-month exhumed body

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**Objective:** Outbreaks of nosocomial transmission of hepatitis C virus (HCV) have been linked to interventions such as surgery and dialysis and breaches in failure to follow standard procedures. In January 2006, acute hepatitis C was diagnosed in six patients who were hospitalized in the two adjacent wards. Investigation of other patients hospitalized in the same period identified two patients with chronic hepatitis. We report an outbreak of patient-to-patient transmission of HCV through the use of a multidose vial during the rinsing of central venous catheters.

**Methods:** A forensic investigation was started to identify the mechanism which posed a risk factor of transmission to the six patients. Forensic investigators retraced the route used by the two ward nurses, when saline flushes were given to 14 patients with each nurse administering to seven patients.

As part of the forensic investigation blood samples were taken from each of the case patients for the comparative analysis of their HCV RNA strains. No samples were available for one of the patient's, who died before the investigation started, and regardless of the known lability of HCV, the cadaver was exhumed 4 months after burial. HCV RNA was amplified, identified and genotyped in liver and spleen samples.

Genotyping of HCV strains was performed by sequence analysis of the 5'NC UTR and E1 Core conserved regions and from the E1E2 hypervariable region.

**Results:** The only shared event involving all patients was blood collection from the indwelling central venous catheters. Forensic investigators demonstrated that the only period in which the unique event causing HCV infection could occur, was between 13 and 14 December 2005 and interviews with the two nurses showed that the transmission event could only have taken place between 7:00 and 7:30 AM on 14 December 2005. The comparative phylogenetic analysis of the strains identified in the patients studied allowed identification of the source of contamination, which was the same for five patients.

**Conclusion:** This study highlights the value of sequence analysis as a tool for solving medical-legal conflicts. In January 2011, the high court of justice sentenced that a health worker's reuse of a contaminated needle resulted in the transmission of HCV. As in multiple historical situations no definitive confirmation of the mode of transmission was possible and it was impossible to identify which of the two health worker's was responsible.

## Improving the detection of beta-lactamases

**P2314** Evaluation of laboratory methods used for detecting carbapenemase-producing Enterobacteriaceae from surveillance specimens

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**Background:** Although halting the spread of CRE has become an international infection control priority, optimal methods for detecting CRE from surveillance specimens have not been established. Using a panel of well-characterized multidrug-resistant (MDR) Enterobacteriaceae (ENT), this retrospective study compared three approaches to CRE detection: (i) CRE screen agars, (ii) ESBL screen agar followed by CLSI ETP disc diffusion (DD), and (iii) ESBL agar followed by the ETP agar spot method.

**Methods:** The 265 study ENT included 116 (43.8%) CRE (90 blaKPC-2/3; 46 *K. pneumoniae* [KPN], 32 *E. cloacae* [ECL], 7 *E. coli* [ECO], 3 *E. aerogenes*, 1 *C. freundii*, 1 *K. oxytoca*; 20 blaNDM-1:

12 KPN, 4 *M. morgani*, 2 ECL, 2 ECO; 4 blaOXA-48: 3 KPN, 1 ECO; 2 blaSME *S. marcescens*) and 149 (56.2%) non-CRE (PCR-confirmed). Isolates were subbed from -80°C onto MacConkey plain agar (Oxoid) and ESBL (Oxoid MacConkey#3 [MAC3] plus 2 mg/L cefpodoxime) agar with ETP discs (Oxoid) to maintain selective pressure. Suspensions equivalent to 0.5 McFarland STD were prepared from growth following to the discs, 10 µL of which was then inoculated to each of the following CRE agars and streaked for isolated colonies: Colorex KPC (Alere), Brilliance CRE (Oxoid), MAC3 (Oxoid) supplemented with 0.5 mg/L and 1 mg/L ETP, MAC3 with 1 mg/L imipenem (IMI). The same suspensions (derived from ESBL agar) were then spotted to Mueller-Hinton (MHA, Oxoid) supplemented with 0.5 and 1 mg/L ETP, and finally inoculated as per CLSI to MHA for ETP DD testing. After 37°C incubation for 18–20 hours, screen agars were examined simultaneously by independent readers blinded to each others results; MHA-ETP spot and ETP DD were read at 16–18 hours after 35°C incubation as per CLSI.

**Results:** Notably, as seen in the Table, all CRE screen agars missed CRE: blaNDM-1 CRE by MAC-ETP 0.5 mg/L, blaNDM-1 and blaKPC-2/3 CRE by MAC-ETP 1 mg/L, and MAC-IMI, blaNDM-1, blaKPC-2/3 and blaOXA-48 CRE by Colorex KPC, and blaNDM-1, blaKPC-2/3, blaOXA-48 and blaSME CRE by Brilliance CRE. In contrast, spot and DD methods prepared from ESBL agars detected all CRE.

Screening methods for detecting carbapenem-resistant Enterobacteriaceae among 265 multidrug-resistant isolates	% Carbapenem-resistant Enterobacteriaceae (CRE) detected (95% CI)				% Overall CRE detected (95% CI) (n=116)	% Non-CRE detected (95% CI) (n=149)
	NDM (n=20)	KPC (n=90)	OXA-48 (n=4)	SME (n=2)		
Colorex KPC agar (Alere)	91.7 81.5-96.8	98.9 86.9-99.8	91.7 62.5-99.9	100 55.7-100	97.4 95.1-98.7	32.1 28.1-36.7
Brilliance CRE agar (Oxoid)	95.7 89-99.8	92.2 88.4-94.9	91.7 62.5-99.9	0 0-44.3	91.4 87.9-93.9	62.6 58.1-67.7
McConkey3 agar with imipenem at 1mg/L (in-house)	95 74.7-99	96.6 89.8-98.6	100 45.4-100	100 29-100	95.7 90.1-98.4	36.2 29.4-44.2
McConkey3 agar with entapenem at 0.5mg/L (in-house)	90 68.7-98.4	100 95.1-100	100 45.4-100	100 29-100	98.3 93.6-99.9	51.7 43.7-59.6
McConkey3 agar with entapenem at 1mg/L (in-house)	90 68.7-98.4	96.7 90.3-99.3	100 45.4-100	100 29-100	95.7 90.1-98.4	35.6 28.3-43.5
ESBL agar (Oxoid) then entapenem 1mg disc diffusion on Mueller Hinton agar (Oxoid)	100 81-100	100 95.1-100	100 45.4-100	100 29-100	100 96.2-100	32.9 25.8-40.8
ESBL agar (Oxoid) then Mueller Hinton entapenem 0.5mg/L spot plate (in-house)	100 81-100	100 95.1-100	100 45.4-100	100 29-100	100 96.2-100	39.6 32.1-47.6
ESBL agar (Oxoid) then Mueller Hinton entapenem 1mg/L spot plate (in-house)	100 81-100	100 95.1-100	100 45.4-100	100 29-100	100 96.2-100	38.9 22.2-56.6

**Conclusions:** Only the ESBL agar followed by ETP DD (most readily available method) or ESBL agar followed by MHA ETP spot detected all CRE, with the MHA ETP 1 mg/L spot showing best performance growing the least non-CRE. However, both methods result in a 24 hours reporting delay compared to the CRE screen agars. But crucially, all CRE screen agars missed some CRE while growing comparable or higher numbers of non-CRE.

**P2315** Strategy of detection of carbapenemase-producing Enterobacteriaceae

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**Objectives:** The rapid dissemination of carbapenemase producers in Enterobacteriaceae strengthens the necessity of their accurate identification. We evaluated the performance of several tests for early detection of those carbapenemase-producing isolates.

**Methods:** Fifty enterobacterial isolates being resistant or of reduced susceptibility to carbapenems were tested. They expressed KPC (n = 10), VIM, IMP, and NDM (n = 11), OXA-48 (n = 21) Non-carbapenemase producers expressed an ESBL or overpressed AmpC associated with outer-membrane porin defect (n = 8). MICs of carbapenems were determined by Etest with and without EDTA. Modified Hodge test (MHT) was performed on Mueller Hinton agar with or without zinc sulfate (100 µg/mL). The Rosco kit (Rosco Diagnostica) including meropenem disks supplemented with inhibitors of class A (aminophenylboronic acid, APAB), class B (dipicolinic acid, DPA), or class C (oxacillin) beta-lactamases was evaluated. The lowest detection limit of the carbapenemase producers was determined using three selective culture media; ChromID ESBL containing a cephalosporin (bioMérieux), CHROMagar KPC containing a

carbapenem (CHROMagar company), and Brilliance CRE containing a carbapenem (OXOID).

**Results:** Class A and D carbapenemase producers were detected by the MHT, whereas only 40% of the NDM-producers were detected ( $n = 2/5$ ). Addition of zinc sulfate improved the performance of the MHT, but the overall sensitivity and specificity of this test was low (77% and 39%, respectively). The Rosco test was efficient for detection of MBL and KPC producers, but failed to identify OXA-48 producers since it does not contain class D inhibitors. Sensitivity and specificity of detection of carbapenemase producers for the ChromID ESBL medium (77% and 12.5%) were comparable to that of Brilliance CRE medium (69% and 25%). The sensitivity of CHROMagar KPC (26%) was lower but its specificity (50%) was higher. Those poor sensitivities were mostly due to difficulties in detection of OXA-48 producers.

**Conclusion:** None of these tests showed an excellent sensitivity for detecting all types of carbapenemase producers. The use of inhibitors such as APAB, EDTA, or DPA is a powerful tool for detecting Ambler class A and B carbapenemases. Selective culture media for detecting all types of carbapenemase producers remain to be significantly improved in particularly for detecting the emerging OXA-48 producers that are spreading in North Africa, Turkey, Middle East and Europe.

**P2316** Evaluation of various culture media and procedures recommended for isolation of Enterobacteriaceae that produce NDM-1 carbapenemase

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**Objectives:** A number of procedures and/or culture media have been recommended for isolation of carbapenemase-producing Enterobacteriaceae (CPE) and new chromogenic media designed for their isolation have been commercialised. We sought to compare the performances of the available media and the recommended methods for isolation of NDM-1 producing Enterobacteriaceae.

**Methods:** Sixty-four previously identified NDM-1 producing Enterobacteriaceae were inoculated at  $10^5$  and  $10^2$  colony forming units (CFU) per 1  $\mu$ L spot on to media designed or recommended for isolation of CPE: Brilliance CRE (B-CRE, Oxoid), Colorex KPC (C-KPC, E&O), chromID ESBL (ID ESBL, bioMérieux), chromID CARBA (ID CARBA, prototype provided by bioMérieux) and MacConkey agar plus 1 mg/L imipenem (MC-I). Tryptone Soya Broths (TSB) plus 2 mg/L ertapenem (TSB-E) or 2 mg/L meropenem (TSB-M) were also challenged with 7500 CFU of each isolate and then subcultured onto MacConkey agar (CDC recommended method for rectal swab screening). Hundred local stool samples were also inoculated onto all media. All Gram-negatives from the 100 stool samples on any of the media were investigated for carbapenemases, AmpC and ESBLs by phenotypic methods.

**Results:** Using ID ESBL and ID CARBA, respectively 98% and 97% of the NDM-1 producers were recovered at both inocula. At high inoculum, B-CRE, C-KPC and MC-I recovered 80%, 98% and 49% of NDM-producers respectively. At low inoculum, B-CRE, C-KPC and MC-I recovered 77%, 73% and 14% of NDM-producers respectively. CDC method with TSB-E or TSB-M recovered 89% and 61% of isolates respectively. No CPE were recovered from the 100 local stool samples. A number of non-CPE recovered from the 100 stool samples required processing from the various media including C-KPC ( $n = 4$ ), B-CRE ( $n = 6$ ), ID CARBA ( $n = 14$ ), ID ESBL ( $n = 38$ ), MC-I ( $n = 59$ ), TSB-E ( $n = 167$ ), TSB-M ( $n = 155$ ). A confluent growth of enterococci was frequently recovered from the selective TSB broths. Further data evaluating Brilliance CRE and ID CARBA in two Pakistan hospitals using 400 stool samples will be presented.

**Conclusion:** In the present study, the methodologies described in the literature for isolation of CPE present important limitations to their sensitivity and/or specificity when attempting to isolate NDM-1 producing Enterobacteriaceae, particularly if low numbers of organisms are present. The higher performances of chromogenic media remain to be confirmed with naturally contaminated samples.

**P2317** How to improve detection of ESBL in ICU patients?

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**Objective:** In order to improve the yield of different screening policies for Enterobacteriaceae with extended-spectrum beta-lactamase (ESBL), from sample frequency to optimal selective medium, we performed a study with a daily sampling during the entire stay of intensive care units (ICU) for all patients (Pt).

**Methods:** All admissions in medical and surgical ICUs between 3 April and 3 July 2011 were included. They were screened daily from the first 24 hours (AS: admission sample) to the last day (DS: discharge sample) using eSwab<sup>®</sup> (COPAN) containing 1 mL of modified liquid Amies (MLA). Fifty microlitre of MLA were inoculated to 3 mL of BHI (COPAN) and incubated at 37°C for 24 hours. For each sample, 10  $\mu$ L of MLA and 10  $\mu$ L of BHI were plated on five different selective agar medium: M1 = CHROMagar ESBL<sup>®</sup> (CHROMagar), M2 = ChromID<sup>®</sup> (Bio-merieux), M3 = Brilliance ESBL<sup>®</sup> (Oxoid), M4 = Drigalski agar ceftazidime<sup>®</sup> (Becton Dickinson), M5 = Drigalski + cefotaxime 5% (home medium), via a system for automatic planting (WASP<sup>®</sup>, COPAN/Siemens).

**Results:** Two hundred and seventy-eight patients were included in the study corresponding to 2449 patient-days (PD). The global screening completeness was 97.3% (2384/2449): 99.6% and 96.4% for AS and DS respectively. At least one positive ESBL sample was found in 52 patients (EPT), (prevalence, 18.7%; incidence, 21.2/1000 PD). From those 2384 samples, 26 224 Petri dishes were plated by the WASP<sup>®</sup>, the equivalent to the activity of a full time technician. For the 552 positives samples, 355 were ESBL only, the others cases were ESBL associated with high level cephalosporinase or high level cephalosporinase only or *Pseudomonas aeruginosa*. The rate of positive ESBL-PD was 14.9% (355/2384). The mean length of stay was 8.8 and 11 days, for Pt and EPT respectively. Among the 63 ESBL isolated, 37 were *Escherichia coli* and 20 *Klebsiella pneumoniae*. The variation of correlation between two by two media were 76.4–89.2% (kappa score 0.61–0.81). Among the 355 ESBL, 19 (5.5%) were only positive with BHI. We determined the sensitivity and the specificity of those media taking M5 as reference.

	Se (%)	Sp (%)
M1	99,2	97,1
M2	99,3	99,4
M3	99,1	97,6
M4	99,1	95,9

**Conclusion:** Increasing the number of sample is needed to allow better detection of ESBL with, at least two screening. All media tested have very good sensitivity but M2 have the best specificity. If BHI was not contributive in this study, the use of the WASP was particularly appropriate for assuming a large number of samples.

**P2318** Broth microdilution test to detect KPCs and metallo-beta-lactamases in clinical isolates of Gram-negative bacilli by use of beta-lactamase inhibitors

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**Objectives:** The present study was designated to evaluate the performance of the broth microdilution (BMD) test to detect production of KPCs and metallo-beta-lactamases (MBLs) in clinical isolates of Gram-negative bacilli by using various beta-lactamase inhibitors.

**Methods:** A carbapenemase detection test, comprising Mueller–Hinton broth containing serial twofold dilutions of imipenem or meropenem with and without aminophenylboronic acid (APBA), phenylboronic



acid (PBA), cloxacillin (CLX), dipicolinic acid (DPA), or EDTA, was evaluated against 31 *Klebsiella pneumoniae* with KPC, 21 MBL-producers (1 *K. pneumoniae* with NDM-1, 2 *Enterobacter cloacae* with VIM-2, 14 *Pseudomonas aeruginosa* with VIM, three *P. aeruginosa* with IMP, and 1 *Acinetobacter genomospecies* 3 with SIM-1), and 16 Enterobacteriaceae with AmpC hyperproduction in combination with porin loss. The test organisms comprised clinical isolates previously characterized by appropriate biochemical, phenotypic, and molecular procedures determine their types of carbapenemase production. An eightfold or greater decrease in the MIC of imipenem or meropenem containing beta-lactamase inhibitors, as compared with the imipenem or meropenem alone, was considered to be a positive results for APBA, PBA, CLX, DPA, and EDTA.

**Results:** Imipenem with and without PBA had most comparable sensitivity (94%) and specificity (95%) for detecting *K. pneumoniae* with KPC enzymes if additional criterion of a negative CLX result was included. Both DPA and EDTA had excellent sensitivity (imipenem with and without DPA vs. EDTA, 90% vs. 95%; meropenem with and without DPA vs. EDTA, 95% vs. 86%) and specificity (imipenem with and without DPA vs. EDTA, 98% vs. 100%; meropenem with and without DPA vs. EDTA, 98% vs. 98%) for detection of MBL-producing Gram-negative bacilli.

**Conclusion:** The comparative study showed that BMD test by using imipenem with and without PBA, CLX, and EDTA is the most effective results for detection of KPC and MBL enzymes. The BMD test could be applicable for routine use in commercially available semiautomated systems for detection of KPCs and MBLs in Gram-negative bacilli.

## MALDI-TOF in the bacteriology lab

### P2319 Use of MALDI-TOF mass spectrometry for analysis of virus-infected cells: a preliminary report

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**Objectives:** The diagnosis of viral infection traditionally relies on direct methods based on cell culture, antigen or nucleic acid detection. This study aimed to demonstrate the matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) ability in detecting specific biomarkers to discriminate between uninfected and virus-infected cells, not yet investigated for diagnostic purposes.

**Methods:** Confluent Intestine 407 cells (ATCC CCL-6), grown for 48 hours in a 24-well plate, were inoculated with Adenovirus (ADV) (NIAID). After a 45-minutes absorption, the viral inoculum was replaced with a maintenance medium and infected cells were incubated at 37°C. At different times post-infection (p.i.), the uninfected and infected cells were washed twice with PBS, harvested in 300 µL of distilled water and the proteins were extracted following the manufacturer's protocol. After 72 hours p.i. ADV purified particles from cultures were subjected to the same extraction protocol. Finally, 1 µL of all the protein extractions was transferred into the target plate and matrix (saturated solution of a cyano-4-hydroxycinnamic acid in 50% acetonitrile) was added, followed by crystallization and air-drying. Spectra from samples analyzed by Microflex LT mass spectrometer (Bruker Daltonics) were recorded in positive linear mode within a mass range from m/z 2 to 20 kDa.

**Results:** Uninfected and virus-infected cells were examined at 48 and 72 hours p.i. A rate of purified ADV was also analyzed. The spectra obtained from the analysis of uninfected cells were used as the baseline for the detection of any significant protein composition change into the ADV-infected cells, resulting from the inhibitory effect of the viral infection on the cellular protein synthesis or from the synthesis of viral specific proteins. The spectra obtained from ADV-infected cells showed two significant peaks which are completely missing in the spectra of uninfected cells and overlapping those obtained by the analysis of purified viral particles. Moreover, most of the peaks which appeared in control uninfected cells spectra completely disappeared in the infected cells.

**Conclusion:** Although the results obtained in this study are preliminary and should be confirmed using also different virus-cells models, the spectral differences observed between uninfected and virus-infected cells may be a promising basis for the spectroscopic detection and identification of infected cells with different viruses in clinical virology.

### P2320 The effectiveness of MALDI-TOF mass spectrometry for screening bacteriuria compared with an automated-flow cytometry

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**Objective:** Bacteriuria is a main indicator of urinary tract infections. Different rapid screening methods along with urine culture are invented for detection of bacteriuria.

The goal of this study was to compare effectiveness of mass spectrometry (MS) and urine automated-flow cytometry (UFC) for the diagnosis of bacteriuria.

**Methods:** One hundred and eighty-two urine samples were tested in parallel. The measuring of white blood and bacterial cells was done by UFC using Sysmex UF-500i (Sysmex corp., Japan). MS analysis of proteins from urine sediments was performed by AUTOFLEX III MS (Bruker Daltonics, Germany). The resulting MS spectra were compared with MALDI BioTyper 3.0 database for bacterial species identification. Urine cultures were used as a control. "Bacteriuria" was defined if there was bacterial growth in titer equal or more than 10<sup>5</sup> CFU/mL and "contamination" was reported for cultures with 10<sup>3</sup>-10<sup>4</sup> CFU/mL.

**Results:** Bacteriologically bacteriuria was found in 24 samples and was caused by *E. coli* (14), *K. pneumoniae* (7), *P. aeruginosa* (2), *E. faecalis* (2), *E. cloacae* (1), *S. maltophilia* (1). In three samples the mixed growth of two bacterial species was observed. MS identification was in concordance with culture results in 16 cases. In all cases *E. coli* and *K. pneumoniae* were identified with a high reliability (score ≥2.0). In one case of mixed infection the only bacterial species was identified. The other samples were identified with a low score (<1.7). According to UFC analysis in 19 urine samples (19/24, 79%) the titer of bacteria was ≥10<sup>7</sup> CFU/mL, in three ones - 10<sup>5</sup> CFU/mL, and in two ones - 10<sup>4</sup> and 10<sup>3</sup> CFU/mL, respectively. The samples with titer of bacteria <10<sup>5</sup> CFU/mL were not identified by MS. Additionally there were three samples positive by UFC (titer ≥10<sup>7</sup> CFU/mL) with negative urine culture and MS detection. And also one specimen was positive by both UFC (titer 10<sup>6</sup> CFU/mL) and MS (*S. epidermidis*) but negative by cultivation. Perhaps this sample was obtained after treatment.

**Conclusion:** In case of urine infections MS provides a rapid way for species identification of bacteria reducing the time before start of therapy. The diagnosis of bacteriuria by UCF is more reliable when the titer of bacteria found equal or more than 10<sup>7</sup> CFU/mL. Both these methods can be successfully used as aids for diagnosis of bacteriuria supported by further classical bacteriological testing.

### P2321 Evaluation of the Bruker Sepsityper kit and Bruker MALDI-TOF BioTyper for the direct identification of organisms from positive blood cultures

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**Objectives:** Blood cultures are one of the most critical specimens processed by clinical microbiology laboratories. Due to the critical nature of these specimens, rapid reporting of organism identification has the potential to beneficially impact patient management. We sought to evaluate the performance and turnaround time of organism identification the Bruker Sepsityper blood culture identification kit and Bruker MALDI BioTyper for the direct identification of blood culture isolates from the BacT/Alert blood culture system.

**Methods:** Blood was inoculated into bioMérieux BacT/Alert SA and/or SN bottles at the bedside and incubated on the BacT/Alert

instrument. For positive cultures, a Gram stain was performed and bottles were subcultured for routine identification. An aliquot of each culture was used for direct identification using the SepsiTyper blood culture kit and MALDI BioTyper. Routine methods included a variety of commonly used rapid (coagulase, indole, latex agglutination) and standard biochemical tests (Vitek 2 and API) depending on the organism isolated. Discordant identifications were further characterized with additional biochemical methods or 16srDNA sequencing. Turnaround time was calculated from culture positivity to reporting identification.

**Results:** A total of 61 monomicrobial and two polymicrobial cultures were evaluated. Among monomicrobial cultures, 52 (85.2%) had a valid score  $\geq 1.7$  for identification. Of these, 100% were concordant with conventional identification. Nine (14.7%) of cultures had a low score ( $< 1.7$ ) or invalid test, of which all those with score  $\geq 1.5$  were concordant with the final identification. Isolates with low scores and invalid tests tended to be likely contaminants or members of the *Streptococcus mitis* group. For polymicrobial cultures, the Bruker BioTyper correctly identified one of the isolates present with a confident score in both cultures. Turnaround time to identification was 6.5 hours from culture positivity for the Bruker BioTyper and 40.8 hours for the conventional methods ( $p < 0.0001$ ).

**Conclusions:** MALDI-TOF MS combined with the Bruker SepsiTyper kit is a rapid and highly accurate method for direct identification of pathogens from positive blood cultures. Allowing for known limitations, the technology has the potential to dramatically reduce time from positivity to definitive identification, potentially allowing for improved patient care.

#### **P2322** Direct identification of micro-organisms in positive blood cultures using MALDI-TOF MS: consequences for advice on antibiotic therapy

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**Objectives:** Identification of micro-organisms by MALDI-TOF MS (MS) is a major recent innovation in clinical bacteriology. Many studies have shown that MS identification directly from positive blood cultures is possible. However, direct identification has to be performed beside culture and is relatively laborious. The question remains whether faster identification of the causative micro-organism has any effect on patient care.

**Methods:** Of 73 consecutive patients with positive blood cultures at the AMC, the clinical history (including empirical antibiotic therapy) and microbiological data were presented anonymously to infectious disease specialists/clinical microbiologists (ID/CM) from academic and peripheral hospitals in the Netherlands and the UK. Cases were presented in two versions. In version 1, 37 cases contained Gram stain results only and 36 cases contained both Gram stain and MS results. In version 2, this was reversed, thus minimizing inter-observer variation. For each case, advice on antimicrobial therapy was asked.

**Results:** Preliminary results are based on two ID/CM evaluating version 1 and two ID/CM evaluating version 2. Advice on therapy differed substantially due to local epidemiology and policy. Identification of *Staphylococcus aureus* vs. coagulase-negative staphylococci, *Enterococcus faecalis* vs. *Enterococcus faecium* and *Candida albicans* vs. other *Candida* spp. had substantial impact on recommended therapy. Recommended therapy after identification of Enterobacteriaceae was more diverse. Aminoglycosides were often switched to cephalosporins if susceptibility was expected based on MS results and local epidemiology, but only if the patient was not critically ill. In 37 of 73 cases antibiotic treatment was started or adjusted. In 21 cases (29% of total), recommendations differed between one or both of the ID/CM who based their advice on MS results and those who based their advice on Gram stain only. In 12 cases, therapy was switched to less toxic or smaller-spectrum antibiotics by one or both of the ID/CM basing their recommendations on MS results. In nine cases, therapy was

switched to broader-spectrum antibiotics. However, some switches may have been the result of epidemiological considerations and/or local policies, not of identification results.

**Conclusion:** Early MS identification of blood culture isolates resulted in the recommendation to change antibiotic therapy in up to 29% of cases and seems a valuable addition to traditional laboratory techniques.

#### **P2323** Rapid and accurate identification of *Campylobacter jejuni* and *Campylobacter coli* isolates using the Vitek MALDI-TOF mass spectrometry system

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**Objectives:** Within the genus *Campylobacter*, *C. jejuni* and *C. coli* are the main causes of human campylobacter enteritis. In routine diagnostic laboratories the differentiation of *Campylobacter* spp. can be a challenge due to their poor biochemical activity, resulting in many laboratories only reporting isolates to genus level. The objective of this study was to show the easy, rapid and accurate identification of *Campylobacter* spp. from clinical samples using the recently launched Vitek MS (Biomerieux, France) matrix assisted laser desorption/ionisation time of flight (MALDI-TOF) mass spectrometry system.

**Method:** Ninety-eight previously identified strains of *Campylobacter* spp. (49 *Campylobacter coli* and 49 *Campylobacter jejuni*) collected in 2010 were subcultured onto charcoal cefoperazone deoxycholate agar (C.C.D.A) Disposable target slides were inoculated with a small amount of a single bacterial colony to provide a thin layer of bacterial growth. This was then overlaid with 1  $\mu$ L of matrix solution and air dried. The resulting slides were then processed in the Vitek MS (Biomerieux, France) instrument using the automatic database analysis of the obtained mass spectra to provide isolate identification. A second target spot was analysed if no spectra or no identification was obtained.

**Results:** The results showed that the MALDI-TOF MS system was correct in 100% of the identifications for all 98 strains tested, including all reference strains.

**Conclusions:** The Vitek MS MALDI-TOF system provides an easy, specific, accurate and importantly rapid identification of the common enteritis causing strains of *Campylobacter* species. MALDI-TOF mass spectrometry provides an accurate alternative to traditional identification methods.

#### **P2324** Identification of rapidly growing mycobacteria by matrix-assisted laser desorption ionisation time-of-flight mass spectrometry

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Nontuberculous mycobacteria (NTM) are responsible for increasingly common opportunistic infections. Differentiation to species level is important in order to determine clinical significance and initiate adequate treatment. Nowadays, identification of mycobacteria is based mainly on standard biochemical tests and molecular methods, including PCR, sequencing and hybridization assays using specific probes. More recently, mass spectrometry, which compares protein profiles, has been introduced as an identification tool for a great variety of microorganisms.

**Objectives:** The purpose of this study was to evaluate the capability of the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) to characterize rapidly growing mycobacteria (RGM) isolated from patients' samples and previously identified through molecular typing methods.

**Methods:** In the present study, strains of RGM isolated during the year 2011 from clinical samples of patients attended at the University Hospital La Fe of Valencia, Spain, were analyzed at the Microbiology Department. NTM were identified by a PCR-based hybridization assay that consists of multiplex amplification and reverse hybridization (GenoType<sup>®</sup> Mycobacterium CM/AS, HAIN Lifescience). For mass spectrometry (MS) identification, an extraction of each isolate was

prepared using formic acid and acetonitrile. This extract was analysed in a microflex mass spectrometer (Bruker Daltonik GmbH, Bremen) and the spectra obtained were compared with the MALDI Biotyper 2.0 database, which includes 23 RGM.

**Results:** A total of 16 strains of RGM were identified using the molecular method described, obtaining three *Mycobacterium abscessus*, 6 *M. fortuitum* and 7 *M. chelonae*. MS identified 10 (62.5%) strains as mycobacteria and was concordant to species level in 5 (31.3%) of them – 2 *M. abscessus* and 3 *M. fortuitum*. The score value was <2.0 in all cases. The identifications and score values obtained are presented in Table 1, together with the best mycobacterial match proposed by the MALDI Biotyper.

	HAIN	MALDI/TOF	Score	Best Mycobacterial Option (Rank)	Score
1			1.503	same	1
2			1.757	same	1
3	<i>Mycobacterium fortuitum</i>	<i>Eleutheromyces unicolor</i>	1.301	none	-
4	<i>Mycobacterium chelonae</i>	<i>Lactobacillus aialumensis</i>	1.398	none	-
5	<i>Mycobacterium chelonae</i>	<i>Cryptococcus neoformans</i>	1.196	none	-
6	<i>Mycobacterium abscessus</i>	<i>Airrobacter castelli</i>	1.694	<i>M. abscessus</i>	(5) 1.581
7			1.243	same	1
8	<i>Mycobacterium chelonae</i>	<i>Gordonia rubripertincta</i>	1.306	none	-
9			1.511	same	1
10	<i>Mycobacterium chelonae</i>	<i>Mycobacterium avium</i>	1.607	same	1
11	<i>Mycobacterium chelonae</i>	<i>Mycobacterium abscessus</i>	1.951	same	1
12	<i>Mycobacterium chelonae</i>	<i>Mycobacterium abscessus</i>	1.666	same	1
13	<i>Mycobacterium fortuitum</i>	<i>Mycobacterium parvum</i>	1.981	same	1
14	<i>Mycobacterium chelonae</i>	<i>Mycobacterium abscessus</i>	1.68	same	1
15			1.497	same	1
16	<i>Mycobacterium fortuitum</i>	<i>Latetia innocua</i>	1.462	<i>M. fortuitum</i>	(7) 1.301

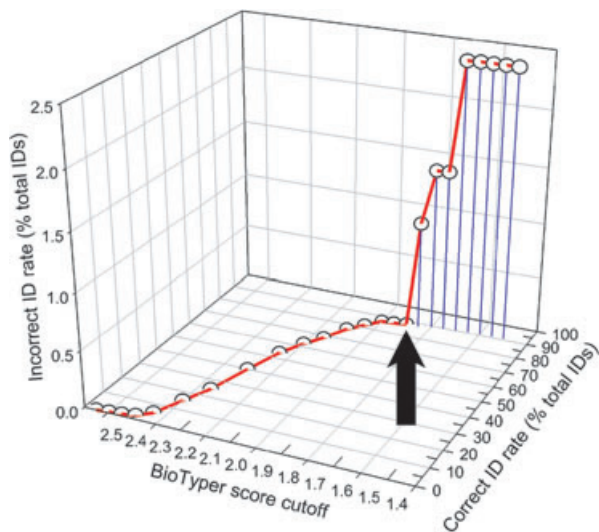
**Conclusions:** MS is not an adequate diagnostic tool for RGM by itself nowadays. Score values obtained showed no reliable identification in most cases. However, we believe that in the near future this technique will further improve as the quality of the available mycobacterial databases or the extraction protocols improve.

**P2325 Systematic evaluation of factors influencing routine clinical identification of Gram-negative enteric bacteria by the Bruker MALDI-TOF MS Biotyper system**

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**Objectives:** We systematically validated an optimized procedure for identification of enteric Gram-negative bacteria using the Bruker matrix-assisted laser desorption/ionization – time of flight mass spectrometry (MALDI-TOF MS) Biotyper by evaluating the effects of common pre- and postanalytical variables on the rate of correct identification.

**Methods:** For each condition tested, colonies were spotted for MALDI-TOF MS analysis in quadruplicate with heavy and light inocula, analyzed directly or overlaid with 1 µL formic acid. Twenty-five isolates of were cultured on six standard media and analyzed after 24 hours at 35°C. These isolates were subcultured to MacConkey agar and incubated at 35, 4°C and room temperature for 24 hours and re-



analyzed. The isolates were subcultured to MacConkey agar, incubated at 35°C, and re-analyzed daily for 5 days. Two hundred and eight clinical isolates comprising 14 genera and 21 species were tested with a Biotyper score of  $\geq 2.0$  as the cutoff for a correct identification. Phenotypic identification was the predicate method, with 16S rRNA gene sequencing used to resolve discrepancies.

**Results:** A formic acid-treated heavy inoculum was superior to the other conditions (difference in median scores significant at  $p < 0.01$  by repeated measures ANOVA). For this condition, the rate of correct identification was 87.0%, 5.8% higher than without treatment. Hektoen Enteric agar decreased this rate to 71% while incubation at 4°C decreased it to 75%. Identifications were stable over 5 days of subculture. There were no misidentifications. For a heavy formic-acid-treated inoculum the Biotyper score cutoff at which clinical isolates were never incorrectly identified was 1.9 (Fig., black arrow).

**Conclusions:** A “heavy” inoculum with formic acid overlay and a Biotyper score cutoff of 1.9 was optimal (Fig. 1). Inoculation at 4°C and growth on Hektoen Enteric Agar were detrimental to the rate of correct identification but there were no misidentifications. We conclude that the Bruker MALDI biotyper is robust when challenged with various preanalytical variables seen commonly in the clinical microbiology laboratory. Optimization of the target spotting and post-analysis scoring procedures was possible for this group of organisms but further study is needed to see if this benefit extends to other classes of isolates.

**P2326 Accurate, fast and affordable: performance of MALDI-TOF mass spectrometry for identification of uropathogens**

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**Objectives:** Urine cultures constitute the single highest volume sample and consequently a substantial expense in most clinical microbiology laboratories. We sought to compare the performance, cost and turnaround time of MALDI-TOF MS for the identification of uropathogens in a hospital clinical microbiology laboratory.

**Methods:** Quantitative urine cultures were plated to chromogenic media (CHROMagar Orientation) and potential pathogens were identified according to an algorithm that accounts for pathogen quantity and clinical symptoms. Conventional identification of pathogens was made using prototypical reactions on the medium for *Escherichia coli*, *Enterococcus* spp. and *Streptococcus agalactiae*. Other pathogens were identified using routine methods, including Vitek 2 and rapid biochemicals. MALDI-TOF MS was performed on all potential uropathogens using a Bruker BioTyper according to manufacturer’s instructions. Discordant identifications were resolved using biochemical panels or 16S sequencing.

**Results:** A total of 418 uropathogens were identified (207 *E. coli*, 69 other Enterobacteriaceae, 66 *Enterococcus* spp., 17 coagulase negative Staphylococci, 16 *S. agalactiae*, 12 *P. aeruginosa*, 11 *Candida* spp., 9 *S. aureus*, 2 *S. saprophyticus* and nine other organisms) in 391 urine specimens. MALDI-TOF correctly identified 100% of the uropathogens while the conventional protocol correctly identified 98.3% of the uropathogens. Errors occurred with the conventional method in identifying *Enterococcus* spp., *E. coli* and *S. aureus*. A significant reduction in turnaround time was observed with the MALDI-TOF MS identification algorithm overall, but most pronounced for organisms where growth-dependent assays were used for conventional identification (mean reduction 23.6 hours). The cost savings incurred by using MALDI-TOF in our laboratory (35 000 positive urine specimens/year) for identification of organisms other than prototypical *E. coli* and *Enterococcus* spp. on chromogenic agar, would be approximately CAN\$30 000/year.

**Conclusions:** MALDI-TOF MS is a rapid, cost-reducing and highly accurate method for identification of uropathogens. The technology is very promising for the identification of uropathogens that traditionally require biochemical panels or cards, or other growth-dependent identification methods.

**P2327** The performance of laboratory methods for the identification of clinical *Enterococcus* isolates

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**Objectives:** *Enterococcus faecalis* is the most frequently isolated enterococcal species from clinical specimens, followed by *Enterococcus faecium*. Human infections due to non-faecalis and non-faecium *Enterococcus* species are emerging. Nevertheless, due to their relatively low prevalence, the information about the performance of various methods in detecting these species is limited. The aim of this study was to compare the performance of two matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) systems, molecular methods and phenotypic testing procedures in identification of clinical *Enterococcus* isolates.

**Methods:** A total of 133 clinical isolates were investigated by (i) a multiplex real-time PCR assay targeting *ddl E. faecium*, *ddl E. faecalis*, *vanC1* and *vanC2/C3* genes, and high-resolution melting (HRM) analysis of the *groESL* gene; (ii) MALDI-TOF MS (Bruker Daltonik); (iii) VITEK MS (bioMérieux); and (iv) VITEK2 system (bioMérieux) complemented by MGP and motility tests. Isolates with discordant identification results were further analyzed by sequencing 16S rRNA gene.

**Results:** The 133 isolates were identified as 32 *E. faecalis*, 63 *E. faecium*, 16 *E. casseliflavus*, 21 *E. gallinarum* and one *E. gilvus*. MALDI-TOF MS (Bruker Daltonik), VITEK MS (bioMérieux), the multiplex PCR assay and VITEK2 correctly identified 100%, 99.2%, 99.2% and 91.0% of the isolates, respectively, at the species level. MALDI-TOF MS (Bruker Daltonik) succeeded in identifying an *E. gilvus* isolate, which was misidentified or not detectable by other methods. HRM-*groESL* assay identified all the *E. gallinarum* isolates and 81.3% of the *E. casseliflavus* isolates. VITEK2 could not differentiate nine isolates of *E. casseliflavus* or *E. gallinarum*. In MGP test and motility test, positive results were observed in 100% of *E. gallinarum* and 87.5% of the *E. casseliflavus* isolates. MALDI-TOF results were available within 1 hour; it took 2.5 and 3–8 hours for PCR assays and VITEK2, respectively. The MGP and motility tests required overnight incubation.

**Conclusion:** MALDI-TOF MS is a rapid and reliable identification technique for Enterococci. The molecular methods are effective in identifying the defined enterococcal species. Phenotypical tests are less efficient in detecting non-faecalis and non-faecium *Enterococcus* species.

**P2328** Value of matrix-assisted laser desorption ionisation-time of flight (MALDI-TOF) for routine identification of Viridans group streptococci causing bloodstream infections

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**Background:** Phenotypic tests do not always unequivocally identify some species of viridans group streptococci (VGS). *sodA* sequence analysis is the most accurate method for identification, although it requires specialized personnel and has not been applied systematically in clinical microbiology laboratory routine. Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) is emerging as a rapid alternative for bacterial identification. This study assesses the ability of MALDI-TOF and the API 20 Strep system to identify VGS isolates recovered from blood cultures using *sodA* sequence analysis as the reference method.

**Materials and methods:** All VGS isolates recovered from blood cultures between January 2007 and January 2010 were identified by *sodA* sequence analysis and API 20 Strep. The strains were then tested by MALDI-TOF. Agreement between API 20 Strep/MALDI-TOF and *sodA* sequence analysis was determined.

**Results:** We examined 124 clinical isolates. Agreement between API 20 Strep and *sodA* sequence analysis was 60.5% at species level and

70% at group level. Agreement between MALDI-TOF and *sodA* sequence analysis was 73.4% at species level and 94.3% at group level. The turnaround times to identify VGS isolates by *sodA* sequence analysis, API 20 Strep, and MALDI-TOF were 8–12, 24–48 hours, and 15 minutes, respectively.

**Conclusions:** API 20 Strep cannot accurately identify all isolates of VGS. MALDI-TOF appeared to be a rapid and reliable alternative for identification of VGS strains to group level, but was not able to discriminate closely related species of certain groups.

**P2329** MALDI-TOF mass spectrometry analysis of Gram-positive, catalase-negative cocci not belonging to the *Streptococcus* or *Enterococcus* genera and benefits of database extension

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**Objectives:** Catalase-negative, Gram-positive cocci, not belonging to the streptococci or the enterococci (of which many taxons may cause serious invasive infection), has become increasingly well characterized and the number of taxonomic entities steadily growing thereby complicating their identification. MALDI-TOF MS were applied on 51 collection strains within this group of bacteria in order to look for benefits of supplementing the existing database with new entries and the identification setups were challenged with 90 well-characterized strains.

**Methods:** MALDI-TOF MS was done with a Bruker Daltronics microflex LT instrument. The database included in the BioTyper v. 2.0.43.1 software was used singly or in combination with a database extension with consensus mass spectra generated on 51 collection strains (representing 16 genera and 51 species). Moreover, 90 well-characterized challenge strains from the Danish national reference laboratory were examined.

**Results:** Collection strains: When using the combined database all collection strains were categorized as obtaining either highly probable species identification ( $n = 39$ ) or secure genus identification, probable species identification ( $n = 13$ ), while use of the BioTyper (v. 2.0.43.1) database singly allocated seven and 21 strains to either obtaining probable genus identification or no reliable identification. Challenge strains: Strains belonging to the genera *Aerococcus*, *Gemella* and *Abiotrophia/Granulicatella* dominated, but 1–5 strains each belonged to eight other genera. By MALDI-TOF MS analysis all strains were allocated to the presumed genera. Strains from the *Aerococcus* genus ( $n = 35$ ) and the following list of genera with 1–5 strains each, were convincingly identified to the species level using both database set ups: *Abiotrophia*, *Lactococcus*, *Leuconostoc*, *Rothia*, *Facklamia*, *Helcococcus* and *Vagococcus*. Strains belonging to the genera *Gemella*, *Granulicatella*, *Globicatella* and *Alloicoccus*, with few exceptions, only obtained convincing identifications when using the combined database.

**Conclusion:** Thus, the method was very useful for quick and reliable identification within catalase-negative, Gram-positive cocci, not belonging to the streptococci or the enterococci. Benefits appeared from adding relevant new entries (consensus mass spectra) into a separate library, when relevant species were not included in the standard database.

**P2330** Clinical evaluation of Bruker Microflex MS vs. bioMérieux Vitek MS, two MALDI-TOF methods, for the identification of clinically significant pathogens

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**Objective:** To evaluate two commercially available MALDI-TOF MS systems, Microflex MS<sup>®</sup> (MMS; Bruker) and VITEK MS<sup>®</sup> (VMS; bioMérieux) for identification (ID) of clinically significant pathogens.

**Methods:** Clinically relevant broad diversity of bacterial and fungal isolates obtained during a 5-consecutive month of routine laboratory

processing of clinical specimens were subjected to ID by the MMS and VMS in parallel with conventional phenotypic system (CPS; VITEK 2). For the MMS the isolates were tested in duplicates directly and after pretreatment. Identification was provided with accompanying scores (<1.7 = no reliable ID; ≥1.7 to <2.0 = ID at genus level; ≥2.0 = ID at species level). For ID with VMS single deposits of the same sets of isolates were tested in duplicates directly on MALDI-plate; yeasts were pretreated with 25% formic acid. Species were separated by a threshold of 50% matching peaks. Interpretation of results was according to the manufacturers' protocols. Discrepant results were resolved one way or the other by double-stranded and 16S rRNA gene sequencing.

**Results:** A total of 1002 pathogens comprising 503 Gram-negative bacilli (GNB), 16 Gram-negative cocci (GNC), 266 Gram-positive cocci (GPC), 20 Gram-positive bacilli (GPB), 188 yeasts and nine molds, made up of 48 genera and 102 species were tested. MMS and VMS correctly ID 72.9% and 87.8%, and 96.8% and 98.7% of all isolates to genus and species levels, respectively. Both systems as well as the CPS correctly ID all species of the family *Enterobacteriaceae*, *Pseudomonas* spp., and *Acinetobacter baumannii*. When isolates not present in the database were excluded in the analyses, both systems performed equally well. VMS performed slightly better than MMS GPC ID, especially with *Streptococcus* spp. Some *S. mitis* isolates were ID as *S. pneumoniae* by MMS. However, when most recently updated MMS database was applied the difference was negligible.

**Conclusion:** Both systems performed excellently well in terms of providing accurate diagnosis 24–48 hours earlier than the CPS. Either of them can be easily incorporated into routine diagnostic procedures because they are user-friendly, have short turn-around time and the cost analysis is low.

**P2331 Application of MALDI-TOF mass spectrometry for rapid pathogen identification in an era of “One Medicine-One Health” microbiology**

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**Objectives:** To evaluate the utility of MALDI-TOF mass spectrometry (MS) as a single platform for pathogen identification (ID) in a regional laboratory practice routinely performing analyses on human- and animal-source clinical specimens. Integration of signature-based proteomic analyses into medical and veterinary laboratories may enhance ID performance, improve clinical service and epidemiologic research, and promote cross-disciplinary collaboration promulgated by the “One Medicine-One Health” concept (Veterinaria Italiana 45[1]; 2009). Here we demonstrate that MS can be as rapid, accurate and cost-effective when testing isolates of veterinary importance as when testing human-source isolates in the same laboratory setting.

**Methods:** Clinically significant pathogens (n = 1502) from humans (573), and domestic and exotic animals (929) were analyzed by MS and included (human/animal isolates): staphylococci (112/193), streptococci (61/112), enterococci (54/110), other Gram-positive cocci (36/0), Gram-positive bacilli (18/56), Enterobacteriaceae (147/251), *Pseudomonas aeruginosa* (61/68), other nonfermentative bacilli (56/58), and other Gram-negative bacilli (28/81). Isolates were identified by standard biochemical methods, and by MS (Bruker Daltonics MALDI Biotyper™ v3.0) according to the manufacturer's recommendations and validation algorithms.

**Results:** Overall, MS provided species- and genus-level IDs, respectively, for 90.0% and 96.7% of 1502 tested isolates. Among human and animal-source isolates, respectively, MS identified 94.4%/87.2% to species-level, 98.8%/95.4% to genus-level and 1.2%/4.6%, no identification (not in database); compared with MS, standard ID approaches identified 68.8%/50.3% to species-level and 94.1%/88.2% to genus-level. Discordant or missing IDs were present in 5.9% of human and 11.8% of animal source isolates.

**Conclusion:** Emerging infection health threats are often zoonotic in origin, and local and regional diagnostic laboratories serve a critical sentinel function in detecting their presence. Utilization of MS for ID of pathogens recovered from human and animal specimens facilitates and

enhances the likelihood of detecting emerging pathogens in both populations. The accuracy and rapidity of MS technology for human (94.4%/98.8%, species-/genus-level) and animal (87.2%/95.4%) pathogen IDs, supports a key component of laboratory infrastructure essential for promotion of the “One Medicine/One Health” concept.

**P2332 Evaluation of the Vitek MS® MALDI-TOF mass spectrometry system in a routine clinical laboratory**

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**Objectives:** Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a rapid method for identification of microorganisms. The Vitek-MS® (Biomerieux, France) is a recently launched MALDI-TOF MS system for rapid identification of bacterial and yeast isolates. Our objective was to evaluate the performance of the Vitek MS® in a routine clinical microbiology laboratory across a wide range of bacteria and yeasts.

**Method:** A total of 617 previously isolated, well characterised strains were tested on the Vitek MS® system. This comprised a total of 40 different genera and 111 individual species of which 55 were reference strains. Disposable target slides were inoculated with a small amount of isolate to provide a thin layer of growth. Yeast strains were overlaid with 0.5 µL of Formic Acid, air dried and further overlaid with 1 µL of matrix solution and air dried. Bacterial isolates were overlaid with 1 µL of matrix solution and allowed to air dry. The resulting slides were then processed in the Vitek MS® instrument with automatic database analysis of the resulting mass spectra. A second target spot was analysed if no spectra or identification were obtained. Discordant isolates were subsequently identified on a Vitek 2® (Biomerieux, France) system or by using extended phenotypic methods and were considered to be the reference identification.

**Results:** Five hundred and seventy-nine out of 617 (93.8%) isolates gave a good identification to species level. Six hundred and one out of 617 (97.4%) isolates gave an identification to group level. Finally, 608/617 isolates gave a correct genus level identification. The remaining nine isolates failed to identify or give any identification profile even after multiple testing.

**Conclusion:** The Vitek MS® MALDI TOF mass spectrometry is a fast, reliable method to identify clinically relevant bacterial and yeast isolates. Although the system failed to differentiate some organisms within the same group for example *S. mitis* and *S. oralis*, the given group identification or slash-line identification proved to be correct and alternative molecular methods often struggle to delineate these strains also. Mass spectrometry is a reliable method to replace traditional bacterial identification methods in routine clinical laboratories.

**P2333 Comparison of costs of microbial identification with and without MALDI-TOF**

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**Objectives:** MALDI-TOF MS is increasingly used in clinical laboratories for routine microbial identification. This technique proved to be rapid, robust and reliable for identification at species level and is expected to be cost saving. To precise the level of cost reduction, we compared costs of reagents and technician-time for microbial identification using MALDI-TOF approach and conventional tests, respectively.

**Methods:** In our laboratory that identify about 60 isolates/day, we compared the costs of (i) identification by MALDI-TOF and when needed additional approaches or (ii) identification using only other approaches. Reagents costs were those effectively supported. Technician time costs was assessed by measuring the time needed to perform each test, assuming a cost of 0.8/minute.

**Results:** A total of 1371 isolates were prospectively identified with and without MALDI-TOF. Overall, the microbial identification was 2.34-fold less expensive with MALDI-TOF (mean cost of 4.03) than without

MALDI-TOF (9.43). Costs were significantly different from species to species. Thus, the cost of *Escherichia coli* identification was often as low as 1.32 and 2.29 with and without MALDI-TOF, respectively. However, the mean cost for *E. coli* identification (n = 258) were 5.31 and 3.02 without and with MALDI-TOF, corresponding to a cost reduction of 1.76. For *Staphylococcus aureus* (n = 278), *Pseudomonas aeruginosa* (n = 107) and *Enterococcus faecalis* (n = 80), we also observed a cost reduction of 1.96, 2.52 and 5.53-fold, respectively, using the MALDI-TOF.

Considering the costs of reagents and technician-time, the extrapolated annual cost of microbial identification in our laboratory was of 168'094 without MALDI-TOF and 71'921 with MALDI-TOF, a 2.34 reduction. When considering also the cost of MALDI-TOF acquisition (208 000 with a 5 years amortization) and an annual maintenance cost (19 000), the annual cost of MALDI-TOF identification was 132 521, a 1.27-fold reduction. Cost savings were mainly due to a reduction of workload and a reduction of reagents costs related to automated phenotypic card-based identification.

**Conclusion and perspectives:** The MALDI-TOF is a cost-saving approach, allowing an annual reduction of identification costs of 1.27-fold. Based on this work, the identification process was optimized by using MALDI-TOF for most isolates and using the phenotypic approach as first-line test only for few species such as *E. coli*. We plan to develop various costs modelling.

#### **P2334 MALDI-TOF mass spectrometry for routine identification of Burkholderia species?**

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**Objectives:** Identification of non-fermenting Gram-negative bacteria such as Burkholderia species is time-consuming, difficult to perform and expensive when molecular tools are required. Infections due to Burkholderia cepacia complex (BCC) in case of cystic fibrosis are life threatening and can contraindicate lung transplantation. In this case, higher rate of mortality is also described with *B. cenocepacia* infection. Identification at the species level is therefore necessary.

**Methods:** Strains collected in our university hospital from respiratory samples (2005–2011) and identified by the French Cepacia Observatory by use of molecular techniques (16s rDNA sequencing and RecA-based PCR) was re-analyzed by MALDI-TOF Mass Spectrometry (MS). This technique was performed with the direct colony method, each deposit being twice realised, using the database from Bruker (June 2011 version) with the MALDI BioTyper 2.0 Software and Flex Control 3.0 (Bruker Daltonics GmbH, Germany). As recommended by the manufacturer, only MS scores above 2.0 at the first rank level were considered. We then compared identifications of 20 strains (15 patients).

**Results:** The MALDI-TOF MS system correctly identified 93.3% (14/15) of the non-redundant isolates, namely nine *B. cenocepacia*, two *B. multivorans*, two *B. gladioli* and one *B. ambifaria*. The unique discrepant case concerns a strain identified as *B. cepacia* by MALDI-TOF MS whereas the French Cepacia Observatory found a new BCC bacteria that remains unnamed presently.

One more strain was identified as *B. cepacia* by MALDI-TOF MS but the final identification is still unknown: the 16s rDNA did not distinguish between *B. cenocepacia* or *B. cepacia*, and RecA-based PCR is currently on progress.

**Conclusion:** Overall, good results were achieved and mass spectrometry is a valuable method of identification for Burkholderia strains. No discrepancies were found for the two majors species (*B. cenocepacia* and *B. multivorans*), but further investigations are needed for *B. cepacia* identification. The case of misidentification highlights the need of an improvement of the database, including hitherto unnamed strains.

The first identification of a Burkholderia strain must be confirmed by a molecular technique if this bacteria may be responsible for an infection or colonisation in a mucoviscidosis patient.

#### **P2335 Excellent identification of biochemically inactive non-fermentative bacteria by MALDI-TOF MS**

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**Objectives:** Sputa from cystic fibrosis (CF) patients often contain multidrug resistant biochemically inactive non-fermentative bacteria rendering standard biochemical tests useless for identification. At our laboratory, costly and time-consuming combinations of biochemical tests are applied for identification of these non-fermentative bacteria. Though MALDI-TOF MS has been used successfully to identify mucoid non-fermentative bacilli from CF patients, its performance for biochemically inactive strains is unknown. We compared MALDI-TOF MS with reference identification tests for the identification of biochemically inactive bacteria derived from sputa of CF-patients.

**Methods:** All non-fermentative bacteria from CF patients were investigated by a set of biochemical reactions, and considered biochemically inactive if acetamide hydrolysis, DNase production and lysine decarboxylation were negative and no growth on C390/phenatrolone agar after 24 hours incubation. Additionally, isolates were sent to a reference laboratory (RIVM, Bilthoven) for identification based on biochemical, fatty acid and 16S sequencing analysis. Isolates were kept frozen at -80°C. The isolates included in this study were investigated by MALDI-TOF MS (Microflex, Bruker) using the most recently provided database.

**Results:** From 73 isolates collected between January 2007 and June 2010, 59 isolates were used for analysis and 14 were excluded, because of absence from our strain collection (n = 5), unculturable (n = 5) or duplicate isolates from the same patient sample (n = 4). Compared to the reference laboratory, 56 isolates (95%) had correct genus identification, three (5%) no identification and none had an incorrect genus identification. Fifty-two isolates were identified to the species level by the reference laboratory, of which 43 (83%) were correctly identified by MALDI-TOF MS, three (6%) isolates had incorrect species, and 6 (11%) had no species identification. Incorrect species identifications were: *P. monteilli* vs. *P. putida* (n = 1); *A. ruhlandii* vs. *A. xylosoxidans* (n = 1); and *B. multivorans* vs. *B. cepacia* (n = 1). Note that *P. monteilli* belongs to the *P. putida* group. *B. multivorans* belongs to the *B. cepacia* complex. All 11 Burkholderia sp. isolates were correctly identified within the *B. cepacia* complex.

**Conclusions:** MALDI-TOF MS can identify biochemically inactive non-fermentative bacteria, although some strains can only be identified to *P. putida* group or *B. cepacia* complex.

#### **P2336 Performance of MALDI-TOF MS for routine identification of non-fermenting Gram-negative isolates from cystic fibrosis patients**

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**Objectives:** Respiratory infections remain a major threat to cystic fibrosis (CF) patients. Non-fermenting Gram-negative bacteria (NFGNB) are ubiquitous environmental opportunists that can chronically colonize the CF lung. The correct identification of these bacteria is essential for both therapeutic management of CF patients and epidemiological implications. Due to the limited biochemical reactivity and different morphotypes displayed by NFGNB, the conventional microbiology methods lack both sensitivity and specificity, with high frequency of bacterial misidentifications. Matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS) is an emerging tool for the fast and reliable identification of microorganisms. Aim of this study was to evaluate the performance of a MALDI-TOF MS technology to identify NFGNB isolates from CF patients.

**Methods:** In the period 2005–2011, we collected and stored 388 NFGNB isolates from sputum samples of CF patients attending Children's Hospital Bambino Gesù (Rome, Italy); 51 of these isolates

were examined and the relative results herewith reported. The study is still ongoing. The bacterial strains were obtained from cultures during routine follow-up visits or exacerbations. Conventional microbiological identification (IDs) using various phenotypic tests at the time of isolation were inconclusive for all collected isolates. ID of the bacterial isolates was performed with a MALDI Bio-typer 2.0 software (Bruker Daltonik GmbH, Bremen, Germany). When necessary, doubtful IDs were uncontroversially assigned by 16S rDNA sequencing (Applied Biosystems, Foster City, CA, USA).

**Results:** Overall, 46/51 examined strains (91%) were correctly identified by MALDI-TOF Bio-typer (score value range: 1.997–2.397), including *Burkholderia cepacia*, *Burkholderia vietnamiensis*, *Pandora apista* (never isolated before in our Centre) and *Pseudomonas aeruginosa*, the latter missed by conventional phenotypic tests. Five isolates were not identified, possibly due to the absence of database entries.

**Conclusion:** These preliminary data show that MALDI-TOF MS technology proves to be fast and reliable, improving dramatically the routine identification of CF isolates. However, it is mandatory to customize the current MALDI-TOF database with rare or infrequent microbial species typical of CF patients. Therefore, the following IDs of the other 337 isolates will be processed by employing an ad-hoc developed database.

**P2337 Identification of fastidious respiratory pathogens by MALDI-TOF mass spectrometry**

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**Objectives:** *Haemophilus influenzae*, *Streptococcus pyogenes* and *Moraxella catarrhalis* are important respiratory pathogens and also cause of significant invasive disease. These common and fastidious pathogens currently require identification methods that are time or cost intensive. We have compared the Matrix-Assisted Laser Desorption Ionization/Time of Flight Mass Spectrometry (MALDI-TOF MS) with the conventional phenotypic methods used routinely in our laboratory for the identification of these microorganisms.

**Methods:** Between August 2010 and October 2011, 64 *H. influenzae*, 70 *S. pyogenes* and 25 *M. catarrhalis* were isolated from respiratory, ear and eye specimens. All isolates were identified by conventional methods: test for bacitracin susceptibility, PYR test and latex agglutination for Lancefield group A antigen detection (Oxoid) for *S. pyogenes*, NH Card Vitek 2 (BioMerieux, Marcy L'Etoile, France) for *M. catarrhalis* and X and V factor requirement, NH Card Vitek 2 or API NH strip (BioMerieux, Marcy L'Etoile, France) for *Haemophilus* spp. Besides, all were tested with MALDI-TOF MS using a Microflex LT mass spectrometer (Bruker Daltonics, Germany) and the standard procedure recommended by the manufacturer, the samples were spotted in duplicate on a MALDI-TOF plate. Scores  $\geq 2.0$  were considered reliable for species level identification, scores of 1.700 to 1.999 were considered reliable for genus level, and scores lower than 1.700 were not considered reliable.

**Results:** With MALDI-TOF, 158 (99.4%) of the isolates were identified to at least the genus level and 139 (87.4%) were correctly identified to the species level. *H. influenzae*: 100% were identified to the genus level and 75% to the specie level. *S. pyogenes*: were correctly identified to the genus level in 98.57% and to the specie level in 95.71%. *M. catarrhalis*: 100% were identified to the genus level and 96% to the specie level.

**Conclusions:** MALDI-TOF MS is a fast and reliable technique and provides an alternative to simplify and accelerate the identification of these fastidious organisms in the clinical microbiology laboratory.

**P2338 Correct identification of *Haemophilus influenzae* and *Haemophilus haemolyticus* by matrix-assisted laser desorption ionisation-time of flight (MALDI-TOF) mass spectrometry**

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**Objective:** Generally accepted laboratory methods that have been used for decades do not reliably distinguish strains of *H. haemolyticus* from strains of *H. influenzae*. Misidentification of commensal *H. haemolyticus* as pathogenic *H. influenzae* results in unnecessary use of antimicrobials. To distinguish *H. influenzae* from *H. haemolyticus* we created a new database on the MALDI-TOF Bio-typer 2 and compared MALDI typing with routine bacteriological and molecular methods, including multilocus sequence analysis.

**Materials and methods:** The bacterial isolates (n = 33) were identified on the basis of the growth requirement for haemin (X-factor) and nicotinamide adenine dinucleotide (V-factor). The used strains were selected, for biodiversity, on the basis of the results of Slide Agglutination Sero Typing and included five capsulated and 28 uncapsulated strains. We created a new database containing reference strains ATCC 49766 (HI), ATCC 33390 (HH) and an in-house reference strain. The results were compared with MLST and species identification by using ompP6 PCR. For cross-identification and spectra quality control all strains were also tested by the Bruker Daltonics Laboratory.

**Results:** Based on colony morphology and the requirements of X- and V-factor all 33 strains were identified as *H. influenzae*. Determination by using MALDI Biotyper 2, 14 (42%) of the 33 strains were identified as *H. influenzae* and 19 (58%) as *H. haemolyticus*. MLST and species identification using the ompP6 gene gave 100% agreement when compared with mass spectrometry identification. The same results were obtained at the Bruker laboratory using the identical set of microorganisms. In a MSP dendrogram the strains of the two species cluster separately, this separation also was supported by Principle Component Analysis.

**Conclusion:** The identification of *H. influenzae* and *H. haemolyticus* based on conventional method is not reliable. MALDI-TOF mass spectrometry is a reliable and rapid technique for distinguishing HI and HH and comparable with molecular techniques. Accurate identification of pathogenic *H. influenzae* is important and may contribute to a reduction in unnecessary antibiotic used for the treatment of misidentified *H. influenzae*.

**P2339 Identification of blood and wound isolates of *Capnocytophaga canimorsus* and *C. cynodegmi* with MALDI-TOF**

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**Objectives:** Infections after animal bites are common and can be related to high morbidity. *Capnocytophaga* spp. are Gram negative bacteria that normally reside in the oral flora of dogs and cats. However, they can cause severe infections in humans ranging from uncomplicated wound infections to sepsis, meningitis and endocarditis. The isolation and identification of these bacteria in routine clinical bacteriology work is complicated due to slow growth of the organism. The routine methods including fermentation and classical phenotypic tests (oxidase and catalase) are insufficient to correctly identify *C. canimorsus* or *C. cynodegmi*. The aim of this study was to compare the performance of modern methods, that is, VITEK and MALDI-TOF in identification of clinical isolates of *Capnocytophaga* spp.

**Method:** Eight blood isolates and 14 wound isolates of *Capnocytophaga* spp. were isolated from patients (n = 21) during 2007–2011. All isolates were analysed with phenotypic tests, VITEK and MALDI-TOF (Bruker Daltonics). Sequencing of the 16S rRNA gene was used as the reference method.

**Results:** VITEK2 could identify 2/8 blood isolates and 8/14 wound isolates correctly. MALDI-TOF analysis of the same strains resulted in

positive identification of 8/8 blood strains and 11/14 wound strains. The mean time to identification with VITEK2 was 6 hours whereas MALDI-TOF required ~10 minutes per sample.

**Conclusions:** Since *Capnocytophaga* spp. has the potential to cause severe infections, reliable and rapid identification of these pathogens is important. Here we show that MALDI-TOF and VITEK2 rapidly identify *Capnocytophaga* spp. and thus constitute valuable diagnostic tools in the clinical laboratory.

**P2340 Identification and differentiation of *Brucella* spp. by whole-cell matrix-assisted laser desorption ionisation-time of flight mass spectrometry (MALDI-TOF MS)**

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**Objectives:** Brucellosis is one of the major bacterial zoonoses worldwide leading to tremendous economic losses in animal husbandry and causing severe multiorgan disease in infected humans. The gold standard in the diagnosis of human disease is the isolation of the bacteria from clinical samples. Suspicious colonies are confirmed by slide agglutination using a polyvalent *Brucella* antiserum or by molecular methods. Species and biovars are subsequently determined by testing CO<sub>2</sub> requirement, H<sub>2</sub>S production, urease activity, agglutination with monospecific sera, selective inhibition of growth on dye containing culture media, and phage typing.

These procedures are inappropriate for clinical microbiology laboratories because they are time-consuming, hazardous and subject to variable interpretation. Therefore, we evaluated the use of matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) for the rapid identification of the genus *Brucella* and differentiation of its species.

**Methods:** A total of 26 *Brucella* type and reference strains as well as 97 field isolates representing all currently known *Brucella* species and biovars were analyzed by whole-cell MALDI-TOF MS. The analysis was performed on a microflex LT and an ultraflex TOF/TOF mass spectrometer (Bruker Daltonik). Spectra were collected in the linear positive mode within the mass range 2000 to 20 000 Da. Reference spectra were generated using at least 20 separate spectra per strain.

**Results:** All strains were identified as members of the genus *Brucella*. Standard bioinformatic tools (composite correlation matrix and polyphasic cluster analysis) were not able to differentiate species and biovars. However, a stepwise decision guidance based on marker peaks allowed species differentiation except for the *B. abortus*/*B. melitensis* group and for *B. canis*/*B. inopinata* strains. Although subgroups could be defined within the species, these did not correlate with the common classification scheme. Interlaboratory comparison revealed that the spectra comprised in the reference database were highly reproducible.

**Conclusion:** In summary, MALDI-TOF MS allowed fast, accurate and reproducible detection and identification of *Brucella* spp. making this new method attractive for routine use in the laboratory diagnosis of brucellosis.

**P2341 Use of the MALDI-TOF method for the identification of bacterial zoonotic agents**

J. Bardon\*, M. Kolár, V. Husicková, V. Hanulík (Olomouc, CZ)

**Objectives:** Bacterial zoonoses represent a serious medical problem. In Europe, the most common ones include campylobacteriosis, yersiniosis and salmonellosis. The clinical course is known to be severe in tularemia, brucellosis and listeriosis. The objective is to assess the use of MALDI-TOF for the identification of bacterial zoonoses.

**Methods:** Between August 2010 and September 2011, bacterial isolates from clinical and necropsy samples collected from animals, foods of animal origin and foods given to animals were identified. Throughout that time, a total of 11 000 identified bacterial isolates were analyzed using the MALDI-TOF Biotyper Microflex (Bruker).

**Results:** The results of the analysis of selected pathogens causing bacterial zoonoses were as follows: *Campylobacter* spp.: excellent species identification (99%, 78 analyzed isolates). *Salmonella* spp.: excellent genus identification (99%, 468 analyzed isolates), conventional serotype determination in all cases. In some cases, samples incorrectly determined as *S. typhi*. *Yersinia* spp.: good species identification of both *Y. pseudotuberculosis* and *Y. enterocolitica* (only nine analyzed isolates), in some cases, samples incorrectly determined as *Y. pestis*. *Listeria monocytogenes*: good species identification (90%, 629 analyzed isolates). *Escherichia coli*: problems with distinguishing *E. coli* from *Shigella* spp., complementation with conventional tests needed (800 analyzed isolates). *Francisella tularensis*: excellent species identification (only two analyzed isolates). *Brucella melitensis*: excellent species identification (only two analyzed isolates). *Bacillus* spp.: good species identification (80%, 60 analyzed isolates), in some cases, samples incorrectly determined as *B. anthracis*.

**Conclusion:** MALDI-TOF is a very good alternative to conventional methods for the identification of zoonotic bacteria. It provides rapid and relatively accurate results. Given to some misidentifications (e.g. *S. typhi*, *Y. pestis*, *B. anthracis*), the analyses must be interpreted by experienced clinical microbiologists.

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**P2342 Comparison of two MALDI-TOF systems for the identification of anaerobic bacteria**

S. Jeverica\*, U. Dolinar (Ljubljana, SI)

**Objectives:** Matrix assisted laser desorption ionization time of flight mass spectrometry (MALDI TOF MS) is an accurate, cost effective and fast method which has the potential to replace conventional biochemical and molecular identification systems for routine identification of aerobic and anaerobic bacterial isolates. We evaluated two commercially available MALDI TOF systems: Bruker Biotyper (version 2.0) and Vitek MS (bioMérieux) for the identification of clinically important anaerobic bacteria.

**Method:** The identification of 220 consecutive clinically important anaerobic isolates from skin and soft tissue infections was performed by both MALDI TOF systems and compared with the conventional biochemical methods: Vitek 2 (bioMérieux) and Rapid ID 32 A (bioMérieux). Isolates tested comprised the majority of the clinically important anaerobic genera (*Clostridium*, *Peptostreptococcus*, *Finegoldia*, *Peptoniphilus*, *Propionibacterium*, *Actinomyces*, *Bacteroides*, *Prevotella*, *Fusobacterium* and *Veillonella*). The strains were tested using the manufacturers' default recommendations and settings.

**Results:** Among 220 anaerobic isolates identified by the conventional methods, 163 (74%) isolates were correctly identified by Bruker MS and 166 (76%) isolates with Vitek MS. Both MALDI TOF systems performed equally well for the identification of various species and genera (*Finegoldia magna*, *Prevotella* spp., *Veillonella* spp.). Bruker

**Table 1:** The identification of 220 consecutive clinical anaerobic isolates obtained by two MALDI TOF systems in comparison to the conventional biochemical test methods

Anaerobes (Conventional ID)	No. of isolates	Vitek MS (Correct level ID)		Bruker MS (Correct level ID)	
		n	%	n	%
<i>Clostridium</i> spp.	15	8	53%	14	93%
Gram positive anaerobic cocci	49	29	59%	18	37%
<i>Finegoldia magna</i>	24	16	67%	16	67%
<i>Peptostreptococcus anaerobius</i>	3	1	33%	2	67%
<i>Peptostreptococcus</i> spp.	2	0	0%	0	0%
<i>Peptoniphilus asaccharolyticus</i>	20	12	60%	0	0%
<i>Actinomyces meyeri</i>	3	0	0%	0	0%
<i>Propionibacterium acnes</i>	7	7	100%	7	100%
<i>Bacteroides</i> spp.	87	80	92%	84	97%
<i>Prevotella</i> spp.	31	23	74%	23	74%
<i>Fusobacterium</i> spp.	21	13	62%	11	52%
<i>Veillonella</i> spp.	7	6	86%	6	86%
Σ Anaerobes	220	166	76%	163	74%

ID identification



MS performed better for the identification of *Bacteroides* spp. (97% vs. 92%) and for the identification of members of the *Clostridium* genus (93% vs. 53%). Vitek MS performed better for the identification of Gram positive anaerobic cocci group (59% vs. 37%). The majority of isolates not identified with the tested MALDI TOF systems to either the genus or the species level belonged to the Gram positive anaerobic cocci group (Table 1).

**Conclusion:** The identification with mass spectrometry revolutionized the processing of anaerobic cultures in the microbiology laboratory. It offers faster detection of anaerobes, lower inoculum for the identification and shortens the time needed to report the results. We compared both commercially available MALDI TOF systems for the identification of anaerobes. Correct identification of isolates occurred in 74% and 76% using Bruker MS and Vitek MS, respectively. The current database in both systems needs further improvement.

**P2343 Evaluation of MALDI-TOF MS Biotyper for anaerobic bacteria identification from paediatric clinical samples**

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**Objectives:** Anaerobic bacteria are a common cause of infections in children which can be serious and life-threatening. Because of their fastidious nature, their isolation requires adequate methods of collection, transportation, cultivation and dedicated personal. In fact, they are often overlooked. Treatment of anaerobic infection is complicated by the slow growth of these organisms and by their polymicrobial nature.

In this study, we exploited the Matrix-assisted laser desorption/ionization-time of flight-mass spectrometry (MALDI-ToF MS) Biotyper to evaluate the capability of an accurate and rapid identification of anaerobes from clinical specimens in comparison to 500 bp 16S rDNA sequencing, considered the "gold standard" to identify bacterial species.

**Methods:** Specimens retrieved from patients recovered at "Bambino Gesù" Children Hospital were cultured on Schaedler 5% sheep blood agar in an anaerobic cabinet at 35°C and isolates grown were identified by 500 bp 16S rDNA sequencing. Results were compared to identification provided by MALDI-ToF MS Biotyper.

MALDI-ToF database was enlarged collecting for each species absent in the database or present with few spectra a multiplicity spectrum consisting of at least eight replicated. The spectra selected were inserted into the database or added to the existing for optimization of identification.

**Results:** A total of 486 isolates belonging to 19 genera and 53 species were collected from pus, stool samples, abscesses, blood cultures, body fluids, biopsy and other materials. MALDI-ToF MS provided a correct identification showing a 96.09% of concordance, compared to 16S rDNA based sequencing analysis, for all the analysed isolates. 0.41% were correctly identified only at the genus level but not at species level. In 0.2% of strains was observed incorrect genus identification and in 3.49% no identification was observed at all.

**Conclusion:** In our clinical routine, 500 bp 16S sequencing allows a correct identification of anaerobic pathogens but is time-consuming, expensive and requires personal dedicated. Our data provides the evidence that MALDI-ToF MS may be an optimal diagnostic approach to overcome culture-based methods for a fast and reliable identification of anaerobic bacteria.

**P2344 Identification of anaerobes in a routine setting: performance and pitfalls of matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry as compared to conventional techniques**

M. Knoester\*, S.Q. van Veen, E.J. Kuijper (Leiden, NL)

**Objectives:** Comparison of MALDI-TOF mass spectrometry with conventional biochemical methods to identify anaerobic bacteria in a routine clinical setting.

**Methods:** Two hundred and ninety-six clinically relevant anaerobic bacteria cultured from patient materials between 1 January 2010 and 4 February 2011 were tested by both MALDI-TOF (Bruker Daltonik, Bremen, Germany) and conventional identification methods (API Rapid ID 32, bioMérieux, Marcy-l'Étoile, France). The results of MALDI-TOF and conventional identification were categorised as (i) identical species identification, (ii) identical genus identification (if either or both techniques identified to the genus level only), (iii) discrepant results at the genus or species level or (iv) no reliable MALDI-TOF identification. In the case of discrepant results, 16S gene sequencing was performed and considered as a gold standard.

**Results:** Of the 296 isolates, 23% was finally identified as *Bacteroides* spp., 14% as *Propionibacterium* spp., and 12% as *Prevotella* spp. Table 1 depicts the study outcomes. Among the 28 isolates with discrepant results and 16S identification, two incorrect species identifications were obtained by MALDI-TOF: *Anaerococcus hydrogenalis* was identified as *A. vaginalis* and *Fusobacterium nucleatum* was named *F. nucleatum/ naviforme*. Conventional methods resulted in significantly more incorrect genus (n = 16) and species (n = 8) identifications (p = 0.009). Performance of MALDI-TOF MS did not vary between Gram-positive and Gram-negative bacteria. Results for *Bacteroides* spp., *Clostridium* spp., *Propionibacterium acnes*, *Finnegoldia magna*, and *Prevotella* spp. were good; the identification results for *Fusobacterium* spp., *Propionibacterium non-acnes* spp. and *Actinomyces* spp. need some improvement.

Table 1. Results of MALDI-TOF MS and conventional methods compared

Number of isolates tested	296
Identical identification to species level	143 (48%)
Identical identification to genus level	82 (28%)
Discrepant results*	33 (11%)
MALDI-TOF correct species identification	21
Conventional methods correct species identification	1
MALDI-TOF correct genus identification	3
No species identification by 16S	3
No 16S performed	5
No reliable MALDI-TOF identification	38 (13%)

\*16S gene sequencing as gold standard

**Conclusion:** MALDI-TOF MS is superior to conventional techniques for the identification of anaerobic bacteria in a clinical setting. Further development of the database will be needed to optimise MALDI-TOF results.

**P2345 The value of MALDI-TOF MS in the identification of clinically relevant anaerobic bacteria**

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**Objectives:** To evaluate the value of a new MS-based identification system for the frequently isolated, clinically significant anaerobic bacteria, to compare the MALDI-TOF MS results with the results of the regularly used identification kits and to use the 16S rRNA gene sequencing for strains with low log (score) or discrepant results.

**Methods:** Between 2010 and 2011 clinically relevant non-duplicate anaerobic isolates (196) were identified – if possible – by different traditional methods such as growth on different selective media, presumptive identification according to the Wadsworth Manual and rapid ID 32A ATB and API20 ANA (BioMérieux) kits in the anaerobic reference laboratory of the Institute of Clinical Microbiology of the University of Szeged. Incubation was carried out in an anaerobic chamber (Bactron, USA). Immediately after isolation, an ethanol extraction was carried out on isolated colonies and the stabilized

samples were sent to the Bruker Laboratory in Bremen, Germany, where the identification was done by using the standard protocol of the MALDI-TOF MS (Microflex) and the spectra were imported into the Biotyper software (version 2.0).

**Results:** Out of the 196 non-duplicate anaerobic clinical isolates from different genera including *Bacteroides*, *Prevotella*, *Fusobacterium*, *Clostridium*, *Peptostreptococcus*, *Fingoldia*, *Propionibacterium* and some unidentified Gram-negative and Gram-positive anaerobic bacteria were investigated by MALDI-TOF MS. The threshold of log (score) >2.000 was used for the species level identification and >1.700 for genus level identification. MALDI-TOF MS identified 166 (84.6%) strains at a species level and 184 (93.8%) at a genus level. After 16S rRNA gene sequencing, it turned out that for 10 isolates the species was not included into the database. In cases of discrepant phenotypic identification 16S rRNA gene sequencing supported the MALDI-TOF identification in 97% of the cases. Even species, which are difficult to be distinguished by commercially available identification kits, such as *B. fragilis* and *B. capillosus*, were correctly identified. By including newly sequenced anaerobic species from our strain collection into the database, the "missed" results could be minimised.

**Conclusion:** MALDI-TOF MS seems to be a very promising identification method especially in the case of anaerobic bacteria, which need a special culture condition, a longer incubation time to get proper growth and are biochemically often inactive.

#### P2346 Clinical testing of bacteria and yeast from paediatric patients by using MALDI-TOF/Vitek MS system

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**Background:** Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) can be used to detect microorganisms rapidly from culture isolates. Vitek-MS System with SARAMIS database by bioMérieux is a commercially available MALDI-TOF MS system for rapid detection of bacterial and yeast isolates. This study is designed to use the Vitek-MS to detect clinical isolates from pediatric patients seen in one children's hospital in the Southeast and another in Midwest of United States.

**Methods:** Clinical isolates including yeasts and bacteria from two children's hospitals were used for testing by using the Vitek-MS System. The results generated from MALDI-TOF MS that gave the definitive identification to genus level were used for comparison with results from conventional culture methods and additional 16S rDNA sequencing methods for challenging organisms.

**Results:** Total of 137 non-duplicated clinical isolates including 64 isolated from one hospital and 73 isolated from another one, were collected from blood, spinal fluid, respiratory, wound, stool, and urine cultures, and were used for MALDI-TOF MS testing. Of 64 isolates including nine challenging organisms from one hospital that were identified correctly by using the Vitek-MS methods as by the conventional culture method, only two organisms could not be further identified at species level, one was *Achromobacter xylosoxidans* from sputum, which was identified correctly at genus level. The similar organism from CSF was identified correctly at species level. Another organism was *Enterobacter cloacae* from tracheal aspirate, though the similar organisms from other patients were identified correctly at species level. Among 73 isolates from another hospital, 62 were routine isolates including yeasts and 11 were *Shigella* isolates. Of 62 isolates, only one *Streptococcus pyogenes* from throat culture was identified incorrectly at species level as *S. mitis*, though similar isolates from other patients were identified correctly. Among 11 *Shigella* isolates tested, all were misidentified as *E. coli*, which was consistent with 12 isolates from adult patients.

**Conclusion:** With the exception of *Shigella* isolates, the clinical testing data demonstrate the capability of MALDI-TOF Vitek-MS method in correct and rapid identification of pathogenic bacteria and yeasts in pediatric patient populations.

#### P2347 Identification of bacteria and yeasts with the MALDI-TOF mass spectrometer Vitek<sup>®</sup> MS

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**Objectives:** Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF) is suitable for rapid diagnostics at low costs for routine microbiological laboratory. Of the two systems currently commercially available, most performance data on pathogen identification have been obtained for the MALDI-TOF system from Bruker Daltonics (Billerica, MA). Here, we evaluate the VITEK<sup>®</sup> MS system (bioMérieux, Nürtingen, Germany) for which only very few data are available so far.

**Methods:** We compared the identification results obtained with the VITEK<sup>®</sup> MS with results from standard biochemical techniques (VITEK<sup>®</sup> 2 and Api<sup>®</sup>, bioMérieux) using a collection of 628 clinical isolates previously cultured from patient materials with discrepancies being resolved by 16 S rDNA sequencing.

**Results:** 6.7% of the isolates were not identified by VITEK<sup>®</sup> MS, 1.3% were identified incorrectly and 92% were correctly identified to genus level and 85.2% also to species level, respectively. Correct species (genus) identification was obtained for 80.8% (91.3%) of Enterobacteriaceae, 79.3% (84.5%) of nonfermentative Gram-negative bacteria, 95% (96.7%) of fastidious bacteria, 100% of *Campylobacter*, 96.4% (98.2%) of staphylococci, 94.1% (97.1%) of streptococci including *S. pneumoniae*, 100% of enterococci, 41.7% (66.7%) of Gram-positive rods including *Bacillus* spp., 91.3% (95.7%) of anaerobic bacteria, and 89.7% (91%) of yeasts. In particular, VITEK<sup>®</sup> MS had a significantly better identification performance than conventional methods for nonfermentative, fastidious and anaerobic bacteria, enterococci, staphylococci, and yeasts. However, some Enterobacteriaceae und *Salmonella* could only be identified to the genus level. Actinomycetes and *Nocardia* could not be identified by VITEK<sup>®</sup> MS, and the identification of Gram-positive rods and in particular of *Bacillus* spp. was also not satisfying but still better than conventional methods. Non- as well as misidentifications by VITEK<sup>®</sup> MS were clearly associated with an absence of sufficient spectra from suitable reference strains in the database.

**Conclusion:** Identification by VITEK<sup>®</sup> MS is a rapid, inexpensive and easy method for identification of bacteria and yeasts in a routine microbiological laboratory.

#### P2348 Performance of MALDI-TOF mass spectrometry in comparison with classical diagnostic methods for the identification of yeast species at a mycology reference laboratory

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**Objectives:** Matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) has proven a valuable tool for the identification (ID) of microorganisms and we thus evaluated the performance of MALDI-TOF MS for the ID of yeast species in direct comparison to classical diagnostic methods.

**Methods:** Clinical yeast isolates were simultaneously identified during a 3 month period by MALDI-TOF MS and classical methods including macro and micromorphology studies, temperature tolerance, carbon assimilations and various rapid ID tests (*C. krusei*, *C. dubliniensis* latex agglutination and *C. glabrata* RTT test). Sample preparations for MALDI-TOF MS were carried out using ethanol fixation prior to protein extraction with formic acid and acetonitrile as described by Marklein et al. (*J Clin Microbiol* 2009). Mass spectra were obtained and compared to the Bruker database including 4005 reference spectra (updated March 2011).

**Results:** A total of 244 clinical isolates were collected and mass spectra were successfully obtained from 224 (92%) isolates (Table), however, less successful for *C. parapsilosis* (73%), *C. dubliniensis* (64%) and *C. tropicalis* (4/8). Obtained spectra were evaluated against the spectrum database yielding 221/224 (99%) isolates with correct best

match compared to classical methods, 86% of which had a score  $\geq 2$ . In one case *C. albicans* was misidentified as the close relative *C. africana* with a log-score above 2. Applying the Bruker pre-defined cut-off for reliable genus ID ( $>1.7$ ) yielded 216/217 (99.5%) correct best match, while a score  $>2$ , generally considered as cut-off for reliable species ID gave 192/193 correct best match.

Among common *Candida* species, MALDI-TOF MS performed best for *C. albicans*, *C. glabrata* and *C. krusei*. All Saccharomyces cerevisiae isolates gave correct best match but 44% of those had scores below 2.

Table: Summary of the performance of MALDI-TOF for identification of yeasts.

Species	No. of isolates		No. of (distinct) isolates with log-score of			Comparison	Incorrect ID
	Total	No spectrum (%)	$< 1.7$	1.7 - 2.0	$\geq 2.0$		
<i>C. albicans</i>	32	2 (2%)	2	77	1*	80/81	* <i>C. africana</i>
<i>C. glabrata</i>	40		1	59	60/60		
<i>C. parapsilosis</i>	22	6 (27%)	2	3	11	16/16	
<i>S. cerevisiae</i>	16		7	9	9	16/16	
<i>C. trusei</i>	13				13	13/13	
<i>C. dubliniensis</i>	11	4 (36%)	1	2	4	7/7	
<i>C. tropicalis</i>	8	4 (50%)	1	3	4	4/4	
<i>C. guilliermondii</i>	2			1	1	2/2	
<i>C. lusitana</i>	3	1 (33%)		1	1	2/2	
<i>C. lusitana</i>	7	2 (29%)		1	4	5/5	
<i>C. orthomorphus</i>	1			1	1	1/1	
<i>C. lusitana</i>	3		1	1	1	3/3	
<i>C. pelliculosa</i>	3		1	1	1	3/3	
<i>C. kefyr</i>	3			1	2	3/3	
<i>C. parapsilosis</i>	1			1	1	1/1	
<i>C. neoformans</i>	1			1	1	1/1	
<i>D. hansenii</i>	1			1	1	1/1	
<i>Epiflorasp.</i>	4	1	1*	1	1	2/3	* <i>D. castellani</i>
<i>Cryptococcus sp.</i>	3		1*		2	2/3	* <i>Arthroascus parvulus</i>
<b>Total</b>	<b>244</b>	<b>20 (8%)</b>	<b>7 (3%)</b>	<b>24 (10%)</b>	<b>193 (79%)</b>	<b>223 (224/99%)</b>	<b>3 (1.3%)</b>

\*Isolate with incorrect best match as indicated in right column

**Conclusion:** Overall, MALDI-TOF MS performed well for the ID of *Candida* species enabling cheaper, faster and easier diagnostics in a clinical routine laboratory. Successful protein extraction is a significant criterion for MALDI-TOF MS analyses, and in our lab attention is needed for *C. parapsilosis*, *C. dubliniensis* and *C. tropicalis* for optimal reproducible results. In-house reference strains, such as for *S. cerevisiae* may be manually added to the spectrum database to level log-score values, which are often borderline for reliable species ID.

#### P2349 The use of MALDI-TOF MS in identification of *Candida albicans*

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**Objectives:** Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) was recently evaluated as a useful tool for the rapid identification of a variety of microorganisms. For identification of bacteria, whole cells can be analyzed very quickly and their spectra are compared with reference spectra. We report the results of the study focused on the identification of clinical isolates *Candida albicans* species by MALDI-TOF MS with confirmation by species-specific polymerase chain reaction (PCR).

**Methods:** Three hundred and nineteen strains of yeasts were investigated. All were originally identified as *C. albicans* due to the colour of pigmentation on CHROMagar *Candida* (CHROMagar, France). For both methods, each strain was incubated on CHROMagar *Candida* for 2 days at 37°C. Samples for MALDI were prepared and investigated due to the protocol of Bruker Daltonics Inc. Species-specific PCR allowing differentiation between three species with the use of single primer pair was chosen for the verification of results. Different sizes of band were made – 941 bp for *C. albicans*, 700 bp for *C. africana* and 569 bp for *C. dubliniensis*.

**Results:** From 319 strains, 299 (93.7%) were classified to the species level by the mass spectrometry. This method was not able to analyze 20 of all isolates (6.3%), even if the whole process was repeated twice. According to the PCR most of isolates were identified as *C. albicans* (236), 78 were classified as *C. dubliniensis* and five as *C. africana*. In contrast to PCR, MALDI identified 32 isolates minimally once as *C. africana*, though only five of them were confirmed by PCR, the rest

were found to belong to *C. albicans* species. From all *C. dubliniensis* isolates 10 were not classified by MALDI (12.8%).

**Conclusion:** Mass spectrometry was found to be highly reliable method. The most important advantages of this method are its simple use and fast obtaining of results, which is very useful for routine identification in clinical laboratories. The only two disadvantages which we found was the high percentage of misidentification of *C. africana* species and the inability to identify some *C. dubliniensis* isolates. By comparison with bacterial identification by MALDI, identification of yeasts is more complicated and lasts longer.

#### P2350 Identification of *Candida* sp. using conventional and biochemical and mass spectrometry methods

E. Stefaniuk\*, A. Baraniak, M. Fortuna, W. Hryniewicz (Warsaw, PL)

**Objectives:** Fungal infections are more common today than ever before thus accurate and timely diagnosis is of great importance. Matrix-assisted laser desorption ionization-time of flight identification is emerging as a potential tool for organism identification. The mass spectrometry is based on analysis of intracellular proteins profile characteristic for particular genus and species. The aim of the study was the comparison of three methods of identification of *Candida* spp. – conventional method, biochemical method and mass spectrometry.

**Methods:** The identification of 84 clinical isolates of *Candida* sp. isolated from various biological materials from hospitalized patients was performed by Sabouraud Agar and ChromAgar culture, semi- and automated method (ATB Expression, VITEK 2 Compact) and mass spectrometry (MALDI-TOF). The strains were obtained from blood, respiratory tract specimens, skin and wound.

**Results:** Concordant results of identification to the species level by all three methods were received for 77 (91.7%) clinical isolates (*C. albicans* n = 54; *C. glabrata* n = 12; *C. tropicalis* n = 9; *C. krusei* n = 2). Discrepancies in identification of *Candida* sp. strains were observed for six of *C. parapsilosis* (automated methods as *C. parapsilosis*/n = 6), ChromAgar – *C. tropicalis* n = 2, *C. glabrata* n = 4; and MALDI-TOF as *C. tropicalis*) and for one of *C. kefyr* (automated method and MALDI-TOF as *C. kefyr*, but ChromAgar as *C. glabrata*). The ATCC reference strain tested was correctly identified to the species level by mass spectrometry method and the other methods.

**Conclusion:** The conventional and automated methods for identification of *Candida* species are accurate, but time to obtain the results is longer than using mass spectrometry method. MALDI-TOF appeared to be a rapid and accurate mode of *Candida* sp. identification. Rapid turnaround time and low cost make this an appealing new option in microbiological laboratories.

#### P2351 Rapid identification of *Candida* species by MALDI-TOF technology

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**Objectives and background:** Rapid identification of yeast isolates is important in order to adjust the antifungal treatment and to reduce patient morbidity and mortality.

In this study we investigate whether Matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) can (i) replace current routine methods in first line identification of *Candida*, (ii) examine differences in generated results by two mass spectrometry instruments situated in two routine laboratory settings, (iii) measure time from entry of sample in the instrument to results. In East Denmark, the Microflex LT (Bruker Daltonics, Germany) and in West Denmark, the Vitek® MS (bioMérieux, France).

**Method:** Retrospective analysis of *Candida* isolates with identification in parallel by two MALDI-TOF instruments and API 20 C AUX (bioMérieux, France) was used as the standard method.

**Results:** Ninety-three isolates were examined on Microflex LT with the Biotyper 2.0 software according to the manufacturer's manual.



30 minutes. Protein extracts were clarified by centrifugation and separated by 1D PAGE, followed by in-gel trypsin digestion to obtain tryptic peptides. Peptides were further separated on a C18 reverse phase column and analysed on the LTQ Orbitrap (Thermo Fisher, Classic). The resultant MS/MS mass spectra were analysed using Mascot against an order specific database (NCBI deposited). All peptides identified, with an ion score >20 were selected for marker selection, validation and virulence factor identification. All species with unique peptides and virulence related signatures were used to populate a "new" microbial identification database, comprising peptide sequences, molecular weight and function.

**Results:** A total of 79 organisms from the family Enterobacteriaceae were analysed, including *Salmonella*, *Shigella*, *Escherichia*, *Yersinia*, *Klebsiella*, *Enterobacter*, *Citrobacter*, *Campylobacter*, *Serratia*, *Proteus*, *Morganella*, *Providencia* and *Hafnia*. We were able to identify markers for all of the species in this study. The identified markers were used to populate a "new" MS/MS microbial identification database.

**Conclusion:** This study revealed that genus and species-specific biomarkers can be deduced for each taxon making it one of the high resolution and high accuracy tools available to date. An added advantage of this approach is its potential to obtain functional characterisation of the expressed proteome including virulence related proteins.

#### **P2355** Lack of *Staphylococcus aureus* delta-toxin production as detected by whole cell MALDI-TOF MS in routine practice is associated with chronicity of infection

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**Objectives:** The aim of the study was to detect the *S. aureus* delta-toxin using Whole-Cell (WC) Matrix Assisted Laser Desorption and Ionization – Time of Flight (MALDI-TOF) mass spectrometry (MS), correlate delta-toxin expression with accessory gene regulator (agr) status, and assess the prevalence of agr deficiency in clinical isolates with and without resistance to methicillin and glycopeptides.

**Methods:** The delta-toxin peak in the mass spectrum was detected by using purified delta-toxin and isogenic strains for agr-maIII, which encodes delta-toxin, as controls. Correlation between delta-toxin production and agr RNAIII expression was assessed by northern blotting. A series of 168 consecutive clinical isolates and 28 unrelated glycopeptide intermediate *S. aureus* strains were then tested by WC-MALDI-TOF MS.

**Results:** Delta-toxin peak was detected at  $3005 \pm 5$  Thomson, as expected for the natural formulated-delta toxin. In clinical isolates, agr (and hence delta-toxin) deficiency was significantly associated with chronic infections, methicillin and glycopeptide resistance. However, in multivariate analysis chronicity of infection remained the sole factor significantly associated with delta-toxin expression ( $p = 0.001$ ).

**Conclusion:** The MS peaks corresponding to *S. aureus* delta-toxin was identified in the WC-MALDI-TOF spectrum produced during routine identification. This biomarker of agr status provides useful information with respect to chronicity of infection and, to a lesser extent, glycopeptide resistance.

## **MRSA – detection and prevention**

#### **P2357** Relative risk of transmission of various MRSA strains

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**Objectives:** The Netherlands is still in control of methicillin-resistant *Staphylococcus aureus* (MRSA) using a strict infection prevention strategy. Despite this sporadic outbreaks do occur. The aim of this study

was to estimate the relative risk of transmission of MRSA belonging to various MLVA complexes (MCs).

**Methods:** A prospective, observational study was performed in 2009 and 2010. All newly identified MRSA positive patients and health care workers (HCW) in 17 hospitals were included. If an MRSA-positive individual could be linked in time and place to an index patient or HCW and the MRSA isolate had a MLVA type identical to that from the index, the source was classified as nosocomial transmission. To estimate the relative risk of transmission of a MRSA belonging to a specific MC (or a group of MCs), the number of transmission events per total number cases with MRSA belonging to this specific MC was compared to the number of transmission events per total number of cases with MRSA belonging to MC398, also called livestock-associated MRSA. MC398 is known for its low transmission rate in hospitals.

**Results:** One thousand and twenty-three patients and 65 HCW were found to be MRSA positive. The MC was not available for 51 strains. In the resulting 1037 strains, 15 different MCs were found. Comparing all non-MC398 (59 transmission events/366 isolates) with MC398 (six transmission events/671 isolates) yielded a relative risk for transmission of 18.0 (95% CI 7.62–46.09,  $p < 0.001$ ).

A stratified analysis of known epidemic MC (49 transmission events/240 isolates) vs. MC398 showed a relative risk for transmission of 28.4 (95% CI 11.5–74.9,  $p < 0.001$ ). The non-epidemic strains (10 events/126 isolates) were 9.6 times more likely to spread than MC398 (95% CI 3.1–30.2,  $p < 0.001$ ). The single MC with the highest proportion of transmission events was MC8 (e.g. USA300) having 26 transmission events per 92 isolates (RR = 31.6 95% CI 12.9–84.1,  $p < 0.001$ ).

**Conclusion:** This study shows that there are huge differences in the transmissibility of various MRSA clones. Especially livestock-associated MRSA rarely spreads in the Dutch hospitals participating in this study. Control strategies could be adapted to these intrinsic differences of various MRSA strains.

#### **P2358** Modelling the impact of discharge screening and community decolonisation on the transmission of health-care associated methicillin-resistant *Staphylococcus aureus*

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**Objectives:** To use a multi-ward individual based model (IBM) of health care associated methicillin-resistant *Staphylococcus aureus* (HCAI MRSA) transmission and control to assess the impact of discharge screening and subsequent community decolonisation.

**Methods:** An IBM of HCAI MRSA transmission in a 900 bed hospital with ICU and general medical wards was developed. Patients were assigned a specialty on admission (Elderly Care or General Medicine) which were associated with distinct patient admission, discharge, transfer and readmission parameters estimated from data collected from a large UK hospital trust. Patient movement characteristics such as length of stay and hospital readmission have been associated with an increased risk of MRSA colonisation, thus risk groups were identified as targets for MRSA screening and treatment. Multiple screening combinations were considered, comparing strategies which screened all patients or identified risk group of patients on admission, discharge and routinely during the hospital stay and using conventional culture, chromogenic agars or PCR. Screening was combined with decolonisation which was applied to MRSA screened positive patients or to a risk group of patients without pre-screening.

**Results:** We identified three risk groups (Gp) for MRSA intervention: (Gp 1) patients in an ICU ward; (Gp 2) patients admitted to an elderly care specialty; (Gp 3) patients with a previous history of hospitalisation. These groups were shown to have a longer than average individual ward and hospital stay (Gp 1 and 2) or an increased probability of readmission (Gp 2 and 3). Over 5 years decolonisation in the ICU ward only reduced hospital wide MRSA infection rates by 55% but did not significantly reduce colonisation rates. Expanding decolonisation to all patients on admission reduced MRSA infection rates by 80% and colonisation rates by 60%. Additionally screening patients in Gp 2 or 3 (30% of the population) on discharge and

decolonising in the community, reduced MRSA infection rates by 23% and colonisation rates by 30% compared to decolonisation in the ICU alone.

**Conclusion:** Mass screening and treatment, although effective in reducing MRSA, results in a large care burden on admission. It also may select for MRSA strains with reduced susceptibility to chlorhexidine and mupirocin. We suggest that targeted decolonisation within the hospital and community may be an alternative to mass screening and decolonisation outside the ICU setting.

#### **P2359** Statistical inference of MRSA transmission in hospitals using whole genome sequence data

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Despite a recent decline in infection rates in some European countries, methicillin-resistant *Staphylococcus aureus* (MRSA) remains one of the most important nosocomial pathogens globally due to the wide dissemination of highly virulent pandemic lineages. The transmission dynamics of MRSA in healthcare settings have been widely studied, but until recently, large-scale studies which take into account the genetic diversity of the pathogen have not been feasible. Technological advances have meant that the collection of whole genome sequence (WGS) data is now easier, faster and cheaper than ever before, but statistical methods to utilize this information in transmission dynamic models are still in the early stages of development. Analyses relying on routinely collected epidemiological data can derive the rate and relative importance of transmission routes, but the specific transmission network remains unclear, since all MRSA isolates are necessarily regarded as identical. We aimed to develop new methods to model within-hospital MRSA transmission, using genetic information in addition to epidemiological data.

We built a transmission model which incorporates genetic information where available, where measures of genetic similarity allow us to estimate who infected whom. Using both real and simulated data, where the WGS for the majority of MRSA isolates was available, we analysed MRSA transmission under various assumptions, and attempted to reconstruct the transmission network. We compared our outcomes with a model excluding genetic data, to determine the benefits of this new approach.

Initial results show that incorporation of WGS data considerably reduces uncertainty surrounding transmission. While our knowledge of true colonisation routes remains imperfect, particularly due to the role of healthcare workers in spreading MRSA, the utilisation of genetic data allows us to estimate the source of each colonisation.

The collection of WGS data, in combination with new modelling methods, allows an unprecedented insight into patient level transmission dynamics. This is of much interest to policy makers, as it allows us to investigate heterogeneity in patient infectivity and the effectiveness of infection control methods. With WGS data likely to become abundant in the near future, the development of sophisticated analytical tools is of great importance.

#### **P2360** Clonal complex 398 MSSA: a frequent unrecognised human pathogen and a probable reservoir for clonal complex 398 livestock-associated-MRSA emergence

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**Background:** Clonal complex 398 Livestock-Associated-MRSA (CC398 LA-MRSA) clone has been described as major animal pathogen, that can also colonize and infect humans. Little is known about its CC398 Methicillin susceptible *Staphylococcus aureus* (CC398 MSSA) counterpart. Here, we investigated the clinical, phenotypic, genotypic characteristics of a large CC398 MSSA collection of human isolates.

**Materials and methods:** One hundred and twenty-six CC398 MSSA strains of human origin were identified within the collection of the French National Reference Centre for Staphylococci. They were extensively characterized using antimicrobial susceptibility testing, spa typing, DNA microarrays (StaphyType, Alere), specific CC398-specific sequence PCR, encoding macrolides resistance (ermT) PCR. Fifty-three CC398 LA-MRSA collected from French pigs and veals were used as comparators. Phylogenetic relation between human CC398 MSSA and animal CC398 MRSA populations were explored on the basis of spa-typing and DNA microarrays.

**Results and discussion:** CC398 MSSA was able to induce a large spectrum of infections (especially skin, bloodstream and respiratory tract infections) and so appeared as an unspecialized pathogen. Surprisingly, its prevalence rate was high in MSSA population, that is, 24.7% (n = 21/85) in a local prospective study on nasal colonization, and 7.5% (n = 10/132) in a national prospective study on infective endocarditis. CC398 MSSA isolates frequently (89%, 112/126) presented an isolated erythromycin resistance, due to the presence of the ermT gene. Of note, the ermT gene was never detected in erythromycin resistant CC398 LA-MRSA strains. CC398 MSSA and CC398 LA-MRSA populations were closely related based on spa-typing and DNA microarrays. Genetic variability was associated with the acquisition of antibiotic resistance genes, including bla genes and a type IV or V SCCmec. Noteworthy, 26 CC398 MSSA strains isolated outside of mainland France (Switzerland, Algeria, Denmark, Dominican Republic, India, Madagascar, US, French Islands) showed similar features and clustered with French isolates.

**Conclusion:** In human, CC398 MSSA is a frequent colonizing bacteria and unspecialized pathogen, that has specific phenotypic and genotypic signatures. The genetic background of this population appears homogenous, and close to CC398 LA-MRSA population. It may constitute the reservoir from which LA-MRSA CC398 emerged.

#### **P2361** A cohort study on the import of *Staphylococcus aureus* from the tropics and subtropics through nasal carriage in travellers

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**Objectives:** Acquisition of nasal colonization abroad and subsequent import into the domestic population of bacteria may promote the dissemination of exchangeable virulence factors and thus the evolution of more virulent *Staphylococcus aureus* strains. This study investigated whether travel to the tropics and subtropics leads to nasal carriage and import of *S. aureus*.

**Methods:** The nasal carriage status (non-, intermittent, persistent carriage) of 503 travellers and 620 non-travellers was ascertained at two time points. New acquisition of *S. aureus* nasal carriage (main outcome) was analyzed by exposure to travel during follow-up (main exposure). Risk factors for nasal carriage at baseline, their influence on change in nasal carriage status and risk factors during follow-up were studied for a potential confounding effect.

**Results:** Of 1123 individuals included at baseline 943 were available for follow-up (loss 16.0%). Loss to follow-up was higher among travellers (21.5% vs. 11.8%). Persistent nasal carriage at baseline was more likely in men, non-smokers, pet-owners, with increasing age and decreasing time period between swabs. Smoking, inpatient stay within 3 months before enrolment, hormonal contraception, follow-up time and antibiotic intake during follow-up were found to be associated with a change in *S. aureus* nasal carriage during follow up. Travel did not have an effect on *S. aureus* -import (OR 1.23, 95%CI 0.70–2.15, p = 0.5) and showed only a weak and non-significant trend towards such an effect after adjusting for antibiotic use during follow-up (adjusted OR 1.35, 0.76–2.41, p = 0.3). There was evidence for interaction of an association of travel with *S. aureus* -gain and follow-up time (likelihood ratio test p = 0.06): the OR comparing import of *S. aureus* in travellers to non-travellers with long follow-up was 1.75 (0.79–3.84, p = 0.2) while the OR for a similar comparison in individuals with short follow-up was 0.49 (0.14–1.72, p = 0.3). Loss

of *S. aureus*-carriage was more common in travellers and partially confounded by antibiotic use during follow-up.

**Conclusions:** This study does not provide conclusive evidence for the acquisition of *S. aureus* nasal carriage during travel to the tropics and subtropics. A trend, however, indicates, that such import may exist in the subgroup of long-term travellers. Genotypic characterisation of pre- and post travel isolates may provide additional evidence to further support this hypothesis.

**P2362** Detection of methicillin-resistant *Staphylococcus aureus* ST398 in mozzarella cheese in Italy

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**Objectives:** Methicillin-resistant *Staphylococcus aureus* (MRSA) is a pathogen of increasing importance in hospitals, the community, and in recent years also in livestock. MRSA associated with livestock (LA-MRSA) have been reported worldwide in many species, but mainly in pigs.

LA-MRSA isolates are non typeable by pulsed-field gel electrophoresis (PFGE) following *Sma*I restriction and belong to multilocus sequence type ST 398. MRSA ST398 has been described as a cause of infection in individuals occupationally exposed to direct or indirect contact with pigs. Clonal lineage ST398 has been isolated also in foods of animal origin, especially retail meat. No data have been published so far about isolation of MRSA ST398 in food of animal origin in Italy.

**Methods:** During 2008–2009, 630 samples from milk and cheese produced in Apulia region (South Italy) were examined for the presence of MRSA. *S. aureus* was isolated from 110 samples (17%). Strains were stored at -20°C in vials containing sterile glycerol (30%) until use. The antibiotic resistance profile was determined using the disk diffusion method (Kirby-Bauer). The presence of the *me*C gene was investigated by PCR. MRSA strains were characterized by PFGE, multilocus sequence typing (MLST), spa- and staphylococcal cassette chromosome *mec* (SCC*mec*) typing.

**Results:** Two MRSA strains were identified among the 110 *S. aureus* collected (1.8%), both from mozzarella cheese taken from different dairy farms during the same period. In addition to oxacillin and the other beta-lactams, the two strains were resistant to tetracycline and were not typeable by PFGE. Genotyping of the two MRSA strains confirmed that they were ST398, spa type t108 and carried SCC*mec* type V.

**Conclusion:** The use of antimicrobials in food animal production can contribute to the spread of antimicrobial resistant microorganisms, including MRSA. Transmission of resistant bacteria from animals to humans can occur also through ingestion or contact with food of animal origin. Although the presence of MRSA in food is low, it has to be monitored because it can contribute to the spread of MRSA. To our knowledge this is the first time that MRSA ST398 has been isolated from food of animal origin in Italy and the first time that it has been isolated from dairy products. This finding is very worrisome because transmission of MRSA from food to humans can be promoted by the consumption of uncooked foods, such as dairy products (Work supported by IZSPB 06/08 – IZSPB 07/09).

**P2363** Consumption of meat as a risk factor for MRSA carriage: a case-control study

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**Objectives:** Although the Scandinavian Countries and the Netherlands have managed to keep their methicillin-resistant *Staphylococcus aureus* (MRSA) prevalence rates low by applying the Search and Destroy strategy, in recent years a rapid increase in MRSA has been observed in patients without known risk factors, that is, MRSA of unknown source

(MRSA-US). The aim of this case-control study was to identify risk factors for MRSA-US in the Netherlands.

**Methods:** Cases and controls were selected from July 2009 until July 2011 from 16 Dutch hospitals. A case was defined as a hospitalised patient or patient visiting the outpatients' clinic that was colonised or infected with MRSA-US. A control was defined as a hospitalised patient without MRSA admitted to the same ward or visiting the same outpatients' clinic as the case on the day the MRSA positive culture was reported. Both cases and controls were aged 1 year or older, had no previous history of MRSA colonisation or infection and had no known risk factor(s) for MRSA as described in the Dutch MRSA guideline. Cases and controls were visited at home to take an extended questionnaire on potential risk factors for MRSA, including country of origin, attendance of day care centre, profession, contact sports, diving, visiting sauna, travel, getting pierced/tattoos/acupuncture, meat consumption and smoking. Nasal and throat swabs were taken, both from cases and controls and from their household members.

**Results:** In 2 years, 96 cases and 96 controls were enrolled. Consumption of pork, beef and chicken, all at least once a week was found to be a risk factor for MRSA carriage (cases: 57/96, controls: 40/96, OR = 2.05 95% CI 1.11–3.79). No other risk factors could be identified.

**Conclusion:** Consumption of meat was identified as a statistically significant risk factor for carriage of MRSA of unknown source in patients admitted to the hospital. Where the contamination of different types of meat with MRSA has been reported previously, the results of this study suggest that consumption of MRSA contaminated meat may indeed result in the acquisition of MRSA by humans.

**P2364** Genetic characterisation of community-acquired methicillin-resistant *Staphylococcus aureus* isolated in a low-endemic area using DNA microarray analysis

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**Objectives:** Methicillin-resistant *Staphylococcus aureus* (MRSA) is a well known nosocomial pathogen that has spread worldwide. Sweden is still a low-endemic area and the prevalence of MRSA has continued to be low, ~1%. However, a changing epidemiology has recently been observed, and community-associated (CA) MRSA is emerging. In 2010 a total of 1580 cases of MRSA were reported in Sweden and >60% were CA-MRSA.

The aim of the present study was to investigate the molecular epidemiology as well as the distribution of genetic characteristics such as virulence genes and resistance genes among CA-MRSA isolates obtained in a low endemic country using DNA microarray analysis.

**Methods:** CA-MRSA isolates (n = 114) obtained from clinical infections as well as from screening procedures (nares, throat and perineum/groin) in Örebro County, Sweden, (population ~280 000 inhabitants) were analyzed.

DNA microarray-based typing was performed by the Alere StaphyType DNA microarray that includes 334 target sequences corresponding to ~170 distinct genes and their allelic variants.

**Results:** The CA-MRSA isolates obtained from 114 index patients displayed 34 distinct clonal complexes (CC) or STs containing 1–26 isolates. The three dominating CCs were CC80-MRSA-IV European caMRSA Clone (n = 26), ST8-MRSA-IV USA300 (n = 10), and CC5-MRSA-IV Paediatric clone (n = 10), comprising together 46/114 (40%). The distribution of the SCC*mec* types were type I (n = 2), type II (n = 3), type IV (n = 95), and SCC*mec* type V (n = 14).

PVL was found in almost all isolates. The genes encoding staphylococcal enterotoxins A, B and C were found in 14, four and three isolates, respectively, and nine isolates harboured the *tst*-1 gene. In general, the prevalence of genes encoding various antimicrobial resistance genes was low. The genes *aacA*–*aphD* encoding resistance against gentamicin and *aaD* encoding resistance against tobramycin were found in 14 (12%) and 9 (8%) isolates, respectively. The gene

*mupR* encoding resistance against mupirocin was not found in any isolate.

**Conclusion:** The CA-MRSA found in our low endemic area are heterogeneous and diverse displaying numerous genetic backgrounds. However, the known geographic background of some of the CCs found suggest a multiple and random importation of CA-MRSA from epidemic regions into Sweden.

**P2365** Duration of methicillin-resistant *Staphylococcus aureus* bacteraemia and impact on patient mortality within methicillin resistant *Staphylococcus aureus* strains in a retrospective cohort

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**Objectives:** This study aims to describe the association between methicillin resistant *Staphylococcus aureus* bacteremia (MRSAB) duration and patient mortality at 30 days of onset of the infection within different methicillin resistant *Staphylococcus aureus* (MRSA) strains: ST5 (USA100), ST8 (USA300), ST45 (USA600) and all other strains.

**Methods:** Seven hundred and sixteen consecutive MRSAB patient data was collected over a 5 year period, from January 2006 to December 2010 in a 900 bed teaching hospital in Detroit. Patients were identified through review of microbiology laboratory records and PFGE was performed on all isolates. Risk factors and outcome data was obtained by chart review. Duration of MRSAB was categorized in two groups, 3 days or less of duration and more than 3 days of duration. The association between duration of MRSAB and mortality at 30 days from onset of MRSAB was compared within each MRSA strain group using relative risk as a measure of association.

**Results:** Seven hundred and sixteen patients were evaluable for strain type, duration of MRSAB and mortality. The MRSA strains were distributed among the patients as follows: 291 (40.6%) ST5, 295 (41.2%) ST8, 21 (2.9%) ST45 and 109 (15.2%) of all other existing MRSA strains. 17.1% (n = 123) of the patient sample died within 30 days from onset of MRSAB. Patients that died within 30 days of onset of the infection had a mean age of 64.4 + 1.7 and were predominantly male, 59.5% (n = 353). Patients that remained alive after 30 days had a mean age of 57.2 + 0.7 and were also predominantly male 58.54% (n = 72). There was a statistical difference in age between the groups (p = 0.0001) but not in sex (p = 0.83). A relative risk calculation was made between the duration of MRSAB and the mortality within 30 days of onset of MRSAB finding that there was no association within any of the strain groups (ST5 (RR 0.05 CI -0.03–0.13 p = 0.25), ST8 (RR 0.03 CI -0.03–0.11 p = 0.33), ST45 (RR 0.08 IC -0.33–0.49 p = 0.69) and all other strains (RR 0.02 CI -0.15–0.19 p = 0.82)).

**Conclusions:** No difference in mortality at 30 days was observed when the duration of MRSAB is longer than 3 days within any of the evaluated MRSA strain groups (ST5, ST8, ST45 or all other MRSA strains).

**P2366** Comparison of composite clinical outcome in USA600 (ST45) vs. USA100 (ST5) methicillin-resistant *Staphylococcus aureus* bacteraemia patients

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**Objectives:** The aim of this study is to evaluate and compare clinical outcomes in patients with Methicillin-Resistant *Staphylococcus aureus* (MRSA) blood infections by strain type- USA600 (ST45), a predominantly hospital acquired strain, which has a reported incidence of about 5% with reported mortality rates as high as 50% and failure rates of 80% vs. USA100 (ST5), which has a reported incidence ranging from 13% to 54% and mortality rates of 18%.

**Methods:** Patients were identified through review of microbiology laboratory records over a 5-year period, from January 2006 to December 2010 in a 900-bed teaching hospital in Detroit. Clinical failure was defined as: presence of either (i) 30 day mortality, (ii) recurrence of bacteremia within 30 days at end of therapy, or (iii) a positive blood culture of MRSA for  $\geq 7$  days after collection of the index blood culture. PFGE was performed on all isolates. Medical history, risk factors, antibiotic exposure and outcome were obtained by chart review.

**Results:** Seven hundred and sixteen consecutive MRSA bacteremia patients were evaluable for composite clinical failure. The prevalence of USA600 (ST45) was 2.9% (n = 21) and 40% (n = 289) for USA100 (ST5). USA600 (ST45) patients had a mean ( $\pm$ SD) age 66 years  $\pm$  20.5 with 13 (62%) females, and 8 (38%) males. Mean ( $\pm$ SD) age for the USA100 (ST5) patients was 61  $\pm$  21.1, with 171 (59%) males and 118 (41%) females. The vancomycin minimum inhibitory concentration (MIC) by E-test was available for 679 of the 716 subjects, and 5 (5%) of the USA600 (ST45) had a MIC  $\geq 2$   $\mu$ g/mL, while 16 (76%) USA600 (ST45) had a MIC < 2  $\mu$ g/mL. Mean ( $\pm$ SD) duration of bacteremia for all patients was 4  $\pm$  4.1 days, and no differences in means of duration were seen within the CDC groups separately. The difference in composite failure amongst the two groups was 13 (62%) of the USA600 (ST45) patients vs. 98 (34%) USA100 (ST5), (p = 0.01). Mortality and recurrence rates amongst USA600 (ST45) was 38% (eight pts) died, and 14% experienced recurrence, and in the USA100 (ST5) group, 14% (three pts) died and 6% were observed as having relapse.

**Conclusions:** This is the first comparative observational study of its kind. The results from this study show a statistically significant difference in clinical outcome between patients with USA600 (ST45) and USA100 (ST5) MRSA bacteremia, as there is evidence of a much higher failure rate within the USA600 (ST45) patients. These findings have important implications for control of further spread of isolates.

**P2367** Comparison of two commercial PCR methods for detection of methicillin-resistant *Staphylococcus aureus* (MRSA) in a clinical setting

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**Introduction:** Methicillin-resistant *Staphylococcus aureus* (MRSA) is a growing problem in health care environments leading to increased, costs, lengths of stays, morbidity, and mortality. Screening of new admitted patients is an important step in the containment of MRSA spreading. Especially molecular methods in combination with classical culture methods are a powerful tool in the detection of MRSA-colonized patients. In our study we compared two commercially available PCR test systems for the detection of MRSA with the classical identification via selective MRSA agars.

**Materials and methods:** Double-nasal/throat-swabs from 813 patients admitted to three intensive care units and emergency rooms of our hospital were screened for MRSA using CHROMagar MRSA (BD, Heidelberg, Germany) for each swab with subsequent MRSA confirmation by our microbiological laboratory. One swab each was screened with the LightCycler Advanced MRSA (Roche, Mannheim, Germany) test and the Detect-Ready MRSA (MDI, Kent, UK) test, respectively. Results were compared to the CHROMagar MRSA results, which were set as gold standard.

**Results:** MRSA was detected in 3.44% of the patients (28/813), in 15.91% of the *S. aureus* positive patients (28/176) with CHROMagar MRSA. The LightCycler MRSA Advanced test (82.1% clinical sensitivity) was more sensitive in the detection of MRSA than the Detect-Ready MRSA test (54.2%), but the positive predictive value was better for the Detect-Ready MRSA test (81.3%) than for the LightCycler MRSA Advanced test (71.9%). The specificities and the negative predictive values of both tests were high (>98%).

**Conclusion:** For routine screening of MRSA the LightCycler MRSA Advanced test proved to be more efficient in our clinical setting as the sensitivity was much higher than the sensitivity of the Detect-Ready MRSA Kit. CHROMagar MRSA detected more true MRSA positive



samples than both PCR methods, leading to the conclusion that the combination of PCR with cultural screening is still the most reliable way for the detection of MRSA. LightCycler MRSA Advanced test was faster and needed less hands-on time, but Detect-Ready identified possible methicillin-sensitive *S. aureus* in 35.5% (289/813) of the patients in addition to the identification of MRSA, which is important additional information for the attending physician.

**P2368** Performance of commercial MRSA real-time PCR assays vary in sensitivity when testing nasal samples in a large control programme

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**Objectives:** Real-time PCR is emerging as the optimal diagnostic test for detection of MRSA colonization in the clinical laboratory. We reported that the Roche LightCycler® MRSA Advanced test has a sensitivity, specificity, PPV and NPV of 98.3%, 98.9%, 86.7% and 99.9% respectively, when tested on 5838 nasal swab samples (IDSA 2011). To further assess this technology we evaluated the BD GeneOhm™ MRSA Assay (BD MRSA), the Cepheid Xpert® MRSA test (Cepheid MRSA) and the Cepheid Xpert® SA Nasal Complete (that detects MRSA as well as methicillin susceptible *S. aureus*; MSSA; Cepheid SA) on nasal samples using the same hospital population.

**Methods:** Admission and discharge nasal swabs were collected from all inpatients at one of our facilities using a double-headed swab. One swab was plated onto CHROMagar™ MRSA (CMA; Becton Dickinson) and then broken off into Tryptic soy broth (TSB) for enrichment. TSB was incubated for 24 hours then plated to CMA. Plates were incubated at 33–35°C for 48 hours. Mauve colonies were subcultured to blood agar (Becton Dickinson) and incubated for 24 hours. *S. aureus* identification was confirmed by performing a Staphaurex agglutination test (Remel). The molecular tests were performed on the second swab according to the manufacturer's instructions. Clinical data review and repeat testing was performed on discordant specimens to resolve discrepancies between culture and PCR. Only PCR positive specimens were cultured with the BD MRSA limiting those results to specificity data. Any positive culture and history of prior MRSA were considered a gold standard (true positive) for this evaluation.

**Results:** A total of 4267 specimens were enrolled for the BD MRSA analysis providing a specificity = 98.5%. For the Cepheid MRSA test 1148 samples were evaluated with a MRSA prevalence of 6.1% and sensitivity, specificity, PPV and NPV of 92.8%, 98.9%, 84.4% and 99.5% respectively. The Cepheid SA test has been tested on 228 samples with a MSSA prevalence of 29%. The overall sensitivity, specificity, PPV and NPV was 81.4%, 98.1%, 95% and 92.3% respectively. There were six MRSA with five detected and one test reporting an invalid result. One MSSA was reported as MRSA and no MRSA were reported as MSSA.

**Conclusion:** Our results suggest that the various molecular assays have somewhat differing performance. The specificity of most assays is good and relatively equivalent, but sensitivity differences may impact outcome when used in a large MRSA control program.

**P2369** Evaluation of potential inhibitory effects of biological and chemical materials on the BD MAX™ MRSA assay, a new fully automated molecular assay

M. Tremblay\*, J.-S. Côté, C. Roger-Dalbert (Québec, CA)

**Objective:** The BD MAX™ MRSA Assay performed on the BD MAX™ System is an automated qualitative in vitro diagnostic test for the direct detection of methicillin-resistant *Staphylococcus aureus* (MRSA) DNA from nasal swabs in patients at risk for nasal colonization. The aim of this study was to evaluate the potential inhibitory effects of biological (biological liquids and organisms) and chemical materials, which may be present on nasal swab specimens, on the BD MAX™ MRSA Assay.

**Methods:** Twenty (20) biological and chemical substances occasionally used in the nares or found in nasal swab specimens were evaluated for potential interference with the BD MAX™ MRSA Assay. MRSA negative specimens and MRSA positive specimens at 2–3 × LoD95% (Limit of detection) were tested with the highest amount of each compound likely to be found at the sampling site or on the nasal swab specimens. Microbial interference was also assessed. MRSA negative specimens and MRSA positive specimens at 2–3 × LoD95% were tested in presence of MSSA and CNS strains (at 10<sup>5</sup> CFU/swab as potentially competing or interfering organisms) were tested with the BD MAX™ MRSA Assay. For all the different tested specimens, a swab was used to absorb the bacterial preparation and was then placed in a BD MAX™ MRSA Sample Buffer Tube containing the evaluated biological or chemical material. The Sample Buffer Tube was vortexed to release cells from the swab into the buffer. The reagents were placed on the racks of the instrument and the BD MAX™ MRSA Assay was run on the BD MAX™ System.

**Results:** Results demonstrated no reportable interference with any of the 20 substances or organisms except for Tobramycin that showed slight inhibition (delay of cycle threshold) in the BD MAX™ MRSA Assay, however, expected assay results were still obtained.

**Conclusion:** No biological (bacteria, blood, mucus) or chemical (nasal spray, nasal gel, etc) interference, which could lead to false-negative or false-positive result, was observed with the BD MAX™ MRSA Assay. These data demonstrate the robustness of this new automated molecular assay.

\*The BD MAX™ MRSA Assay is not available for sale or use in the US.

**P2370** Clinical evaluation of the BD MAX™ MRSA assay for the rapid detection of MRSA directly from nares swabs

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**Objective:** It is well documented that methicillin-resistant *Staphylococcus aureus* (MRSA) is a leading cause of health-care associated infections (HAIs) and that early detection can reduce transmission and infection. Equally, MRSA prevention programs that include active surveillance are increasingly common in health care settings. Sensitive molecular amplification techniques capable of detecting extremely low bacterial burden in clinical specimens within a few hours allow for prompt isolation of colonized individuals and thus may assist preventing the spread of MRSA. This study evaluated the fully automated sample-to-result platform of the BD MAX™ in comparison to conventional culture for direct detection of MRSA from nasal swab specimens.

**Methods:** A total of 892 dual swab nares specimens were analyzed from four clinical sites. Swabs were inoculated onto a BBL CHROMagar™ SA plate (CSA), broken into a sample buffer tube (SBT), vortexed, and analyzed using the BD MAX™ instrument/assay. A 200 µL aliquot of the SBT was inoculated into an enrichment broth (TSB with 6.5% NaCl) to maximize culture sensitivity. Results from the BD MAX™ were compared to direct and enriched culture using standard and chromogenic media incubated for up to 48 hours (reference method). Identification as MRSA was performed using a latex agglutination test, and Kirby-Bauer cefoxitin disk diffusion according to CLSI.

**Results:** Compared to reference culture methods, BD MAX™ identified 72 of 75 culture positive specimens. Of those, three were positive by direct culture/enrichment culture only. An additional 29 specimens were positive for MRSA by BD MAX™ alone which yields an overall sensitivity of 96% (88.9%, 98.6% CI) and overall specificity of 96.5% (94.9, 97.5 CI).

**Conclusions:** The BD MAX™ exhibited excellent sensitivity/specificity for direct detection of MRSA from nasal swabs specimens compared to reference culture methods. The BD MAX™ is a fully automated sample-to-result molecular platform that can provide MRSA test results for up to 24 specimens in ~2.5 hours, allowing rapid decisions affecting patient management.

**P2371** Evaluation of the analytical sensitivity (Limit of Detection) of the BD MAX™ MRSA assay, a new fully automated molecular assay

M. Tremblay\*, F. Hamel, C. Roger-Dalbert (Québec, CA)

**Objective:** The BD MAX™ MRSA Assay performed on the BD MAX™ System is an automated qualitative in vitro diagnostic test for the direct detection of methicillin-resistant *Staphylococcus aureus* (MRSA) DNA from nasal swabs in patients at risk for nasal colonization. The aim of this study was to determine the analytical sensitivity (Limits of Detection) of the BD MAX™ MRSA Assay (in colony-forming units [CFU] per swab) for six Methicillin-resistant *Staphylococcus aureus* (MRSA) genotypes (mec Right Extremity Junction [MREJ]). The study was performed with negative nasal specimens.

**Methods:** The analytical sensitivity (LoD95%) for the BD MAX™ MRSA Assay was determined as follows: simulated positive specimens were prepared by soaking swabs in a wide range of MRSA bacterial suspensions prepared and quantified from cultures of six MRSA strains representing six MREJ genotypes (i–vi) and four SCCmec types (I–IV). The swabs were then eluted in pooled negative clinical nasal specimens. Each MRSA strain was tested in replicates of 24 per concentration by two different operators using three different production lots of the BD MAX™ MRSA Assay and nine different BD MAX system. The LoD95% value was determined using a method that models the positive response (expressed in percentage) as a function of Log(CFU) per swab. The logistic model equation of the fitted curve allows the computation of the LoD95% by inverse prediction using the parameter estimates and its 95% confidence interval.

**Results:** Analytical sensitivity (LoD95%) values, defined as the lowest concentration at which 95% of all replicates tested positive, were determined for six MREJ genotypes (four SCCmec types) in the presence of nasal specimens. The LoD95% values ranged from 273 to 645 CFU/swab. More precisely, the obtained values for the MRSA MREJ type i–vi were respectively 645, 400, 346, 490, 273, and 357 CFU/swab.

**Conclusion:** Analytical sensitivity ranged from 273 to 645 CFU/swab. Since the average clinical load of MRSA in the nares corresponds to 10<sup>6</sup> CFU/swab, the BD MAX™ MRSA Assay is sufficiently sensitive to detect low MRSA load in clinical specimens.

\*The BD MAX™ MRSA Assay is not available for sale or use in the US.

**P2372** Experiences with RT-PCR methicillin-resistant *Staphylococcus aureus* screening in a low-prevalence population

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**Objectives:** Evaluation of an in-house dual RT-PCR strategy for detection of methicillin-resistant *Staphylococcus aureus* (MRSA) carriers.

**Methods:** Selection of persons and sampling from two or more anatomical sites were done according to national guidelines. Samples were incubated over-night in a selective enrichment broth, then extracted and analysed for presence of *nuc* and *mecA* gene using two in-house RT-PCRs. Samples that tested negative for ≥1 gene were concluded negative, presence of both genes were further investigated by plating the broth out on blood agar and chromID™ MRSA chromogenic agar.

**Results:** In the period March through October 2011 we examined 7530 screening samples from 1046 healthcare workers and 1227 patients, in total 2273 persons. Of these tested 6299 (84%) samples negative and 1580 (75%) persons were cleared. Culture detected MRSA in 138 (1.8%) samples from 62 (2.7%) persons. Median *nuc* RT-PCR cycle threshold values (C<sub>T</sub>) in MRSA culture negative samples were 28.8 (SD 6.0), in culture positive samples 22.4 (SD 4.0). Of 7530 samples, 5513

(73%) tested negative for *nuc* gene, while 1176 (52%) persons had no samples positive for *nuc* gene.

**Conclusion:** In a low MRSA prevalence setting, rapid evaluation of carrier status is important. In our hands the *nuc* RT-PCR alone would have cleared 73% of the samples, but only 52% of the persons. Addition of a *mecA* RT-PCR increased our person clearance rate to 75%. This illustrates the danger of evaluating a strategy on per sample alone. Differences in C<sub>T</sub> values between culture negative and culture positive sample groups suggest that “CT-breakpoints” may be applied to further increase RT-PCR clearance rate.

**P2373** Twenty-minute identification of enterococci and *Staphylococcus aureus* causing bloodstream infections by QuickFISH™, a novel assay based on PNA technology

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**Objectives:** The identification of bloodstream pathogens in a clinically relevant timeframe is critical to improved patient management and strong antibiotic stewardship programs by impacting the prudent selection of appropriate empiric therapeutic agents. This study evaluated the performance of two novel, fluorescence in situ hybridization (FISH) assays differentiating; *S. aureus* (SA) from coagulase-negative staphylococci (CoNS) and *Enterococcus faecalis* (E) from other non-faecalis enterococci (NEF), from newly Gram-positive blood cultures (BC) compared to culture.

**Methods:** From July to October 2011, 124 patients with BC bottles smear positive for Gram-positive cocci in clusters (GPCC) were tested as well as 58 patients with BC positive for GPC in pairs and chains (GPCPCH) using two FISH assays, the *Staphylococcus* QuickFISH BC and the *Enterococcus* QuickFISH BC (AdvanDx). The peptide nucleic acid (PNA) fluorescent probes in the tests target rRNA specific sequences and appear either green or red when examined by fluorescence microscopy as follows: SA (green)/CoNS (red) and E (green)/OE (red). Culture ID was performed for comparison using the MicroScan system (Siemens), coagulase and Staphaurex (Remel). 16S sequencing was used to resolve discordant results.

**Results:** Of 124 GPCC from BC, 87 were CNS, 34 SA (10 methicillin-resistant) and three *Micrococcus* species. One BC was positive for SA and CoNS, which resulted in both red and green fluorescence. Of 58 GPCPCH, 17 were E, 12 OE and 29 streptococci. The sensitivity, specificity, positive and negative predictive values for both FISH assays compared to culture or 16S sequencing were 100%. One BC isolate, *Lactococcus garvieae* by 16S sequencing was misidentified as *E. faecium* by MicroScan and was FISH test negative for OE. The time to results for the QuickFISH assays were 20 minutes as opposed to 1–2 days by conventional culture methods.

**Conclusions:** The 20 minutes QuickFISH tests are highly sensitive and specific assays for identifying enterococci and staphylococci from BC in real-time. The ability to distinguish SA from CoNS and micrococci is clinically useful in evaluating true infection from contamination. In addition, identification of E alerts the clinician to the high probability of ampicillin and vancomycin susceptibility, whereas NEF alerts to the high probability of vancomycin resistance, when targeting appropriate pathogen specific therapy.

**P2374** Identification of methicillin-resistant staphylococci by PNA FISH directly on smears made from positive blood cultures from EU and USA

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**Background:** Rapid identification of *Staphylococcus aureus* (*S. aureus*) vs. coagulase negative staphylococci (CNS) and determination of resistance to methicillin (MR) of positive blood cultures containing Gram-positive cocci is crucial for optimal patient therapy.

*S. aureus*/CNS PNA FISH (AdvanDx) is a rapid method for routine identification staphylococci using ribosomal RNA (rRNA) as target. In this study, we evaluated a *mecA* PNA FISH assay targeting *mecA* messenger RNA (mRNA) in staphylococci. *mecA* PNA FISH was evaluated alone or in parallel with *S. aureus*/CNS PNA FISH on smears made from positive blood cultures.

**Methods:** The clinical performance of *mecA* PNA FISH in parallel with *S. aureus*/CNS PNA FISH (AdvanDx) was assessed using 140 Gram-positive cocci in clusters (GPCC) positive BD BACTEC™ and bioMérieux BacT/ALERT® blood culture bottles. Results were compared to routine identification obtained following subculture and determination of resistance to methicillin using cefoxitin disk diffusion. In addition, was the clinical performance of *mecA* PNA FISH assessed using 46 *S. aureus* -positive TREK® blood cultures bottles. The *mecA* PNA FISH results from this study was compared to routine susceptibility testing determined by Microscan (Siemens Healthcare Diagnostics)

**Results:** Table 1: MRSA PNA FISH vs. reference culture method 100% MRSA Positive agreement, 100% MRSA Negative agreement, 73% MR-CNS Positive agreement, 100% MR-CNS Negative agreement, 100% MRSA Positive predictive value, 100% MRSA negative predictive value, 100% MR-CNS Positive predictive value, 62% MR-CNS negative predictive value.

**Table 1: MRSA PNA FISH vs. reference culture method**

MRSA PNA FISH	Culture				
	MRSA	MSSA	MR-CNS	MS-CNS	
MRSA	18	0	0	0	18
MSSA	0	72	0	0	72
MR-CNS	0	0	49	0	49
MS-CNS	0	0	18	29	47
	18	72	67	29	186

**Conclusions:** In this study we have shown that *mecA* PNA FISH is a promising tool for identification of MRSA and potentially MR-CNS directly from positive blood cultures (2 hours). The <100% sensitivity for MR-CNS, may be explained by delayed expression of *mecA* which has been reported in the literature for some MR-CNS and that the assay are missing some strains in charcoal bottles.

## Controlling *S. aureus* infections in hospitals

### P2375 Methicillin-resistant *Staphylococcus aureus* in the elderly: a global analysis of trends in occurrence and susceptibility, 2004–2010

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**Background:** Methicillin-resistant *Staphylococcus aureus* is a significant pathogen worldwide affecting people of all ages. In this report, occurrence of MRSA in the elderly was compared to younger populations, and the susceptibility of MRSA in the elderly was assessed using data from the Tigecycline Evaluation and Surveillance Trial (TEST).

**Methods:** Three thousand nine hundred and fifty-six MRSA were collected between 2004 and 2010 in 52 countries from various infection sites in patients ≥65 years old. MICs were performed as specified by CLSI at each site using custom broth microdilution panels and interpreted per CLSI/FDA guidelines. Linear trends in % MRSA and % susceptible were assessed with the Cochran-Armitage test.

	% Susceptible						
	2004	2005	2006	2007	2008	2009	2010
Levofloxacin	5.0	7.5	8.4	12.0	8.6	7.1	2.4
Minocycline	96.5	97.5	96.2	96.1	92.4	86.3	82.8*
Tigecycline	100	100	99.8	100	100	99.7	100
Vancomycin	100	100	100	100	100	100	100
n	479	509	572	773	688	680	255

**Results:** Overall during 2004–2010, the MRSA rate was significantly higher in the elderly (45% of all *S. aureus*) than in the younger population (<65 years, 36%). The MRSA rate in the elderly decreased over that same time period from 52% in 2004 to 29% in 2010 ( $p < 0.05$ ), with a similarly significant decrease in the younger population. The susceptibility of MRSA in the elderly to several antimicrobial agents is shown below.

\*Statistically significant decreasing trend in susceptibility ( $p < 0.05$ ).

**Conclusions:** MRSA were more prevalent in the elderly than in the younger population but have been decreasing significantly over time. Only minocycline showed a highly significant linear trend of decreasing susceptibility ( $p < 0.0001$ ) from 2004 to 2010. Tigecycline and vancomycin showed excellent in vitro activity against MRSA in the elderly, with only three isolates non-susceptible to tigecycline.

### P2376 Multimodal strategy to control methicillin resistant *Staphylococcus aureus* – the experience of a Portuguese hospital

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**Background:** Methicillin Resistant *Staphylococcus Aureus* (MRSA) is the most frequently multidrug-resistant microorganism isolated in hospitals in many parts of the world, including Portugal, with a national rate of 49.1% reported in 2009. This agent is associated with a increased morbidity, mortality risk and costs.

**Objectives:** Evaluation of a multimodal strategy to control the emergence of MRSA, in a hospital with high endemic levels, after 4 years of its implementation.

**Methods:** Application of procedure based on active surveillance cultures (nasal swab using RT-PCR detection technique) and isolation measures in a selected population (patients from other health institutions and nursing homes or with a history of hospitalization/ MRSA, hospitalized patients in intensive care and, in other inpatient services, direct contacts of new MRSA patients). Other parallel activities: (i) review of isolation and standard precautions policy, (ii) reinforcement of alcohol-based handrubs at the point of patient care, (iii) information sessions to health professionals, (iv) targeted information flyer for health professionals, (v) information leaflet for patients and visitors; (vi) procedure monitoring by audit (vii) patient decolonization only in intensive care, with follow-up screenings.

**Results:** Between 2007 and 2011, there was a decrease in the proportion of MRSA from 66% to 56% and of density of incidence from 1.70 to 0.95 cases per thousand days of hospitalization. This decrease was not constant with an increase of cases in 2009. This may be explained by failures in implementing the protocol established. In fact, audits identified: omission of screening in patients with indication for it; screening without contact isolation measures and failure in signaling patients in isolation (observed in audits and subsequently corrected). These audits also allowed identifying haemodialysis patients as a risk population to be colonized with this microorganism.

**Conclusion:** A multimodal strategy proved effective in reducing cases of MRSA. Continuously monitoring of the procedure, with feedback to health professionals, is the key for the success of the strategy as well as to identify and adjust changes in the target population for screening.

### P2377 MRSA PCR spearheads a successful health-care associated infection programme at a teaching hospital in northwest England: a cost economic model supporting government aims of cost saving, improving quality, driving efficiency and safety in patient care

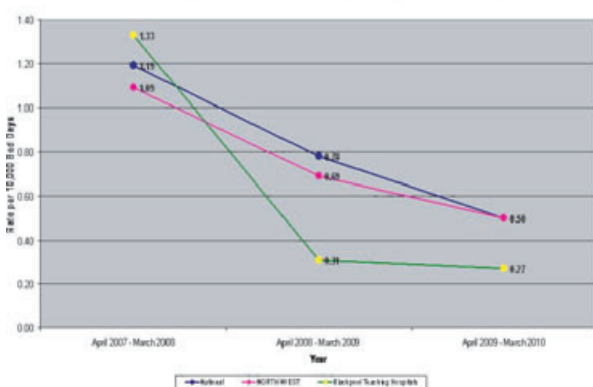
A. Guleri\*, R. Sharma, S. Bloor, S. Staff, A. Jones (Blackpool, Old Malton, UK)

**Background:** Rapid and accurate identification of MRSA in hospital admissions is essential for timely decisions on optimal treatment, isolation/bio-burden reduction, and reducing the potential for cross

transmission/self-acquisition, patient harm/mortality. Significant reductions in MRSA infections including bacteraemias can be used to realise cost savings. Blackpool Teaching Hospitals has a successful HAI programme spearheaded by MRSA PCR. Significant reductions [80%] in MRSA bacteraemia and non-bacteraemic infections have been reported in last 3-years. We present a case for improving efficiency, productivity and quality outcomes by using health informatics and statistical process control (SPC)-based analysis of all MRSA infections including key indicators – re-admissions, mortality and length of stay. Details to be presented.

**Methods:** SPC based analysis, data entry and testing, analyses of key indicators and cost modelling using local costs with relation to MRSA infections **Results:** Preliminary data available at time of submission shows: Reduction in total MRSA infection bed-days by 73% (827 in 07/08 to 222 in 10/11); MRSA surgical site infections bed-days by 69.8% (691 in 07/08 to 208 in 10/11). Reduction in readmissions with MRSA infection from 3 to 1/month (07/08 to 10/11). Infection control database results: MRSA bacteraemias in Blackpool Teaching Hospital (BTH) reduced from 1.33 to 0.27/10 000 bed-days (2007–2008 to 2010–2011) compared to National (1.19 to 0.5/10K bed-days) and northwest (1.09 to 0.5/10K bed-days). Optimisation of glycopeptide usage with >50% reduction.

**Figure 1. MRSA Bacteraemias per 10,000 Bed Days**



**Conclusions:** Savings of £50M over 3–4 years must be made by BTH as part of the Government's aim to deliver £20 billion (4%) efficiency savings in the NHS by the end of 2014–2015. Reduction in HAIs and other quality initiatives have been used to close a 24 bedded ward driving savings of approx £970K. The SPC-based analyses and health informatics project is set to analyse in detail the savings from reductions in HAIs using local costs.

**P2378** Can high-level mupirocin resistance reporting be relied upon to ensure patients are prescribed appropriate treatment?

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**Objectives:** To determine whether patients found colonised with mupirocin resistant Methicillin Resistant *Staphylococcus aureus* (MRSA) on screening are prescribed effective treatment for nasal decontamination as per the MRSA policy.

**Methods:** All acute and planned adult admissions are screened by nose, axilla and groin swabbing for MRSA and decolonised where necessary. Eleven thousand six hundred and ninety-two MRSA screens were processed over 6 weeks. All cases of high level mupirocin and neomycin resistance were followed up to see if patients were prescribed appropriate treatment.

**Results:** Two hundred and forty (2.1%) MRSA positive results were reported. Thirty (12.5%) had high level mupirocin resistance, and eight

(3.3%) also had neomycin resistance. Thirteen (43%) were new MRSA cases.

The cases notes were accessible for 22 (73%) of patients. Five patients were seen in pre-assessment clinic. One (20%) had the correct treatment recommended in a letter to the family doctor, two had mupirocin recommended and two had no letter sent.

Of the 17 in-patients, three were discharged before results were available. None had letters sent. Five new MRSA patients were not prescribed appropriate treatment until after intervention by the pharmacist (average delay = 2 days). Of the nine previously MRSA positive patients, seven were either mupirocin sensitive or had no sensitivities available at time of admission. All except one were correctly started on mupirocin treatment, but none were switched to the correct treatment when the new results were available, until intervention. Average delay 4 days. The two remaining patients with known mupirocin resistant MRSA were prescribed the correct treatment, but one took 3 days to start. So only one (7%) was correct.

**Conclusion:** There is no standard approach at ward level to check MRSA screens. Few patients have their treatment adjusted accordingly where high level mupirocin is detected, and most remain on treatment with mupirocin. Patients discharged before the results are released are rarely getting pre-printed letters sent to their family doctor or new hospital as described in the MRSA policy. Overall it can be concluded that the current web-based MRSA screen reporting process is ineffective in ensuring that patients are prescribed an appropriate agent where high level mupirocin is detected. This 0.3% of patients require different communication processes to improve this. The telephone follow up is a possible solution. It is possible that such a delay could contribute to MRSA acquisition within the Trust and possibly MRSA bacteraemia.

**P2379** Impetigo bullosa outbreak at a neonatal ward due to a methicillin susceptible *Staphylococcus aureus* strain, the Netherlands

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**Background:** Here, we describe an outbreak of impetigo bullosa (IB), caused by a (methicillin susceptible, clindamycin resistant) *Staphylococcus aureus* (SA) strain, spa-type t408, at the neonatal/gynaecology ward of the Jeroen Bosch hospital, the Netherlands. The first case presented with IB was in March 2011. Subsequently, similar cases were observed in April (2), May (11), June (3), July (4) and August (1) 2011.

**Methods:** A case was defined as a neonate with a blistering skin disease within 1 week after birth. We performed a retrospective outbreak investigation, including methicillin susceptible *Staphylococcus aureus* (MSSA) colonization surveillance, environmental sampling for MSSA, detailed molecular typing of MSSA isolates, and spa sequencing was performed to discriminate between the SA isolates. In addition, Raman-typing was performed on the clustered spa-types. We performed an infection control audit of the neonatal and gynaecology premises and practices in May, June, September and October.

**Results:** Fourteen out of 21 cases yielded a SA positive culture, from seven possible cases no positive SA culture could be collected. Spa-typing revealed a cluster of seven cases with SA t408, these strains were Meca-, PVL-, ETA-, ETB+, ETAD-, all were fusidic acid-resistant and methicillin susceptible. Raman typing of the seven t408 strains yielded four Raman types: three related types I (n = 4), II (n = 1) and III (n = 1) and an isolated Raman type IV (n = 1). All others were fusidic acid susceptible, no spa-typing could be performed on seven SA positive samples.

In addition, 482 samples of healthcare workers (HCW) were taken and three were found to be a t408 carrier, two nurses and one paediatrician. Two were treated and tested negative in subsequent screenings, the last HCW was recently diagnosed still needs to be treated.

In addition several hygienic protocols in the delivery rooms and operation room were updated and revised. Also, all staff was reminded of the importance of hand hygiene and other hygienic protocols.

**Conclusion:** Treatment of HCW carrying SA t408 and re-implementing and emphasising hygienic measures seem to be effective to control the outbreak of SA t408 among neonates. Fusidic acid resistant strains isolated from neonates should be typed and analysed for possible clustering with other isolates.

## New aspects of toxoplasmosis, Chagas, malaria and leishmaniasis

### **P2380** Seropositivity rate of *Toxoplasma gondii* infection in renal transplant recipients using IFA method

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**Objectives:** *Toxoplasma gondii* is a coccidian parasite of the phylum Apicomplexa that can infect humans and a broad spectrum of warm-blooded animals serving as intermediate hosts. Toxoplasmosis is a wide distributed opportunistic infection around the world, which might be influenced by several factors including host immune system conditions. Because of the continuous administration of immuno-suppressive drugs among renal transplant recipients, they are prone to acquire many opportunistic parasite infections; one of the most common organisms among them is *T. gondii*. In a cross-sectional study, *T. gondii* antibody titers were measured on serum samples from renal transplant recipients in Iran.

**Methods:** Patient's information was recorded in a questionnaire before sampling. A total of 551 serum samples were obtained from randomly selected population referred from different areas all over the country to a central clinic in Tehran. Two samples of finger-prick blood were collected from each person in heparinized microhematocrit tubes. Serum samples were isolated by centrifugation and stored at -20°C until being examined. Antibody titer against *Toxoplasma* was assessed by indirect fluorescence antibody (IFA) technique using fluorescein conjugated anti-human globulin antibody (AHG). Serum dilutions of 1:20, 1:100, and 1:200 were prepared from each sample to be tested. Parasite antigen were prepared from  $1 \times 10^5$  tachyzoite whole parasite bodies which were fixed on glass slides.

**Results:** Totally 39 cases (7.1%) including 7.7% (25/325) of male and 6.2% (14/226) of female patients were positive for antibody by a titer of 1:20 (cut off) or higher. Among seropositive patients 5.6% had close contact with pet animals, 8.7% had under-cooked meat, and 7.8% had chronic diseases. On investigation of odd's ratio, no significant difference was found between people who had and people who had not risk of consumption of under-cooked meat, close contact with animals and drinking unfiltered water in the seropositivity rate of toxoplasmosis.

**Conclusion:** The regular surveillance through serological screening of *Toxoplasma* antibody in kidney transplant recipients is advisable. Awareness of transplant recipients about the potential risks of acquisition of infectious diseases due to regular administration of suppressive drugs will be helpful in reducing the rate of infections.

### **P2381** First isolation of a *Toxoplasma gondii* strain from a symptomatic human case of congenital toxoplasmosis in south-east Europe

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**Objectives:** Isolation and partial characterization of the first strain of *Toxoplasma gondii* isolated in South-East Europe from a symptomatic human case of congenital toxoplasmosis.

**Methods:** We present the case of a premature (32 weeks) neonate born in cranial presentation with Intrauterine Growth Restriction (IUGR): 2000 g, skull perimeter – 31 cm, anterior fontanelle 2/2 cm under tension, microphthalmia, axial hypotonia and average respiratory

distress. Congenital hydrocephalus was diagnosed at 26 weeks of gestation and mother's serology diagnosed an acute toxoplasmosis, treated with spiramycin in the last 4 weeks of pregnancy. Transfontanelar ultrasonography (TFUS), MRI, neurologic and ophthalmologic examination were indicated and blood and cerebrospinal fluid (CSF) were collected 4 days after birth. CSF sample was analyzed by PCR and bioassay. Amplification of *T. gondii* DNA was made with specific primers Tox 4 and Tox 5 that amplify a sequence of 528 bp and for bioassay three mice were used. After 4 weeks the mice were sacrificed and the brains checked for *T. gondii* cysts.

**Results:** TFUS (4 hours after birth) result: dilated lateral ventricle compressing the brain mass, biventricular diameter: 33.8 mm, dilated ventricle III, reduced cerebral parenchyma with hyperdense punctiform images. MRI results: complex brain malformation with agenesis of the corpus callosum, right frontal schizencephaly and obstructive hydrocephalus. Ophthalmologic exam: acute central chorioretinitis (right eye) and sequelae of anterior and posterior uveitis, retinal detachment and microphthalmia (left eye). Specific serology against *T. gondii* (ELISA) showed positive IgG and IgA while IgM remained negative. CSF serology was positive for IgG and IgA and equivocal for IgM. *T. gondii* was detected by PCR and isolated in one mouse (*T. gondii* cysts – Fig. 1). The rest of mice are still alive 4 months after inoculation with positive serology. The diagnosis of congenital toxoplasmosis is established and treatment is initiated with spiramycin followed by pyrimethamine.

**Conclusions:** Infection evolution in neonate and mice indicates appurtenance to types II or III but final characterization of the isolated *T. gondii* strain by molecular methods is still in work. The originality of the case resides in the fact that it is the first *T. gondii* strain isolated from CSF inoculated in mice from symptomatic congenital toxoplasmosis, even though mother was treated with spiramycin 1 month before birth.

### **P2382** Management of congenital toxoplasmosis in countries of low seroprevalence in general population: the Greek experience

I. Katsantoni\*, A. Katsihti, N. Kastrinios, E. Charvalos on behalf of the The IASO toxoplasmosis group

**Aim:** Congenital toxoplasmosis is a matter of concern in countries like France where the tradition of eating raw meat leads to a high seroprevalence in the general population. A recent survey in France (FS) (Villena et al. 2007; Eurosurveillance), evaluated the risks of transmission of toxoplasmosis in newborns, using a screening protocol (toxoplasma IgG, IgM and PCR) and treatment of the mothers and their newborns. In Greece, a country with low rates of seroprevalence it is critical to collect data and depict the situation.

**Subjects and protocols:** We used a modified French protocol for the management of congenital toxoplasmosis at IASO Maternity Hospital (IM). Six cases out of 5500 pregnancies leading to births were confirmed as congenital toxoplasmosis. The three of them were in the first trimester of pregnancy with positive PCR in blood. Mothers decided to end pregnancy after informed consent (IC). Three cases were at the third trimester when seroconversion was detected. After IC, all refused the PYR/SULF treatment, they took spiramycin and they all deliver earlier.

**Results/conclusions:** All neonates were asymptomatic at birth. One of them was diagnosed with congenital toxoplasmosis in the first year of life and developed chorioretinitis at 3 years of age. The other two discontinued the screening after the first 2 months as it seemed that the IgG titers were going to diminish. We compare here our results to the FS: The percentage of seroprevalence for women in childbearing age in FS and in IM is 44%, and 4.6% respectively. The percentage of confirmed congenital cases ante natal was 3.3/10 000 cases in FS, whereas in our study was 10/10 000 cases. The percentage of abortion due to positive toxoplasmosis was 2.3% in FS and 50% in IM respectively, probably due to the fear of Greek mothers for congenital toxoplasmosis. The births of newborns with suspected congenital toxoplasmosis in France were in 98% whereas in IM, 50%. The

asymptomatic neonates with congenital toxoplasmosis in FS were 82%, whereas in IM was 66%. Comparison of results shows that the low seroprevalance in the general population increases the chance of getting toxoplasmosis during pregnancy. Without any treatment a toxoplasmosis seroconversion leads to abortion and the incidence of congenital infection increases. We need, first, to investigate more cases from 1st and 2nd trimester and analyse socio-economic parameters for the adoption of a new protocol.

**P2383 Genetic and virulence characterisation of *Toxoplasma gondii* strains isolated from pigeons in Lisbon region**

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**Objectives:** The aim of this study is to help the understanding of the global portuguese reality that concerns *Toxoplasma gondii* typing and virulence strains.

**Methods:** The sample consists in 1507 pigeons weekly euthanized by the Lisbon Municipal Council. In all pigeons with positive (1:20) Direct Agglutination brain tissues were removed. These tissues were homogenized with PBS and divided into two parts one to mice inoculation and other to typing by molecular Biology. The mice were monitored serologically at 15 days 1, 2 and 3 months. In the last serology they were euthanized and macroscopic and microscopic observed by cytological procedures in different organs. To perform the analysis of molecular biology, the DNA was extracted by Tissue Quiagen column method. Strains were genotyped by sequencing of the two ends of *SAG2* gene and by multiplex PCR of five microsatellites.

**Results:** Forty one brain tissues were collected and inoculated in mice. The isolation rate was 61% (25). All strains were not virulent to mice. Thirteen strains of *T. gondii* are type II and one are type III by sequencing of the two ends of *SAG2* gene. PCR multiplex confirmed all the *SAG2* gene typing. In eight strains we can't perform the "3' end" of *SAG2* gene, maybe because the low DNA concentration, however these strains were typed by *SAG2* "5' end" belonged to type I or type II.

**Conclusions:** Pigeons are considered a good model to evaluate the environment contamination and the risk factors of infection to public health. Unfortunately, few studies have been done in pigeons and the majority concern serologic prevalence. With this work we can observe that the majority of strains belong to type II which is also found in humans in most European countries, including Portugal. On the basis of the results of this study, the zoonotic agent is present in the urban environment and reveals same geographical infection risk points to humans. It is important to continue the study in order to identify different infection sources, more geographical risk infection points to humans and animals and finally to correlate the genotype with phenotype *T. gondii* strains (Project financed by Foundation for Science and Technology).

**P2384 Monitoring salivary IgG to rule out congenital toxoplasmosis**

E. Chapey, V. Meroni, F. Kieffer, M. Wallon, F. Peyron\* (Lyon, FR; Pavia, IT; Paris, FR)

**Objective:** The diagnosis of toxoplasmosis currently relies on the examination of humoral immunity through serological investigations. Because *Toxoplasma*-specific immunoglobulin (Ig) G crosses the placenta, infants born to women who seroconverted during pregnancy and who have a negative medical evaluation at birth must undergo serological tests until the disappearance of serum IgG in order to rule out a congenital infection. Detection of salivary Ig has been suggested as an alternative for the diagnosis of various diseases caused by micro-organisms or parasites, and we hope to develop a non-invasive, painless, simple and rapid method to detect anti-*Toxoplasma gondii* IgG and monitor its evolution.

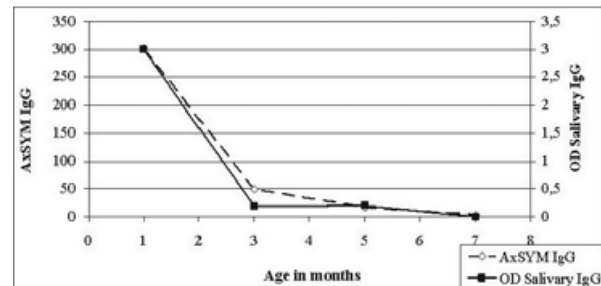
**Methods:** Saliva was collected using micro-sponges placed in the gingival crevice for 2 minutes. Salivary IgG was detected using a

home-made indirect enzyme-linked immunosorbent assay whereas serum IgG was quantified by the AxSYM<sup>®</sup> Toxo IgG assay (Abbot Laboratories, USA).

**Results:** Pilot study: Three hundred paired human saliva and serum samples were collected and catalogued according to their level of serum IgG. The correlation coefficient between salivary and serum IgG was 0.51 ( $p < 0.01$ ) for 258 samples from 214 patients with serum IgG. In 42 samples from 36 negative patients, the mean optical density in saliva was  $0.15 \pm 0.26$ .

For five children who were free of congenital toxoplasmosis, at least three paired saliva and serum samples were analysed. During follow-up, four of these showed a parallel pattern of evolution of serum and salivary anti-*T. gondii* IgG (Fig. 1). However, the profiles of the fifth child were discordant, suggesting the presence of interfering factors in the saliva.

Figure 1. Evolution of serum and salivary anti-*Toxoplasma gondii* IgG in a child free of congenital toxoplasmosis.



**Conclusions:** Saliva collection is painless, well accepted by patients and parents and inexpensive. Given the correlation between anti-*Toxoplasma* IgG levels obtained in saliva and serum, quantification of antibodies in saliva appears to be a good substitute for serological monitoring of toxoplasmosis, but further studies will be needed before blood sampling can be replaced by salivary test in this clinical setting.

**P2385 Comparative evaluation between ARCHITECT<sup>®</sup> and VIDAS<sup>®</sup> toxoplasmosis IGG avidity in pregnant women**

C. Pomares\*, A. Smets, J. Quetel, F. Ajmia, T. Fauchier, P. Delaunay, P. Marty (Nice, FR)

**Objectives:** The French national program of congenital toxoplasmosis prevention recommends a monthly follow-up of seronegative pregnant women. As the first serum is in most of cases sampled during pregnancy, several methods have been developed to date contamination. Indeed, in case of IgG with IgM antibodies seropositivity observed on this first serum, IgG avidity could represent an aid to date contamination. The aim of this study is to compare two avidity assays: The ARCHITECT<sup>®</sup> toxoplasmosis IgG avidity and the Vidas<sup>®</sup> toxoplasmosis avidity assays.

**Methods:** One hundred nineteen sera obtained from 119 different pregnant women were prospectively tested. For ARCHITECT<sup>®</sup> toxoplasmosis IgG avidity and Vidas<sup>®</sup> toxoplasmosis IgG avidity a high index is a strong indication of a primary infection dating back more than 4 months. Measures of inter-rater agreement were made using Cohen's kappa coefficient.

**Results:** Past infection was found in 93 cases with an infection drawn later than 4 months (IgG Vidas<sup>®</sup> > IgG ARCHITECT<sup>®</sup>, with IgM and more or less IgA). In 26 cases, toxoplasmosis was acquired in the preceding 4 months with an infection drawn within 4 months (IgG ARCHITECT<sup>®</sup> > IgG Vidas<sup>®</sup> with IgM and IgA). A good agreement was found between ARCHITECT<sup>®</sup> toxoplasmosis IgG avidity and Vidas<sup>®</sup> toxoplasmosis IgG avidity results. Both tests give statistically identical results but those from Vidas<sup>®</sup> IgG avidity are more agreed with the biological conclusions than results from ARCHITECT<sup>®</sup> IgG

avidity (Kappa equal 0.838 against 0.724 respectively). However a borderline avidity in both ARCHITECT<sup>®</sup> and Vidas assays does not permit to rule out a recent infection within the preceding 4 months.

**Conclusion:** ARCHITECT<sup>®</sup> toxoplasmosis IgG avidity gives statistically similar results to those obtained with Vidas<sup>®</sup> toxoplasmosis avidity and could be routinely used to rule out a primary infection date back more than 4 months.

**P2386 Performance of the BioPlex<sup>®</sup> 2200 flow immunoassay (Bio-Rad) in critical cases of serodiagnosis of toxoplasmosis**

Y. Garin, J. Menotti\*, S. Hamane, N. Guigue, F. Derouin (Paris, FR)

**Objectives:** Serological screening and diagnosis of toxoplasmosis requires robust and specific IgG and IgM assays. The recently developed BioPlex<sup>®</sup> 2200 ToRC IgG and IgM immunoassays already met these criteria in a prospective study on 600 sera submitted for routine testing (Binnicker, 2010). To complement this study, we examined sera which have been selected for their individual peculiarities and critical relevance for diagnosis or screening.

**Methods:** A unique panel of 193 individual sera (101 pts) or sequences was selected over 5 years from our routine practice of Toxoplasma serology, using Platelia<sup>®</sup> toxo IgG and IgM immunoassays (Bio-Rad) combined with a sensitized agglutination (AGG) assay (Toxo Screen-DA, Biomérieux). Sera met one of the following criteria: (i) evidence of a recent infection, (ii) Apparent long-lasting IgM, based on the presence of IgM (Platelia) and high avidity (Bio-Rad), (iii) Discrepant IgG results between Platelia-G and AGG; in this case the definite diagnosis was assessed by western-blot (LDBIO). Sera were tested by BioPlex blindly to any other data.

**Results:** Among 45 sera from presumed recent infections (Platelia M+, IgG avidity <0.4), 43 were IgM positive with BioPlex; in one of the remaining sera, the ISAGA score was 7, and a past infection with persisting low avidity was likely. Analysis of serum sequences showed a trend to a more rapid decrease of IgM titres by BioPlex than by Platelia or ISAGA. In one case of proven seroconversion with no IgM with Platelia, IgM was positive with BioPlex (confirmed by ISAGA). Among 23 serums from probable past infection with long lasting IgM, (Platelia M+ and IgG avidity >0.5), 11 (47.8%) were positive by BioPlex IgM.

Discrepant Platelia/AGG IgG results were available for 16 pts. In eight cases of false positive Platelia G, BioPlex IgG was positive in 2. None of the two sera giving a false positive AGG was positive by BioPlex. In 5/6 cases of false negative Platelia G and 2/2 false negative AGG, BioPlex IgG was positive.

**Conclusion:** Most false positive or false negative Platelia G or AGG results were corrected by the BioPlex test, assessing for its high specificity, reducing uncertainty on patients' serological status and limiting the use of additional confirmatory tests. For IgM, the performance of BioPlex for diagnosis of acute infection was comparable to Platelia. The negative BioPlex IgM results in sera with positive Platelia M and high IgG avidity suggest a lower sensitivity to residual IgM antibodies.

**P2387 Prevalence of antibodies to *Trypanosoma cruzi* in Latin American immigrants in Madrid**

M. Gutiérrez\*, N. Iglesias, P. Trevisi, B. López, P. Rivas, M. Baquero (Madrid, ES)

**Objectives:** Chagas' disease, also known American trypanosomiasis is major public health problem in Latin American, affecting nearly 10 million people. Outside of endemic areas, Chagas' disease may be transmitted through the transfusion of the infected blood components, organ transplantation and congenital infection. Our study aims to determine the prevalence of antibodies to *Trypanosoma cruzi* in a community sample of Latin American immigrants in our hospital.

**Methods:** A total of 465 serum samples from Latin American individuals attending in our hospital between January 2010 and

October 2011 were studied. Sixty-five percent (302) were women and 35% (163) were men. The median age was 34.2 (range 1–69) years and 12.7% (59) of patients were children. Serological screening was performed using a commercial enzyme immunoassay (EIA) (Chagas ELISA, Vircell). Samples reactive by EIA were confirmed by indirect immunofluorescence (IFA) (Inmunofluor Chagas, Biocientífica) and lateral flow immunochromatography (IC) (Chagas Ab Rapid Test, Biotech).

**Results:** The geographic distribution of the patients was: 277 Bolivia (59.6%), 52 Colombia (11.2%), 42 Ecuador (9%), 26 Peru (5.6%), 16 Argentina (3.4%), and seven other countries of Latin American (11.2%).

One hundred seventy-seven patients (38%) were reactive for all three tests. Of these, 170 (96%) were from Bolivia, 3 (1.7%) Argentina, 2 (1.1%) Ecuador and 2 (1.1%) Paraguay. All serum samples from children were non-reactive.

A non-reactive serum sample by EIA or by IC was reactive by IFA. It was for a woman from Colombia with leishmaniasis.

**Conclusion:** High rates of *T. cruzi* seropositive patients among Latin American immigrants, mainly among Bolivian population (170/277, 61.4% in our study), underscore the importance of serological screening in this population to prevent transmission by blood transfusion or organ transplantation and vertical transmission. The rate of transplacental transmission from mothers with chronic *T. cruzi* infection to their newborns is 2–10%.

**P2388 Association between seropositivity for Chagas' disease and blood parasitaemia with markers of metabolic syndrome**

C. Toro\*, A. Amor, S. Puente, N. Iglesias, M. Baquero (Madrid, ES)

**Background:** Adipose tissue represents an important target and reservoir of *Trypanosoma cruzi*. In fact, recent reports have studied the metabolic consequences of *T. cruzi* infection on basal glucose levels in murine model. However, there is a lack of data about the significance of this finding in the clinical setting. The aim of this study were to investigate the markers of metabolic syndrome in subjects with Chagas disease (CD).

**Methods:** From December 2008 to April 2011, subjects with suspicion of CD were included. Two different methods for determination of antibody anti- *T. cruzi* (IFI and EIA) were performed. Samples were considered seropositive when reactivity in both tests was detected. A real-time PCR was made in seropositive samples to determine parasitemia. Biochemical determinations were also performed. Patients who had been treated for CD were excluded. In addition, only the most recent sample in seronegative subjects was included for the analysis.

**Results:** A total of 802 samples corresponding to 776 patients were studied. Four hundred and twenty-two (54.4%) were immigrants from endemic regions (mainly Bolivia) and the remaining were travellers. The mean age of the study population was 32.9 (SD 14.6) years and 459 (59.1%) were women. One hundred and eighteen (17.5%) samples were seropositive for Chagas disease and all but four travellers were immigrants. Seropositive subjects in comparison to seronegative ones had higher levels of total-cholesterol (194 vs. 182.7 mg/dL,  $p = 0.004$ ), glycemia (96.2 vs. 92.4 mg/dL,  $p < 0.0001$ ), tryglicerides (126.4 vs. 91.5 mg/dL,  $p = 0.001$ ), and lower HDL values although not significant (47.8 vs. 51.2 mg/dL,  $p = 0.075$ ). Similar results were obtained when the analysis was performed only among seropositive immigrants: total-cholesterol (193.8 vs. 182.6 mg/dL,  $p = 0.013$ ), glycemia (96.4 vs. 93.4 mg/dL,  $p = 0.022$ ) tryglicerides (126.7 vs. 107.2 mg/dL,  $p = 0.09$ ) and HDL (47.6 vs. 47.4 mg/dL,  $p = ns$ ). A real-time PCR could be performed in 107 out of 118 seropositive subjects. Thirty-four subjects showed parasitemia but with a very low load (median, 0.9 epimastigotes/mL; interquartile range, 0.2–4.5). Seropositive subjects with parasitemia in comparison to those with no parasitemia showed similar trend although only for glycemia were significant (99.8 vs. 94.8 mg/dL,  $p = 0.027$ ).

**Conclusion:** Patients with CD showed blood levels of glycemia and lipids that may influence in the development of metabolic syndrome.

The levels of glycemia were special higher in those CD subjects with blood parasitemia.

#### **P2389** Diagnosis of Chaga's disease in Navarra, Spain

I. Polo Vigas, C. Martin Salas, I. Tordoya Titichoca, Y. Lopez Sanchez, V. Martinez de Artola, C. Ezpeleta Baquedano\* (Pamplona, ES)

**Objective:** The three main transmission routes of *Trypanosoma cruzi* in non-endemic regions are transfusion of blood products, organ transplantation and vertical transmission from a seropositive mother to her child during pregnancy or delivery. In Navarra (Spain) with 630 578 inhabitants it is estimated that there are 11.2% immigrants (42% from Chagas endemic areas). There isn't regulation for screening of pregnant women. In March 2011 the Microbiology laboratory established three methods for the diagnosis of Chagas disease and we began a systematic screening of pregnant women from endemic areas, of newborns from infected pregnant women and the other children from these women. The aim of this study is to review the results of the first 8 months of this program.

**Methods:** The study was carried out at the Complejo Hospitalario de Navarra, the reference hospital in the region of Navarra (Spain). The microbiology laboratory has established Chemiluminescence (Abbott), IFA (Vircell, Granada, Spain) and PCR (Ingenie molecular, Valencia Spain) for diagnosis of Chagas disease. The pregnant women from endemic countries are studied and they are diagnosed of *T. cruzi* infection if CLIA and IFA are positives. PCR is carried out for diagnosis of children born from infected mothers. Other patients also have been studied: children from infected mothers, and adults with cardiac or digestive symptoms.

**Results:** One hundred and sixty-eight sera have been studied. Twenty-two out of them have been positives corresponding to 17 patients. One hundred and thirty-four sera were from pregnant women and there nine positives among them (6.71%), seven women were born in Bolivia, one was from Ecuador and the other one was from Brazil. There were also two children one of them had a congenital Chagas disease who's mother was born in Ecuador and the other one was a 9 years old boy from Bolivia. The remaining six cases were adults from Bolivia (five women, one man), two out of them had digestive symptoms and one of them had cardiac disease.

**Conclusion:** The WHO report from 2009 that take into account the prevalence of *T. cruzi* infection in Latin America suggest that between 40 000 and 65 000 affected people currently reside in Spain and only 3600 have been diagnosed. There is a need of easy accession to laboratory diagnosis. The detection of Chagas disease relies on the initiative of health care workers. Our experience has been positive with 17 new cases diagnosed in the first 8 months after implementation of ELISA, IFA and PCR at the microbiology laboratory.

#### **P2390** Screening of *Trypanosoma cruzi* infection with a chemiluminescent microparticle immunoassay in a Spanish university hospital

M. González-Domínguez\*, R. Benito, J. Gil, M.J. Gude, J. Arribas, R. Cebollada, A. Garrido, M.C. Rubio (Zaragoza, ES)

**Objectives:** *Trypanosoma cruzi* infection is endemic in Latin America, but migration flows has expanded outside of these areas. This imported parasitic disease is present in Spain, for this reason, the aim of the study was to evaluate the new chemiluminescent microparticle immunoassay (CMIA) for rapid screening of *T. cruzi* infection.

**Material and methods:** A total of 71 sera, maintained at -80°C in our serum bank, were analyzed. Samples were included into three groups. Group 1: 13 samples with positive *T. cruzi* immunofluorescence assay (IFA) (MarDx Diagnostics, Carlsbad, CA, USA). Group 2: 24 samples with negative *T. cruzi* IFA result. Group 3: 34 cases with positive serology for parasites susceptible to produce cross-reaction with *T. cruzi*, such as *Plasmodium falciparum* (n = 11), *Entamoeba histolytica* (n = 13) and *Leishmania infantum* (n = 10).

All groups were analyzed by a chemiluminescent microparticle immunoassay (CMIA) (ARCHITECT Chagas, Abbott GmbH & Co.; Wiesbaden-Delkenheim, Germany) for the qualitative detection of IgG antibodies to *T. cruzi* in serum.

Groups 1 and 2 included 26 patients from endemic countries and 11 patients who had made trips to these countries. In group 3, only one patient was from endemic countries.

**Results:** Into group 1, 12 out of 13 (92.31%) samples were CMIA and IFA reactive. IFA titers ranged from 1/256 to 1/5120 and CMIA result (S/CO) from 5.23 to 17.00. Only one sample, belonging to a Bolivian patient, showed discordant results: IFA titer 1/32 (positive) and CMIA value of 0.78 (non reactive).

In group 2, CMIA results were non reactive (<0.8) and IFA titers were negative (<1/32). IFA titers ranged from IFA <1/8 to 1/16 and CMIA index from 0.01 to 0.39.

In group 3, 33 out of 34 (97.05%) showed negative CMIA result. One sample had CMIA reactive result (index 1.0) but negative IFA titer (1/16). This patient was a native of Ghana who had a positive hemagglutination (1/640) for *E. histolytica* without clinical or epidemiological data related with Chagas disease.

**Conclusions:** Our results suggest that CMIA, which is a rapid and automatic method, can be applicable to emergency *T. cruzi* screening in patients with epidemiological factors related to Chagas disease because shows good correlation with IFA assay. In our experience, discrepant results in both methods occur in samples with low titers. The frequency of cross-reactions with other parasites is low, although further studies should be conducted in patients with *E. histolytica* infection.

#### **P2391** A 12-year epidemiology report of imported malaria in Parma, Italy

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**Objectives:** At present malaria is re-emerging as an imported disease in areas where it has been eradicated, such as Europe, due to the increasing amount of travellers and migratory flows from endemic countries. A surveillance system has been established in Italy where malaria is the most commonly imported disease, in order to prevent its endemic return and to monitor the epidemiology of imported cases.

This study aimed to accurately and promptly diagnose malaria and to describe the occurrence in our area of the different involved species of plasmodia, focusing on patient clinical/epidemiological information.

**Methods:** From 2000 to October 2011, blood samples from 1002 patients with the suspicion of malaria were subjected to microscopy and to different nested- and Real-time PCR assays targeting plasmodial 18S-rDNA, alternatively used during the study period.

**Results:** Two hundred and twenty-seven cases of malaria were diagnosed by microscopy (189 *Plasmodium falciparum* [Pf] [83.3%], 12 *P. ovale* [Po] [5.3%], 13 *P. vivax* [Pv] [5.7%], 10 *Plasmodium* spp. [4.4%], one Pf/P. spp. [0.4%], two mixed infection [0.9%]), whilst 234 were diagnosed by PCRs (190 Pf [81.2%], 23 Po [9.8%], 9 Pv [3.85%], 3 *P. malariae* [Pm] [1.3%], nine mixed infections [3.85%]).

Among the 234 cases, 213 (91%) were foreigners and 21 (9%) were Italian travelling for tourism, business or humanitarian mission. The majority of the patients presented with fever (about 90%) and had no correct anti-malarial chemoprophylaxis (65%); 211 patients (90.2%) became infected in Africa (mostly West Africa), 3 (1.3%) in Indonesia, 2 (0.8%) in India, 1 (0.4%) in Amazonia, and for 17 patients (7.3%) country of origin/visit was unknown.

**Conclusion:** Despite microscopy remains the reference diagnostic method in our experience PCR assays were the only ones allowing a correct diagnosis of malaria, particularly in cases of infections by species other than Pf and in mixed infections, resulting in a reliable description of the epidemiological picture of imported malaria in our area.

In this study a high prevalence (23.3%) of imported malaria is described as an uncommon finding in a non-endemic country and involving



immigrants particularly from West Africa, explaining the highest prevalence of Po cases among non-Pf infections. By the combined use of microscopy and PCR-based methods an accurate diagnosis and description of the epidemiology of imported malaria could be accomplished allowing the administration of a targeted therapy.

**P2392 Risk co-factor in malaria drug resistance selection: frequency of CYP2C8\*2 allele in Uganda**

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**Objectives:** *Plasmodium falciparum* malaria is one of the leading cause of morbidity and mortality in sub-Saharan Africa and Uganda bears a particularly large burden from this parasitic infection. Unfortunately, drug resistance is a problem in the control of *P. falciparum*, its selection being influenced by several factors such as drug usage, transmission intensity, host immune status and pharmacokinetic. Human genetic variation could represent a further cofactor. All antimalarial drugs are metabolized by hepatic cytochrome P 450 enzymes. In particular, cytochrome P450 2C8 (CYP2C8) contributes to the hepatic metabolism of chloroquine (CQ), amodiaquine (AQ) and dapson (DDS). This cytochrome shows a genetic variant (CYP2C8\*2) associated with increased adverse side effects and with higher rate of CQ-resistant parasites in the infected host. Our aim is to describe CYP2C8\*2 frequency in populations from Ugandan areas characterised by high levels of malaria transmission intensity.

**Methods:** The samples analysed in the present study were collected during cross-sectional surveys performed during 2007 in Uganda (Karamoja and Kampala regions). A total of 262 children and adolescents had been enrolled and genotyped for the polymorphism rs11572103 (A/T). The PCR-RFLP technique was used to discriminate the wild-type (A) from the defective allele (T).

**Results:** The frequency  $\pm$  SE of the CYP2C8\*2 in rural sites of Karamoja region (North-Eastern Uganda) was  $0.096 \pm 0.021$ , while was  $0.132 \pm 0.032$  in the Kampala suburbs (central Uganda). Both the genotype distributions are in Hardy-Weinberg equilibrium, and the allele frequencies are not statistically different (Yates corrected  $\chi^2 = 0.89$ ,  $p = 0.346$ ). The overall CYP2C8\*2 frequency in all sites was  $0.105 \pm 0.019$ . Genotype frequencies were in Hardy-Weinberg equilibrium  $\chi^2 = 1.52$ ,  $p = 0.221$ .

**Conclusion:** Our study demonstrated that CYP2C8\*2 allele is present at an appreciable frequency in Uganda, an area of hyperendemic malaria transmission. Here antimalarial treatment is based on artemisinin combination therapies (ACTs), and artesunate (AS) plus AQ is used as second line ACT. Consequently, the presence of the CYP2C8\*2 allele may be a potential co-factor in the onset of adverse side effects associated with AQ administration. Furthermore, we emphasize the risk related to the presence of CYP2C8\*2 in selecting AQ-resistant strains, since the interplay between host and parasite genetic variation could be similar to that of CQ.

**P2393 Malaria, different faces of a permanent threat**

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**Objectives:** The aim of this study was to describe the epidemiological and clinical spectrum of malaria and to identify predictors of severe malaria in adults admitted in our hospital.

**Methods:** Retrospective study conducted from February 2003 to June 2011, in a tertiary hospital in the south of Spain. We investigated 62 cases of malaria. Statistical analysis was carried to identify factors related to severe malaria by univariate analysis.

**Results:** Malaria was acquired in Sub-Saharan Africa in 60 patients (96.7%), South-America (Ecuador) in one patient and one autochthonous case (no travel). 91.9% (57 patients) were immigrants/visitors and 8% (five patients) were Spanish people infected while

travelling for many reasons (three business, two tourist). Only 8.1% of cases had taken chemoprophylaxis with poor compliance. The mean age at the time of admission was  $32.2 \pm 8.23$  years (R: 16–60). Fifty three (85.5%) were male and three pregnancy women with malaria. The most frequent presenting symptoms and signs were fever (100%), myalgia (75.8%), headache (71%), nausea and vomiting (48.4%), hepatomegaly (27.9%), jaundice (12.9%), seizure (4.8%). The latency of symptoms was  $6.19 \pm 3.55$  days ( $r = 2-21$ ). Thrombocytopenia ( $<100\,000/\text{mm}^3$ ) and increase of protein C reactive was the predominant laboratory feature. Parasitological diagnosis was performed by thin and thick Giemsa-stained blood smears (100%) and rapid diagnostic test in 35.5%. *Plasmodium falciparum* was found in 75.4% (patients traveled in Sub-Saharan Africa and autochthonous case), *P. vivax* in 3.2% (patients traveled in Ecuador and Mauritania), dual infection in 6.45% and plasmodia were unspecified in 11.3%. The most of patients (90.3%) were treated with mefloquine-doxycycline and 11.7% with quinine-doxycycline. The mean duration of treatment was  $9.44 \pm 5.29$  days ( $r = 5-35$ ). There were four cases of severe malaria admitted to intensive care unit (ICU). The risk factors related with severe malaria were travelers/non immigrants ( $p < 0.001$ ), longer latency of symptoms prior to treatment ( $p < 0.05$ ), pregnancy ( $p < 0.01$ ), HIV ( $p < 0.01$ ) and renal failure ( $p < 0.05$ ). The attributable mortality was 1.6%.

**Conclusions:** *Plasmodium* infections in our area were imported falciparum malaria in immigrant patients from Sub-Saharan African countries. We report an autochthonous case in the South of Spain, probably related with an imported case of malaria with subsequent onwards transmission to nearby residents by local mosquito vectors.

**P2394 Occurrence of highland malaria in three community hospitals in rural Burundi located 2000 metres above sea level**

S. Seckova\*, L. Gaston, E. Smerkova, M. Philippe, P. Kisac, J. Sokolova, V. Krcmery (Bratislava, SK; Rutovu, Rutovu, Gasura, Murago, BI; Tmava, SK)

**Objectives:** Malaria has been observed within last 10 years also increasing frequency in above sea level (highlands malaria) but was not imported from down country by local travellers. The objective of this study was to assess occurrence of highland malaria in three community hospitals in rural Burundi located 2000 m and high above sea level.

**Methods:** We studied occurrence of microscopically confirmed highlands malaria in Burundi (Tropical Programme of St. Elizabeth University in Murago, Rutovu and Gasura). All community hospitals are in attitudes from 2000 to 2280 m in rural Burundi. Each hospital had between 40 and 80 beds and outpatients department. Hospital staffs was composed of two doctors, 8–12 nurses, 1–2 lab technicians and pharmacist, with a patient follow daily 40–120 in the outpatients department and 2–10 inpatients per day admissions.

**Results:** Of 42 342 outpatients visits in 2011, 4203 (9.9%) was clinically suspected and 3244 (7.7%) microscopically documented malaria. Most of patients in Gasura and Murago do not travel apart of 5–10% male, traveling since 2005 weakly to work near lake Tanganyika or Bujumbura (1050 m above sea level), what means that about 2500 patients of confirmed malaria have not acquired *P. falciparum* (94% of cases) outside Burundian highlands. Similar observation we had in the Eldoret (Kenya) in an attitude of 2250 m and Bissero (Rwanda) – 2400 m above sea level from community health care centres visiting within St. Elisabeth University Tropical Programmes.

**Conclusion:** Increasing traveling to work within last 5 years after more security is observed in Rural Burundi and global warming may be the reason of increased occurrence of microscopically positive *P. falciparum* malaria in upcountry in Burundi in attitude of more than 2000 m above sea level. Therefore preventive strategies for foreign travellers and tourists to highland or mountain areas of East African should be probably reconsidered and revised.

**P2395** Assessing the skill of medical doctors on appropriate management and treatment of severe malaria

B. Paktinat jalaly\*, M. Moghadami (Tehran, Shiraz, IR)

**Background:** Malaria is the most deadly vector-borne disease in the world. The physicians working in emergency wards should have a high index of suspicion if a history of fever is accompanied by suggestive symptoms in a patient with a history of travel to an endemic region. Failure to consider malaria in the differential of a febrile illness following such travel, even if seemingly temporally remote, can result in significant morbidity or mortality, especially in children and pregnant or immunocompromised patients.

**Methods:** A cross-sectional survey was conducted to assess the knowledge and perceptions of Iranian medical doctors on severe malaria management and treatment choice and clinical treatment decisions. Medical doctors who are involved in management of malaria patients in Iran were recruited and a self-administered questionnaire was used to obtain information: years of experience, speciality, private/governmental job and knowledge on severe malaria and methods used to decide on the management and treatment of patients with severe malaria.

**Results:** Thirty-nine (82%) of the participants knew at least one form of ACTs. Ninety-six percent reported that they know the importance of surveillance for malaria disease 42% of them work in public sector and the rest were working in private sector. The 38% mentioned first line ACT is Artesunate (AS) and 96% of the participants indicated that they notice availability of severe malaria management tools in their working place. Ninety percent of participants believed that continuous training on management of malaria and using ACT are essential.

**Conclusion:** This study shown that most of the participants are familiar with importance of disease surveillance, availability of severe case management requirements, but did not believe that ACTs is first line treatment for malaria. However, most of the participants have not received any training on severe malaria management and how to use ACT and report final result of disease management. There is need for more training of health care professionals to ensure correct and effective severe malaria case management and use of ACTs.

**P2396** Assessment of malaria identification methods in clinical blood samples

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**Objectives:** This study describes a comparison of three malaria identification methods; microscopy, antigen detection and PCR at the Scottish Parasite Diagnostic and Reference Laboratory (SPDL) over a 2-year period. In addition, the sensitivity of two commercial antigen detection kits is assessed.

**Methods:** Blood from 57 cases were subjected to (i) microscopy (thick and thin films), (ii) antigen detection (Binax Now and OptiMal commercial kits). PCR was performed using a nested approach on blood from a further 11 cases for the identification of *Plasmodium* species. Travel history and clinical symptoms, where available, were recorded.

**Results:** Seventeen of the 68 bloods were positive for microscopy and malaria antigens (25%). Microscopy was positive in two bloods which were antigen negative using both kits. Seven of the 11 samples subjected to PCR in addition to microscopy and antigen detection were PCR positive. All PCR positive samples were positive by antigen detection, however only five were microscopy positive. The two microscopy negative, PCR positive samples were only positive using Binax, not the Optimal antigen kit. Four samples were microscopy, antigen and PCR negative.

Comparison of both antigen detection kits demonstrated identical results in 12 of the 24 positive samples. Variation was observed; five samples were only antigen positive by Binax (3 × *P. falciparum*, 1 × *P. ovale* and 1 *P. vivax*) whereas four samples were positive by OptiMal only (3 × *P. malariae*, 1 × *P. ovale* and 1 *P. vivax*). Seven of these nine samples were microscopy positive.

Travel history was available for 12 cases: *P. falciparum*, *P. malariae* and *P. ovale* (Africa n = 9); *P. vivax* (Pakistan n = 3). Clinical

symptoms were only provided for seven cases, the most common being fever (n = 6).

**Conclusion:** PCR is more sensitive than microscopy alone for the detection of *Plasmodium* species. This is of particular importance when examining samples with low parasitaemia which can result in the absence of positive microscopy. Variation between microscopy and antigen detection supports the benefit of performing several tests rather than a single test. Travel history is consistent with *Plasmodium* species endemic to particular regions. Imported cases of malaria in Scotland are being assessed and validation of real-time PCR/sequencing is on-going to replace nested PCR.

**P2397** Imported submicroscopic malaria: can it be a risk for re-emergence in Europe?

A. Rodriguez-Guardado\*, F. Pérez, M. Rodriguez, V. Carcaba, J. Carton (Oviedo, ES)

**Objective:** Submicroscopic malaria can be defined as low-density infections of *Plasmodium* that are unlikely to be detected by conventional microscopy. Such submicroscopic infections only occasionally cause acute disease, but they are capable of infecting mosquitoes and contributing to retransmission. This entity is frequent in endemic countries; however, little is known about imported submicroscopic malaria. The goals of this study were twofold: (i) to determine the frequency of imported submicroscopic malaria; (ii) to describe epidemiological, laboratorial and clinical features of imported submicroscopic malaria.

**Methods:** During the years 2008–2011 we conducted a prospective screening program of malaria in all immigrants patients attending in Tropical Medicine Unit of Hospital Central de Asturias. Routine detection techniques for *Plasmodium* included Giemsa staining and microscopic examination through thick and thin blood smear. A seminested multiplex malaria PCR was used to diagnose or to confirm cases with low parasitemia. All positive patients were treated with quinine and doxycycline during 7 days.

**Results:** We screened 606 patients. Twenty six patients had a malaria diagnosis, 14 of them (53.8%) had a submicroscopic malaria. Mean age was 37 years (23–68) 57% male. No patients come back to countries of origin since their arrival to Spain. The countries of origin were: Equatorial Guinea (43%), Senegal and Ivory Coast (14.3% respectively), Ecuador, Brasil, Nigeria and Mauritania (7% respectively). Fever was present only in four patients (28%) and the rest were asymptomatic. Mean time in Spain were 551 days (15–1825). One patient had leukopenia and other patient had thrombopenia. Nine patients (64.3%) had *P. falciparum* infection (64.3%), three had *P. malariae* infection (21.4%), one patient had *P. vivax* and one patient had *P. ovale* infection.

**Conclusions:** Results from this study suggest that imported submicroscopic malaria should be considered in all patients proceeding from endemic zones. Although it is usually asymptomatic it may be responsible of fever or abnormalities laboratories. The apparition of autochthonous cases of malaria could be favoured by this patients.

**P2398** Evaluation of quantitative gametocyte detection by QT-NASBA using dried blood spots on filter paper

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**Objectives:** Real-time quantitative nuclear acid sequence-based amplification (QT-NASBA) is a sensitive method for evaluation of submicroscopic gametocytaemia by measuring fragile gametocyte-specific RNA. However, performing this method on fresh whole blood samples is not feasible in remote areas that are mostly affected by malaria. For large-scale epidemiologic studies on the infectious reservoir of gametocytes, a more feasible method is urgently needed.

**Methods:** RNA was extracted by the miniMAG<sup>®</sup> method and quantified by Pfs25 real-time QT-NASBA (bioMérieux). Serial

dilutions of whole blood spiked with *Plasmodium falciparum* NF54 gametocytes were spotted on filter paper. Filter papers were subjected to different storage conditions and duration. QT-NASBA was performed of the dilution series from fresh whole blood and filter paper spotted blood as well as of filter paper blood spots of Ethiopian malaria patients. Gametocyte detection sensitivity was compared to microscopy.

**Results:** Quantification by microscopy significantly correlated with QT-NASBA for gametocyte density. Aged 28 days, all DBS filter papers of all storage procedures showed a loss of pfs25-RNA detected in comparison with day 0 and 24 hours aged samples. The mean amount of detected gametocytes in the filter papers aged 28 days was 82.13% (SD 10.7) of the original whole blood dilution series. Differences in detected RNA among storage conditions were not statistically significant at days 1 or 28. Among samples of the last dilution step (0.25 gametocytes/ $\mu$ L), seven out of 15 (46.6%) samples turned negative by QT-NASBA analysis after being aged 28 days. Negative samples were more often observed among warmer storage procedures (+27.6°C, +37.0°C and ambient temperature of the transport simulation) than among the two cold storage procedures (+8°C and -20°C). In 21 out of 31 (65.5%) clinical samples negative by microscopy, low gametocytaemia was detected by QT-NASBA.

**Conclusion:** Our results suggest that dried blood spots on filter papers stored at ambient temperature for up to 28 days are a practical and reliable method for quantification of *Pl. falciparum* gametocytes circulating at low densities by using real-time QT-NASBA. This could be used for gathering epidemiological data on prevalence of gametocytaemia in malaria endemic countries in the light of renewed elimination and eradication efforts.

#### P2399 Retrospective evaluation of malaria patients in Latvia

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**Objectives:** To evaluate epidemiological, etiological and clinical features of the malaria cases in Latvia.

**Materials and methods:** Thirty-eight case reports on 34 patients with malaria who were treated in Infectology Center of Latvia between 2001 and 2010 were included in this study. The cases were analysed retrospectively and epidemiological data, clinical manifestations, complications and treatment regimens were studied. The diagnosis was proved by demonstration of malaria parasites in blood films by light microscopy.

**Results:** Of the 34 patients, seven were female and 27 male, at the age from 19 to 61 year. *Plasmodium falciparum* was causative agent in 23 cases, *Plasmodium vivax* in eight, *Plasmodium ovale* in one, *Plasmodium malariae* in one case. One case to fail specification. The greatest part of patients (82%) has travel history to Sub-Saharan Africa (Sierra Leone, Kenya, Nigeria, Cameroon, Uganda, Ghana), three patients had travelled to South Asia (Vietnam, Cambodia, India), 1 – to Brazil. Appropriate chemoprophylaxis was used by three patients; all others did not use prophylaxis at all or did it in inappropriate way. In 26 patients malaria was suspected before hospitalisation. Nineteen patients were hospitalised lately, after the fifth day of disease. The leading clinical symptoms were fever, anaemia and splenomegaly. Level of parasitemia on admission ranged from 0, 02% to 53%. Nine of *Plasmodium falciparum* cases (39%) had severe course of disease with parasitemia higher than 1%. Two of *Plasmodium falciparum* cases had fatal outcome. Three patients had relapses: two with *Plasmodium vivax* and one with *Plasmodium ovale*. *Plasmodium falciparum* malaria patients were treated with quinine and doxycycline, in three cases quinism developed. Patients with non-falciparum malaria received chloroquine or hydroxychloroquine, in *Plasmodium vivax* and *ovale* cases in combination with primaquine.

**Conclusions:** Malaria could be an actual problem in nonendemic countries, like Latvia, and must be suspected in all returning travellers with febrile presentations. All travellers to malaria endemic areas must be convinced of necessity of appropriate chemoprophylaxis.

#### P2400 Visceral leishmaniasis: clinical and epidemiological features among adult patients in Albania

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**Objectives:** The aim of our study is to explore different features of visceral leishmaniasis (VL) in adults, through the analysis of its epidemiological, clinical and therapeutic profiles, in a cohort of 45 adult patients.

**Methods:** In this observational study we have reviewed retrospectively the medical records of 45 adults diagnosed with VL between January 2005 and December 2009 in Infectious Diseases Service, University Hospital Centre of Tirana, Albania. The diagnosis of VL was based on demonstration of leishmania parasites in bone marrow smears and ELISA serological testing.

**Results:** Forty-five adult patients were included in the study. Age ranged from 15 to 69 years, the average age was 43 years. Eighty-two percent (37 cases) were male and 66% (30 cases) lived in rural areas. The period between onset of symptoms and diagnosis ranged from 10 till 120 days (average 30 days). Main clinical and laboratory findings were: fever (100%), malaise (100%), hepato-splenomegaly (95%), anaemia (82%), leucopenia (80%), thrombocytopenia (40%), hipergamaglobulinemia (100%) and increased liver enzymes (44%). Bone marrow aspirate was performed in all cases with amastigotes identified in 98% of the cases. The ELISA test was performed in five cases, being positive in all of them (100%). Meglumine antimoniate was used in all cases as an initial treatment. Treatment failure occurred in two cases (4%) that were treated subsequently with liposomal amphotericin B. Two patients died (case fatality 4%). The main causes of death were liver and cardiac failure.

**Conclusion:** VL is considered an endemic disease in Albania, so further efforts should be made for an earlier recognition by health care providers. Issues such as treatment efficacy and drugs' availability are the two main factors which influence the choosing of meglumine antimoniate as the first line treatment for VL in adults in Albania.

#### P2401 New anti-Leishmania agents: the potential underlining *Thymus sp. volatile extract* against *L. infantum*, *L. major* and *L. tropica* – major compounds may not be the answer

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Leishmaniasis treatment is still under several limitations, as toxicity, side effects, rate of relapse, cost, length of treatment and resistance of parasites to available drugs, so more attention should be given to the search of new chemotherapeutic options.

The present work focused on the activity of *Thymus sp.* namely *Thymus capitellatus* Hoffmanns. & Link, *T. mastichina* L. and *T. zygis* Loeffl. Ex L. subsp. *sylvestris* (Hoffmanns & Link) Brot. ex Coutinho (chemotype thymol and geraniol) volatile extracts on *L. infantum*, *L. tropica* and *L. major*. *Thymus* extracts were analysed by GC and GC-MS and antileishmanial activity was performed on promastigotes cultures. Viability was assessed by tetrazolium-dye colorimetric method and expressed as concentration that inhibits parasite growth by 50% (IC50). Effects on promastigotes were analyzed by flow cytometry in order to assess mitochondrial transmembrane electrochemical gradient (JC-1), to analyze phosphatidylserine externalization (annexin V-FITC, propidium iodide) and to evaluate cell cycle (DNase-free, RNase, PI). Morphological and ultrastructural modifications were evaluated by light, scanning and transmission electron microscopy.

All *Thymus sp.* volatile extracts revealed anti-*Leishmania* activity. The most prominent activity was observed on presence of *T. capitellatus*: IC50 values of 35, 37 and 62  $\mu$ g/mL on *L. tropica*, *L. infantum*, and *L. major*, respectively. *T. capitellatus* was followed by *T. mastichina* and *T. zygis*. The monoterpenes isolates from the extracts, alpha-pinene, borneol, 1,8-cineole, p-cymene, geraniol, thymol, carvacrol, exhibit anti-*Leishmania* activity with IC50 values ranging from 87 to 277  $\mu$ g/mL.

Modifications on normal cell shape, mitochondrial swelling, an increase number of autophagosomal structures, electron density lost and cytoplasmic vacuolizations were some of the morphologic and ultrastructural alterations shown. The leishmanicidal activity of *T. capitellatus* was mediated partially via apoptosis as evidenced by externalization of phosphatidylserine, loss of mitochondrial membrane potential, and cell-cycle arrest at the G(0)/G(1) phase.

These results provide new perspectives on the development of drugs against *Leishmania*, but nevertheless, data on isolated monoterpenes (major compounds) did not justify the activity of plant volatile extracts, and future research should explore the possibility of the presence of more active compounds.

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#### **P2402 HIV/AIDS-associated visceral leishmaniasis in Georgia**

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The aim of the study was to describe the clinical and epidemiological features of HIV patients with visceral leishmaniasis in Georgia. We performed a retrospective analysis of demographic, clinical and laboratory data from HIV patients admitted to the in-patient Department of Infectious Diseases, AIDS and Clinical Immunology Research Center of Georgia since 2008. Therefore, 18 patients were included in the study. Most of cases came from Eastern Georgia (15 patients).

Diagnose of visceral leishmaniasis was made based on clinical symptoms and detection of amastigotes in the bone marrow punctate. *Leishmania* serology was negative in 15 patients (83%).

The median age of the patients was 42 years (range 29–51 years); 15 patients (83%) were male. Main clinical and laboratory presentations were fever (95%), splenomegaly and hepatomegaly (91%), anemia (86%), pancytopenia (82%). In all patients Meglumine antimonial was used for treatment. Relapses were reported in 67% of the patients. Two patients died. There was correlation between the CD4 T lymphocyte count and clinical course of visceral leishmaniasis. Namely, in the patients with multiple relapses median CD4 T lymphocyte count was very low (median 48 cells/mm<sup>3</sup>, range 11–199 cells/mm<sup>3</sup>, n = 12).

**Conclusions:** (i) Visceral leishmaniasis can be considered as an important etiological agent of the fever of unknown origin in HIV patients, particularly from Eastern Georgia. (ii) Implementation of new regimens and secondary prophylaxis may reduce the number of relapses and improve the surveillance of visceral leishmaniasis-HIV/AIDS patients in Georgia.

#### **P2403 A comparative evaluation of three methods for diagnosis of visceral leishmaniasis in Serbia**

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**Objectives:** Visceral leishmaniasis is a re-emerging disease in the Mediterranean area. A retrospective diagnostic study of VL was carried out from December 2004 to August 2011 and included all patients with suspected VL referred to the Parasitological Laboratory, Clinical Center of Serbia, Belgrade. This study compared efficiency of three methods for the diagnosis of VL.

**Methods:** All patients with suspected VL (n = 44) were examined by Giemsa-stained bone marrow smears, by the rapid dipstick rK39 test (DiaSys Europe, UK) and indirect hemagglutination assay (Siemens, former Behring Diagnostics, Germany). Diagnosis of VL was confirmed on the demonstration of *Leishmania amastigotes* in Giemsa-stained BM smears. If the initial BM smear was negative but the clinical index of suspicion high, parasitological investigation was repeated, or the diagnosis based on the clinical presentation and positive serology. The control group included 62 patients with other diagnoses, who were tested by IHA and strip-test, without BM aspiration.

**Results:** VL was diagnosed in 14 patients (eight male and six female; age, 11–69 years, mean 40). Eleven of them (79%) were treated at the Clinic of Infectious and Tropical Diseases, Belgrade. The infection was contracted in Montenegro (n = 8), Herzegovina (n = 4), southern Serbia and Portugal (n = 1, each). The initial examination of BM smears was successful in 85.7% patients. At the first examination, two patients had negative BM smears. In only one, parasitological investigation was repeated and VL was confirmed. In another patient, diagnosis was based on clinical picture, positive serology and therapeutic effect. Both the strip-test and IHA performed with a sensitivity of 92.9%, specificity 96.7% and a positive predictive value of 92.9%. The density of *Leishmania amastigotes* and antibody titer by IHA were not always in correlation with each other or with the clinical condition. One patient had positive both the strip-test and IHA (1:256), while parasitological investigation was negative; further examination confirmed liver and spleen multi-focal micro-abscesses. All patients in control group tested negative with both the strip-test and IHA.

**Conclusion:** The diagnosis of VL would have been missed in these patients if diagnosis had been solely on one diagnostic method. Inadequate sensitivity of the initial BM smears and rare false-negative reactions of the strip-test and IHA requires introduction of molecular diagnosis.

#### **P2404 A 4-year outcome summary of 20 mg/kg liposomal amphotericin B regime for the treatment of primary KA in Bihar, India**

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**Background:** India reportedly has 70% of the worldwide burden of Visceral Leishmaniasis (VL), with Bihar having 90% of reported cases. With the support of the RMRI, MSF has implemented a VL treatment project in Vaishali district since 2007, using a total dose of 20 mg/kg liposomal amphotericin-B (LAmB) as the first-line drug.

**Methods:** Intravenous LAmB (Ambisome, Gilead) has been administered in four doses of 5 mg/kg/doses to a total dose of 20 mg/kg to all patients with primary VL. Treatment was given at PHC, district hospital or tertiary referral center over a period of 5–10 days depending on severity of disease. The Excel based database of the project was analysed over a 4-year period.

**Results:** A total of 6368 patients with laboratory confirmed VL and no history of previous KA infection or treatment with anti-KA drugs were treated at three facility levels: 18.5% at Primary Health Centers (n = 1178), 79.5% at district Hospital level (n = 5065) and 1.8% at the Tertiary Referral Centre (n = 125). Clinical cure was defined as improvement of symptoms, cessation of fever and recession of spleen immediately following the last dose of treatment and was achieved in 99.7% (n = 6344) with 0.05% (n = 3) defaulting after initiation of treatment and 0.2% (n = 13) deaths. The admission Hb value was <11 g/dL in 88% of the cases and <8 g/dL in 45.3%. The average spleen size at time of admission was 6 cm, reduced by an average of 2.8 cm at time of discharge. Global acute malnutrition was present in 26.5% (n = 1684) and 6.4% (n = 409) had severe acute malnutrition. 0.9% (n = 64) were co-infected with HIV and 0.5% (n = 33) with TB. Treatment was discontinued in 0.05% (n = 4) patients – all due to allergic reactions. No significant difference in initial cure and tolerance was observed under different administration schedules. Up to October 2011 1.3% (n = 80) of this cohort have been readmitted having relapsed following initial treatment, with average time to relapse of 12 months, of whom 17 were HIV positive. Fifteen cases treated have been readmitted after developing PKDL with an average length of time from treatment to diagnosis of 2.1 years.

**Conclusions:** Twenty milligrams per kilogram total dose LAmB has shown a very high cure-rate and a very low rate of adverse reactions using short treatment course under field conditions. Low relapse rate has been identified in immunocompetent patients, however much higher relapse rates in HIV patients and several cases of PKDL post-LAmB have been reported and both cohorts require further study.

## Late Breaking News

### LB2803 Decreasing ventilator associated pneumonia rates: the SKMC experience

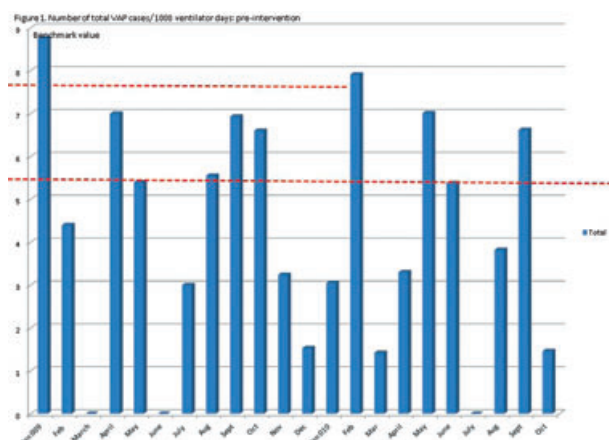
M. Pitout\*, S. Weber, K. Krishnareddy, M. Sara, A. Khallaf, S. Ellaham (Abu Dhabi, AE)

**Introduction:** Patients on mechanical ventilation are at serious risk of developing ventilator associated pneumonia (VAP), a costly complication that impacts negatively on patient morbidity and mortality. We describe an outbreak of multi drug resistant *Acinetobacter c* infections (MDR AB) in our intensive care unit (ICU), the intervention measures taken to address our high VAP rates and the long term impact of the multi-faceted program.

**Background:** Sheikh Khalifa Medical City is a 568 bed tertiary health care facility in Abu Dhabi, UAE. In 2006 our new 30 bed adult intensive care unit (ICU) experienced an outbreak of MDR AB infections that consisted of four cases of bacteremia and one case of ventriculitis. All five isolates produced OXA-23 carbapenemases while four of the five isolates were clonally related. Ever since the outbreak our surveillance data has shown unacceptable VAP rates in spite of the following infection control measures: (i) in-house training on VAP prevention bundles; (ii) in-house training on hand hygiene; (iii) measurement of hand hygiene compliance; (iv) hand cultures of ICU health care workers; (v) re-evaluation of environmental cleaning, type of cleaning agent used and frequency of cleaning.

**Intervention:** A multi disciplinary task force was formed to address the high VAP rates. Meetings involving infection control practitioners, intensive care physicians, microbiologists and nurses were held and the following program was put forward: (i) Keep on implementing the steps of the previous infection control measures (see above); (ii) Screen all patients for MRO AC colonization; (iii) Cohort positive MRO AC patients; (iv) Change the nursing care matrix so that nurses care only for MRO negative or positive patients; i.e. cross care was not allowed.

**Results:** Post intervention measurement of VAP rates was performed over a 13 month period to determine its sustainability and brought to light the following: pre-intervention total VAP rates measured 4.2 cases/1000 patient ventilator days whereas post-intervention rates were on average 1.4 cases/1000 patient ventilator days. MRO AB associated VAP rates decreased from 1.12/1000 patient ventilator days pre-intervention to 0.65/1000 patient ventilator days post-intervention.



**Conclusion:** MDR AB screening, cohorting of positive patients and nursing care matrix adjustment have lowered our total VAP rate by 67% (p value < 0.001), AB MDR related VAP rates by 42% (p value < 0.001) and rates remained well below the benchmark value for the whole study period. It seems that our intervention is effective and sustainable in decreasing VAP rates.

### LB2804 First isolation of New Delhi metallo-beta-lactamase 1-producing *Escherichia coli* ST101-B1 in South Korea

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**Objectives:** Cases of carbapenemase-producing Enterobacteriaceae are rare but their incidence is increasing in Korean hospitals. Various types of carbapenemase-producing Enterobacteriaceae, mostly *Klebsiella pneumoniae*, KPC-2, NDM-1, IMP-1, and VIM-2, were detected during a lab-based investigation in Korea. New Delhi metallo-beta-lactamase 1 (NDM-1) -producing *E. coli* was first detected in one hospital in December 2011.

**Methods:** Antimicrobial susceptibility tests and the MHT were performed according to CLSI methods and an imipenem disk synergy test was performed. NDM-1 genotype and other resistant genes were confirmed by PCR and sequencing using specific primers. For epidemiological analysis, PFGE types were determined by Xba-I restriction, and phylogenetic group PCR and MLST were performed.

**Results:** One NDM-1-producing *E. coli* was isolated from a urine culture, and three isolates were recovered after surveillance of stool cultures from inpatients on the same ward. This hospital performed specific medical examinations for foreign patients. All patients were hospitalized 1–7 months before NDM-1 was isolated without history of overseas travel or medication. All the NDM-1-producing isolates were resistant to imipenem, meropenem, ciprofloxacin, gentamicin, amikacin, ceftazidime, and cefotaxime, but were susceptible to colistin and tigecycline. TEM, SHV, CTX-M-15, DHA-1, OXA-1 genes were detected. PFGE analysis showed 98% similarity, indicating clonality. All isolates belonged to phylogenetic group B1, ST101, which is a rare sequence type in Korea.

**Conclusion:** This is the first case of NDM-1-producing *E. coli* in Korea. All isolates were identified from the four patients sharing the room. The transmission route of the organism was determined to be personal contact among the patients in the hospital, although the origin was not clear. However, *E. coli* B1-ST101 was reported as the major international clone of NDM-1-producing *E. coli* from England, Pakistan, Canada, Australia, and after considering the patients' epidemiological data, it was suspected that this organism was imported. Thus, continuous surveillance and hospital infection control would be important to prevent the spread of this organism.

**LB2805** Pan-European study of real-world treatment patterns and early switch/early discharge opportunities in patients with complicated skin and soft tissue infections due to methicillin-resistant *Staphylococcus aureus*: study methodology and interim results

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**Objectives:** Describe methodology and interim results from the first multinational real-world study of treatment patterns, healthcare resource use, and criteria-based assessment of early switch (ES) and early discharge (ED) opportunities in patients (pts) with methicillin-resistant *Staphylococcus aureus* complicated skin and soft tissue infections (MRSA cSSTI).

**Methods:** This is an ongoing retrospective, observational medical chart review study that will enroll pts from >400 hospitals across 12 European countries. Pts  $\geq 18$  years, with documented MRSA cSSTI admitted between 1 July 2010 and 30 June 2011, discharged alive by 31 July 2011, are randomly sampled to collect data on clinical characteristics/outcomes, hospital length of stay (LOS) in days (d) from cSSTI diagnosis to discharge, intravenous (IV) and oral (PO) antibiotic use, labs, surgeries, and readmissions. Data for evaluating eligibility for ES (from IV to PO antibiotics) or ED are also captured. Literature review and experts' consensus opinion were used to create ES and ED eligibility criteria and algorithms for use in real-world clinical settings, which will be validated in this study. We present results of an interim analysis.

**Results:** Interim data includes 344 pts from nine countries who were 59% male, 88% white, and mean age  $60 \pm 17$  years. Eighty-eight percent listed treatment of MRSA cSSTI as the primary reason for hospitalization, with extensive cellulitis (30%) and infected ulcers (24%) being most common. Forty-two percent of pts required surgical procedures such as debridement/incision/drainage for cSSTI management. Targeted MRSA antibiotic therapy was used in 72% of pts, started  $4 \pm 12$  day after diagnosis, and lasted  $13 \pm 8$  day, with single-agent vancomycin IV (33%), linezolid IV (15%), daptomycin IV (6%), and linezolid PO (5%) as most frequent initial therapy. Seventeen percent of pts began PO antibiotic therapy while in hospital. In IV only pts, 29% met ES criteria and potentially could have discontinued IV therapy  $9 \pm 18$  day sooner. Mean LOS was  $22 \pm 23$  day (median 17 day; range: 3–283 day). Twenty-four percent of IV only and 16% of IV to PO switched pts met ED criteria with a mean potential LOS reduction of  $7 \pm 20$  day. At 30 day post-discharge, all-cause and cSSTI-related rehospitalizations were 5% and <1%, respectively.

**Conclusion:** ES/ED opportunities appear to exist based on interim data from this first pan-European observational study of MRSA cSSTI. Future analysis will also describe country variations and predictors of ES/ED eligibility; validate ES/ED algorithms/tools; and identify opportunities to improve patient care nationally while reducing bed-days and costs.

**LB2806** Reemerging of tick associated bacteria in Slovakia

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**Objectives:** More than 30% of hospitalized human cases with symptoms like high fever, vomiting, diarrhea and headache, cannot be assigned to any of the predictable illnesses. Tick readily bites humans and easily transmits pathogens hence they are the continuous source of infection. During the last 10 years we have succeeded to detect & isolate numerous known or reemerging tick transmitted bacteria circulating in Slovakia, and suggested link from vector to causative agent and the origin of diseases.

**Methods:** Overall seroprevalence was evaluated by immunofluorescence of human sera, followed by PCR (16S rRNA, *gltA*, *sca4*, *ftsY*, *rpoB*, *parC* genes) & sequencing analyses. Tick extracted DNA collected from the areas of interest in Slovakia was used for identification of several bacterial agents by molecular biological means. If positive, a tentative isolation was performed in diverse cell lines (Vero, L929, XTC, BME/CTMV2).

**Results/Conclusions:** We have revealed the prevalence of 12 known and several newly detected bacterial agents in *I. ricinus* ticks. Some of them confirmed as human pathogens, like *Bartonella elizabethae*, *Borrelia afzelii*, *Borrelia garinii*, *Anaplasma phagocytophilum*, *Rickettsia helvetica*, *Rickettsia slovaca*, *Rickettsia raoultii*, *Franciscella tularensis*. For the rest the pathogenicity is either supposed or unknown. First time ever detected in Slovakia was *Bartonella elizabethae*, *Arsenophonus* aff. *Nasoniae*, *Spiroplasma* sp. aff. *Ixodetis*, or *Candidatus Rickettsia* sp. strains IRS3 and IRS4. Even more interesting was a case of a world premiere isolation of *Diplorickettsia massiliensis* from a Slovak tick. They may all well be source of numerous mysterious human cases, people suffering from diseases with unclear origin of infection. The identification of formerly unknown newly detected or first time isolated bacteria shows that the list of tick associated bacteria is not yet complete. The role of these new bacteria for human and animal health is to be evaluated.

**Acknowledgement:** This work was partly supported by grants VEGA No. 2/0031/11, 2/0065/09, and 2/0142/10 from SAS, Bratislava, Slovakia, as well as from bilateral project PICS, CNRS France/ SAS Slovakia.

**LB2807** Clonality and resistance of *E. coli* bacteraemia isolates from 14 institutions

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**Objectives:** To determine the clonality and resistance trends of *E. coli* bacteraemia isolates affecting a population of ~5 million.

**Methods:** Ten percent of *E. coli* blood culture isolates were collected per month from 14 hospital trusts in the Yorkshire & Humber region, UK, between July 2010 and December 2011. Individual laboratories submitted antibiotic susceptibility data and the isolates were tested again centrally (Leeds) by VITEK<sup>®</sup>. Isolates were characterised by the presence of sequences encoding extended-spectrum beta-lactamases (ESBL) and amplified fragment length polymorphism (AFLP).

**Results:** Of 573 isolates, 52 (9%) were ESBL-positive; TEM (50%) and CTX M group 1 (62%) enzymes were common. Resistance to amoxicillin/clavulanic acid (MIC > 8 mg/L) doubled between July 2010 (16%) and December 2011 (31%); results from the central and individual laboratories were consistent and the increase was not driven by a particular AFLP type. Resistance to trimethoprim was frequent (40% July 2010–45% December 2011). Only one isolate was resistant to carbapenems (0.2%). Trends were not driven by single institutions. AFLP of 404 isolates identified 35 distinct types, of which 17 comprised >2 isolates. Most isolates (n = 262) belonged to one of two AFLP types: AFLP 1 (18%) and AFLP 2 (47%). ESBL-positive isolates were predominantly AFLP 1 (53%), which had  $\geq 95\%$  similarity with NCTC13441 (multilocus sequence type ST131). A subgroup of isolates (n = 104) were categorised according to time of onset and presumed source of infection; 65% of isolates were associated with community-onset bacteraemia (i.e. within 48 h of hospital admission) and where stated the presumed sources of infection were the urinary tract (45%), abdomen (n = 30%) or chest (25%). ESBL-positive isolates were commonly associated with hospital-onset bacteraemia (60%) and the urinary tract (33%). AFLP 1 and 2 were associated with both hospital and community-onset bacteraemia. AFLP 1 was associated with 10%, 17% and 35% of abdominal, urinary and chest sources, respectively, the corresponding rates for AFLP 2 were 42%, 57% and 35%, respectively. **Conclusions:** Prospective surveillance of invasive *E. coli* isolates affecting a large population has identified the clonal dominance of two strains and important changes in antibiotic susceptibility. Dominant *E.*

*coli* clones were responsible for both community and hospital-associated bacteraemias. Interventions are needed to address the large health burden represented by *E. coli* bacteraemias.

**LB2808 National 1 week audit of MRSA admission screening: final results of the cost-effectiveness evaluation of MRSA screening strategies in English NHS hospitals**

J. Robotham\*, S. Deeny, C. Fuller, B. Cookson, S. Hopkins, S. Stone (London, UK)

**Objectives:** This Department of Health funded study uses data collected from a national 1 week audit of MRSA admission screening in 144 NHS hospitals to populate a dynamic MRSA transmission model to evaluate the effectiveness and cost-effectiveness of 10 different screening and intervention strategies in various hospital settings to inform future English policy.

**Methods:** A dynamic, economic model was developed describing MRSA transmission in hospital at an individual patient level. Model parameters were derived from audit data and relevant literature. Ten alternative screening and intervention strategies were evaluated, including universal, specialty- and risk-based screening, pre-emptive isolation, as well as a comparison of alternative MRSA checklist-based strategies. Screening was combined with isolation and/or patient decolonisation. Incremental costs and health benefits (measured in quality adjusted life years [QALYs]) were evaluated under different assumptions about MRSA prevalence, transmission potential and Trust type. Probabilistic sensitivity analyses, incorporating uncertainty in model parameters, were conducted.

**Results:** Health benefits associated with competing strategies were similar. Compared with no screening the most intensive screening strategies were able to reduce MRSA infection rates by up to 20%; however, this led to only small reductions in deaths (<5%). Combining effects with costs showed screening of admissions to high risk specialties could be good value for money, having cost/QALY values beneath the usual National Health Service willingness to pay threshold (£30 000 (35 s500)/QALY). Costs were found to be dependent on the valuation of a bed day; QALYs gained depended largely on the estimation of death probabilities. Sensitivity analyses and evaluation of each strategy in different settings will be presented.

**Conclusion:** Models incorporating both economics and the transmission process can be powerful tools to inform policy, particularly when informed by high quality data such as here. We find the current national mandatory policy of screening all elective and emergency admissions is unlikely to be cost-effective at the whole hospital level, although further work is exploring sensitivity to model parameters, setting and long-term effects. Targeted screening of high risk specialties is more likely to be cost-effective, reflecting the higher proportion of infections seen.

**LB2809 Reducing acquisition of resistant bacteria in intensive cares: a European cluster randomised trial**

L.P.G. Derde\*, B.S. Cooper, C. Brun-Buisson, M.J.M. Bonten on behalf of the MOSAR Mastering Hospital Antibiotic Resistance research consortium

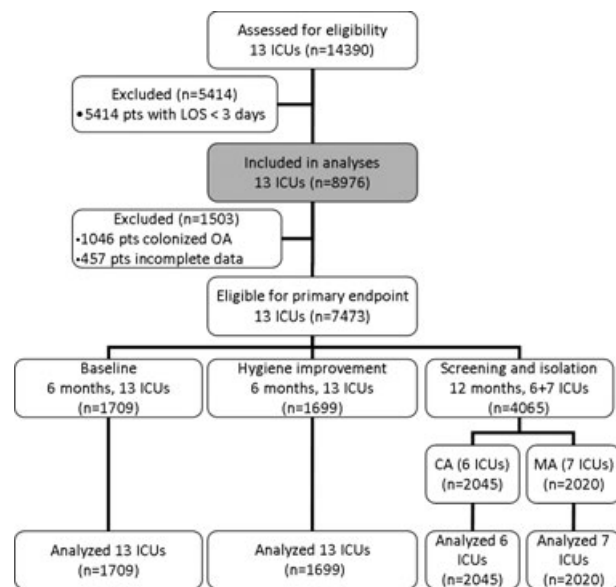
**Objective:** To quantify effects of different infection control measures on acquisition of antimicrobial-resistant bacteria (AMRB; MRSA, VRE or highly resistant Enterobacteriaceae [HRE]) in intensive care units (ICUs).

**Methods:** We conducted a cluster-randomised trial in 13 European ICUs. After a 6 month baseline (P1), unit-wide chlorhexidine body-washing (CBW) combined with the WHO '5 Moments' hand hygiene program (P2; 6 months) was implemented. In phase 3 (P3; 12–15 months) random allocation of ICUs to molecular (MA) or chromogenic-based (CA) screening with contact precautions was added. AMRB acquisition was determined by admission and twice weekly screening. Results were only disclosed in P3. Effects on

stepwise changes in AMRB incidence density and on trends were determined by multilevel Poisson regression.

**Results:** Seven thousand four hundred and seventy-three out of 14 390 included patients were at risk for AMRB acquisition, of whom 18.3% acquired AMRB (Fig. 1). Hand hygiene compliance was 52% in P1, 69% in P2 and 77% in P3. For CBW, median compliance was 0% in P1, and 100% in P2 and P3. In P3, median turn-around times were 23–24 h for CA, and 2 h for MA (MRSA/VRE). There were 21.5% and 91.8% relative increases in contact precautions in the CA- ( $p = 0.387$ ) and MA arm ( $p = 0.002$ ). In all 71 796 surveillance cultures were obtained; 95% of patients had surveillance data for assessment of colonization status. In P1, AMRB acquisition increased by 1.4% per week (95%CI  $-0.4, 3.1$ ). Following P2 there was a reduction in trend (weekly IRR 0.98 [0.95, 1.0]). In P3 neither CA nor MA was associated with further changes in trend. The null hypothesis that CA and MA were equivalent could not be rejected ( $p = 0.06$ , likelihood ratio test). For MRSA, there was a 4.2% (1.0, 7.5) weekly increase in acquisition in P1 and this trend reversed in P2 (weekly IRR 0.93 [0.89, 0.96]), to give a net 3% weekly decrease. MRSA levels plateaued in P3, an increase in trend relative to P2 (weekly IRR 1.06 [1.03, 1.09] for CA and 1.04 [1.01, 1.07] for MA).

No intervention was associated with a significant stepwise change in acquisition rates and no significant effects were demonstrated for VRE and HRE.



**Conclusion:** Unit-wide CBW and hand hygiene improvement reduced AMRB acquisition, mainly by reducing MRSA acquisition. There is no incremental effect of an admission screening, either by CA or MA, on reduction of MRSA and AMRB acquisition.

**LB2810 Transferable plasmid-mediated resistance to linezolid due to cfr in a human clinical isolate of *Enterococcus faecalis***

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**Objectives:** The *cfr* gene encodes a methyl-transferase that catalyzes the post-transcriptional methylation of nucleotide A2503 in the 23S rRNA conferring a multi-resistance phenotype. In enterococci, *cfr* has been described only in an animal isolate of *Enterococcus faecalis* from China, but isolates from human origin have not been characterized. Here, we report an isolate of linezolid-resistant *E. faecalis* (603-50427X) recovered from a patient in Thailand who received prolonged

therapy with the antibiotic for the treatment of atypical mycobacterial disease.

**Methods:** Linezolid MIC of the *E. faecalis* 603-50427X was confirmed by Etest and the Sequence type (ST) was determined by multilocus sequence typing (MLST). The presence of mutations in genes encoding all copies of the 23S rRNA and ribosomal proteins L3 and L4 were investigated by PCR and sequencing. Detection of the presence of the *cfr* gene was performed by PCR and the entire gene was sequenced. In order to determine plasmid localization of *cfr*; S1 digestion, PFGE and Southern hybridization with a *cfr* probe within the genome of *E. faecalis* 603-50427X, were performed. The DNA sequence upstream and downstream of *cfr* was obtained using inverse PCR methodology and sequencing. Conjugative transfer of *cfr* was conducted by filter mating using *E. faecalis* 603-50427X as donor and *E. faecalis* OG1RF, *Enterococcus faecium* GE1 and *Staphylococcus aureus* RN4220-RF as recipients.

**Results:** The isolate lacked mutations in the genes coding for 23S rRNA, L3 and L4 ribosomal proteins and belonged to the ST16. Resistance to linezolid was associated to the presence of *cfr* on a ~97-kb transferable plasmid. The *cfr* gene environment exhibited DNA sequences similar (nucleotide identity 99–100%) to other *cfr* plasmid associated regions previously described in staphylococci. The *cfr* gene was flanked upstream and downstream by IS256-like sequences and was carried on a plasmid that was transferable by conjugation to a laboratory strain of *E. faecalis* (OG1RF) (efficiency of  $2.6 \times 10^{-5}$  transconjugants per donor) but not to *E. faecalis* or *S. aureus*.

**Conclusion:** This is the first characterization of a linezolid resistant *E. faecalis* human isolate carrying the *cfr* gene with evidence of its potential horizontal transferability.

#### **LB2811** First evaluation of a high throughput microfluidic molecular system for the detection of *C. difficile*

A. Salmon\*, R. Farrington, J. Kavanagh, L. Fenelon (Dublin, IE)

**Objectives:** Clostridium difficile associated diarrhoea is a life threatening hospital acquired infection, with an attributable mortality of 6-15%. It is essential to have accurate laboratory diagnosis of *C. difficile* associated disease in order to properly manage patients and to establish appropriate infection control measures. The Microbiology Laboratory in St. Vincent's Hospital receives approximately 5000 samples for *C. difficile* testing per year. All samples are initially screened by glutamate dehydrogenase (GDH) ELISA. Positive specimens (approximately 15% of samples) undergo confirmation testing by PCR using the Cepheid GeneXpert. This 2 step algorithm is time consuming and delays reporting of results.

Our aim was to compare the current testing algorithm to a single test algorithm utilizing the 3M integrated cycler, which is a microfluidic molecular detection system. This rapid real-time PCR thermocycler combines real-time fluorometric detection with direct detection chemistry (that does not require prior nucleic acid extraction) to identify targets within sample wells.

**Methods:** A total of 275 stool samples received in the laboratory for routine testing for Clostridium difficile were tested using the currently used algorithm and the test method. The GDH and GeneXpert assays were run according to manufacturers' specifications, as was the test method. Any positive samples detected by the test method were tested by the GeneXpert PCR methodology. The sensitivity and specificity of the test method was then computed using the current algorithm as the gold standard.

**Results:** The currently used method detected 21 positives by the GDH method, with positive confirmation by GeneXpert, and 254 samples were found to be negative. The test method using the Integrated Cycler detected 25 positive samples, with 250 negatives.

Overall, using the 3M integrated cycler as a stand alone diagnostic test for detection of *C. difficile* provided a sensitivity, specificity, positive predictive value, and negative predictive value of 100%, 98.4%, 88%, and 100% respectively.

The time to result for Integrated Cycler is less than 2 hours for 96 specimens. The GeneXpert cannot run a high throughput of samples so each positive sample takes approximately 2 hours to confirmation using the 2 step algorithm.

**Conclusion:** The single test algorithm provided comparable results to the multi-test algorithm, and results were available much more rapidly than when using the two tiered approach. Although the use of a non-molecular test to do initial patient screening can provide some cost benefits, the labour requirement is higher, and the delay in providing results may add additional cost to patient care and infection control efforts.

#### **LB2812** A randomised, double-blind study of combination antifungal therapy with voriconazole and anidulafungin versus voriconazole monotherapy for primary treatment of invasive aspergillosis

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**Objectives:** Invasive aspergillosis (IA) remains a major risk in patients with haematological malignancies, including allogeneic stem cell transplant (allo-HSCT) recipients. In vitro, animal and clinical observational studies each support the use of combination antifungal therapy with voriconazole and an echinocandin to treat this infection. We present the results of the first prospective, randomised, double-blind clinical trial to investigate the efficacy of the combination of voriconazole and anidulafungin for the treatment of IA in these patients.

**Methods:** Patients with active haematological malignancies, including allo-HSCT recipients, with a diagnosis of proven or probable IA were eligible, but patients at high risk of death for other reasons (underlying disease and organ function) were excluded. Patients were stratified by host and transplant-related characteristics and randomised to receive initial treatment with the combination of voriconazole and anidulafungin or voriconazole monotherapy (with placebo). Study treatment was administered for  $\geq 2$  weeks, followed by voriconazole maintenance to complete 6 weeks. The primary endpoint was all-cause mortality at 6 weeks. Certainty of diagnosis of IA was adjudicated by an independent, blinded Data Review Committee (DRC).

**Results:** During the period 9 July 2008–8 April 2011, 454 patients were enrolled from 93 sites in 24 countries, and 277 patients with DRC adjudicated proven or probable IA were included in the primary analysis. Mortality at week 6 was 26/135 (19.3%) in patients treated with the combination of voriconazole and anidulafungin, compared to 39/142 (27.5%) for monotherapy; p-value for the difference (using Kaplan–Meier estimates and adjusted for randomisation strata) was 0.09 (95% confidence interval –18.99, 1.51). In a post-hoc analysis of 218/277 (78.7%) patients with probable IA based on detection of galactomannan (GM) in bronchoalveolar lavage or serum, mortality at week 6 was 17/108 (15.7%) for combination and 30/110 (27.3%) for monotherapy; p-value was  $<0.05$  (95% CI –22.69, –0.41). Safety parameters did not show significant differences between treatment arms.

**Conclusion:** This is the first prospective, randomised, double-blind clinical trial of combination antifungal therapy for IA. Although the difference in all-cause mortality in the primary analysis was not statistically significant, the combination was beneficial in patients with a diagnosis of probable IA based on a positive GM.

#### **LB2813** Untreated wastewater urban effluents samples might act as a biosensor of environmental dissemination of carbapenemase producing Enterobacteriaceae: emergence of KPC-2 producers in Madrid, Spain

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**Objectives:** Carbapenemase producing Enterobacteriaceae (CPE) are increasing worldwide, including Europe. We detect the presence of



CPE isolates in a wastewater urban effluents sample and ascertain its potential as biosensor for its dissemination.

**Methods:** A single untreated wastewater urban effluents sample obtained in July 2011 in Madrid (Spain) was plated onto MacConkey agar (MAC), MAC-2 mg/L-cefotaxime and MAC-2 mg/L-ceftazidime. One isolate per morphotype was selected. Bacterial identification and susceptibility patterns were determined using the MALDI-TOF MS (Bruker) and semi-automated WIDER (Fco. Soria Melguizo) systems. Production of carbapenemases was phenotypically confirmed by disk diffusion using meropenem, meropenem + cloxacilin; meropenem + boronic acid and meropenem + dipicolinic acid following the manufacturer instructions (Rosco Diagnostica). Carbapenemases and ESBLs were characterized by PCR and further sequencing. Clonal relatedness was established by XbaI-PFGE.

**Results:** A total of 154 isolates were studied (91 from MAC and 63 from MAC-cephalosporins plates). Six isolates (one isolate from MAC and five isolates from MAC-cephalosporins) (3.9%), presenting a phenotype compatible with carbapenemase production, were identified as *Citrobacter freundii* (n = 4), *Enterobacter asburiae* (n = 1) and *Klyuvera cryocrescens* (n = 1). All of them were confirmed as KPC-2 producers. The MICs for ertapenem, meropenem and imipenem ranged from 4–32, 0.5–12 and 0.5–2 mg/L, respectively. None of them were ESBL producers. *Citrobacter freundii* were demonstrated to be unrelated, but all were resistant to fluoroquinolones.

**Conclusions:** This is the first report of KPC-2 producing *C. freundii*, *E. asburiae* and *K. cryocrescens* from wastewater urban effluents. This finding suggests that untreated wastewater urban effluents samples might act as biosensors of CPE dissemination, particularly in geographic areas with low prevalence of these isolates.

#### **LB2814** Successful control of a large outbreak of OXA-48 producing Enterobacteriaceae in the Netherlands

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**Objectives:** OXA-48 positive Enterobacteriaceae (OXA-E) are an emerging cause of nosocomial infections in Europe. We report the first outbreak of OXA-E in the Netherlands, probably the largest so far.

**Methods:** An OXA-48 CTX-M-15 producing *K. pneumonia* was detected in a single hospital in May 2011. Outbreak investigation included retrospective analysis of stored multi-resistant *K. pneumonia* isolates, implementation of Dutch guideline-adherent infection prevention measures, and labelling of all patients into risk groups (high risk [roommates of OXA-E patients], low risk [admitted during outbreak period]). High-throughput screening of rectal swabs was based on enrichment broth (0.125 mg/L ertapenem), OXA-48 specific PCR, and determination of PCR-positive samples. All high risk patients were contacted for screening, low risk patients were screened when hospitalized.

**Results:** The 'oldest' OXA-E isolate detected among stored isolates originated from September 2010 and based on the prevalence of clinical CTX-M-15 *K. pneumonia* isolates the start of the outbreak period was defined as 1 July 2009. On 18 July 2011 all prevention measures, including labelling and screening, were implemented. By that day, 64 OXA-E patients had been identified. Hereafter, OXA-E carriage was documented in 40 of 3374 (1.19%) high risk and 12 of 3478 (0.35%) low risk patients. There were no new acquisitions after 18 July among patients without risk exposure during the outbreak period (n = 886). Among the 116 patients with OXA-E 63 (54%) were male, median age was 70.4 years and median number of admissions during the outbreak period was 3 (IQR 2–6), with median length of stay of 4 days (IQR 1–14 days). Most admissions were to ICU, surgery and nephrology wards. Eighteen patients (16%) were infected with OXA-E. Mortality 30 days after OXA-E detection was 17% (n = 20). In three patients OXA-E infection contributed directly to death.

OXA-E included *K. pneumonia* (96 patients), *E. coli* (n = 52), *E. cloacae* (n = 6), *K. soxytoca* (n = 6), *M. morgani* (n = 5), *C. freundii*

(n = 3), and nine other species (n = 1 or 2). MICs for imipenem and meropenem ranged from  $\leq 1$  to  $\geq 16$  mg/L.

**Conclusion:** A combined approach of classical infection prevention methods, together with large-scale screening was successful in controlling a hospital-wide outbreak with OXA-48 Enterobacteriaceae, mainly of OXA-48 CTX-M-15 *K. pneumonia*. The outbreak was characterized by the presence of OXA-48 in multiple species of Enterobacteriaceae and widely varying phenotypes.

#### **LB2815** Letermovir (AIC246) for prevention of HCMV infections in patients after human blood precursor cell transplantation: a randomised, double-blind, placebo-controlled trial to evaluate the safety, tolerability and antiviral activity of 12 weeks treatment

H. Zimmermann on behalf of the AIC246 Study Team

**Objectives:** Letermovir (AIC246) is an innovative, highly active and specific inhibitor of HCMV. It stems from a novel chemical class (quinazolines) and addresses a novel target (the viral terminase). Activity in patients was previously shown for pre-emptive treatment in phase IIa and in treating HCMV disease under an individual EIND protocol. The presented trial investigated for the first time the prophylactic use of letermovir in transplant patients.

**Methods:** On hundred and thirty-three HCMV-seropositive allogeneic human blood precursor cell (HBPC) recipients were included in the trial receiving either 60, 120 or 240 mg of letermovir or matching placebo. The efficacy endpoints were incidence and time to onset of 'HCMV prophylaxis failure', defined as development of systemic detectable HCMV replication (viral load above assay cut-off of 42 DNA copies/mL) or HCMV End-Organ Disease.

**Results:** Given orally once daily for 84 days, letermovir meets both primary efficacy endpoints with high statistical significance vs. placebo. In the primary Full Analysis Population, the incidence of failure due to efficacy failure of prophylaxis or due to discontinuation of treatment for any other reason prior to Day 84, is significantly lower in the Letermovir 240 mg/day (29.4%; p = 0.007) and 120 mg/day (32.3%; p = 0.014) groups compared to placebo (63.6%). The incidence of HCMV prophylaxis failure amongst patients receiving treatment for at least seven days prior to HCMV replication was none for Letermovir 240 mg (p = 0.004 vs. placebo) and only two patients for Letermovir 120 mg (p = 0.109 vs. placebo). Similarly, the time to onset of prophylaxis failure among patients receiving 240 mg/day of Letermovir was significantly different (p = 0.002) compared to patients receiving placebo. The analysis of safety demonstrates that – in all Letermovir groups combined – the percentage of patients with at least one treatment emergent adverse event (TEAE) either considered related to the treatment by the investigator, or leading to discontinuation of treatment (17.3% and 25.5%, respectively) is lower than in the placebo group (33.3% and 57.6%, respectively). A summary of all final safety and efficacy results of the trial will be presented.

**Conclusion:** Letermovir showed activity in the prophylaxis against HCMV infections with high statistical significance as well as excellent safety.

#### **LB2816** Comparison of tedizolid phosphate and linezolid in investigator's assessment of clinical response in patients with cellulitis in United States and Europe from a phase 3 ABSSSI study

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**Objective:** Tedizolid (T) is the active moiety of the prodrug tedizolid phosphate (TP), an oxazolidinone with 4- to 16-fold greater activity than linezolid against gram-positive species including methicillin-resistant and linezolid-resistant *Staphylococcus aureus* (MRSA). The FDA and EMA are using different primary efficacy endpoints for the investigation ABSSSI. EMA uses the traditional endpoint of clinical

Clinical Outcome at PTE	Tedizolid		Linezolid	
	US	EU	US	EU
ITT population	N (%) N=74	N (%) N=45	N (%) N=80	N (%) N=46
Clinical Success	61 (82.4)	44 (97.8)	62 (77.5)	42 (91.3)
Clinical Failure or Indeterminate	13 (17.6)	1 (2.2)	18 (22.5)	4 (8.7)
Clinical Failure	7 (9.5)	0	9 (11.3)	2 (4.3)
Indeterminate	6 (8.1)	1 (2.2)	8 (11.3)	2 (4.3)

Clinical Outcome at PTE	Tedizolid		Linezolid	
	US	EU	US	EU
CE population	N (%) N=64	N (%) N=42	N (%) N=61	N (%) N=40
Clinical Success	57 (89.1)	42 (100)	52 (85.2)	38 (95.0)
Clinical Failure	7 (10.9)	0	9 (14.8)	2 (5.0)

investigator assessment at the Post-Therapy Evaluation (PTE) visit. The objective of this review was to compare the investigators' assessment of clinical outcome at the PTE Visit of tedizolid phosphate vs. linezolid in US patients versus European patients with cellulitis.

**Results:** There were 304 patients diagnosed with cellulitis with a minimum total lesion surface area of 75 cm<sup>2</sup> were enrolled in the United States and Europe in the study. The baseline median lesion sizes for patients in the US were 189.4 and 168.9 cm<sup>2</sup> for tedizolid and linezolid patients, respectively. In Europe the baseline median size for tedizolid patients was 442.0 cm<sup>2</sup> and for linezolid patients was 346.7 cm<sup>2</sup>. Clinical outcome at the PTE visit in US patients and European patients are listed in the table below for both the Intent to Treat (ITT) and Clinically Evaluable (CE) populations (see Table 1).

**Conclusion:** The investigator's assessment of clinical outcome at the PTE visit were similar for tedizolid and linezolid in patients with cellulitis in the United States and Europe in both the ITT and CE populations. In spite of much larger lesions in European patients, the clinical success rate was still very high. The size of the lesion did not appear to impact the response rate. Based on investigator outcome at PTE, tedizolid phosphate 200 mg daily for 6 days is as efficacious as linezolid 600 mg BID for 10 days in patients with cellulitis.