Oral presentations

Surveillance of resistance in *M. tuberculosis* and potential impact of epidemiological cut-off values

S6 The critical concentration is important

G. Skenders (Stopini, LV)*

There are two types of drug susceptibility testing – qualitative and quantitative. Quantitative test Minimal Inhibitory Concentration (MIC), the lowest drug concentration that completely inhibits bacterial growth in vitro. Qualitative test gives only a suggested interpretation without MIC. For *M. tuberculosis* qualitative tests have been developed without correlation with MIC.
The critical concentration is defined as the concentration which clearly differentiates resistant clinical isolate from the susceptible one while critical proportion for resistance is the percentage of resistant population of an culture which separates out susceptible cultures from the resistant. Originally these parameters were established by clinical trials and recommended two or three drug concentrations and 1% or 10% critical proportion for establishing resistance. To simplify the procedure modification was recommended with one critical concentration of drug and 1% proportion. A culture which shows less than 1% growth on a medium containing a test drug as compared growth on the control is considered as susceptible while 1% or more growth is considered as resistant for clinical purpose. This principle has been applied to other culture based methods standardized against proportion method and resistance ratio methods.
The proportion method using a defined inoculum, growth on a growth control is compared with the growth on culture medium containing the critical concentration of the test anti-tuberculosis drug and then a percentage is calculated. The test is to be read after 4 weeks.
The resistance ratio method where resistance ratio is defined as ratio of the MIC for the patient's strain to the MIC for the drug susceptible reference strain (H37Rv), both tested in same experiment. Reference strain in this method standardizes the results. Reading after 4 weeks of incubation, interpretation is given by determination of resistance ratio between MIC of H37Rv and test strain as 2 or less for susceptible strains and 8 or more for resistant strains.
In the absolute concentration method critical concentrations used were similar to proposed for proportion method. Only one dilution of inoculum is used for drug containing media and two drug free controls are used. After 4 weeks of inoculation the inhibition of growth is reported if the number of colonies is less than 20 on drug media with confluent growth on drug free control.

Clinical problems from parasitology

S12 *Strongyloides* hyperinfestation

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Current estimates indicate that it affects 30 to 100 million people, but accurate prevalence data are lacking. Although strongyloidiasis is endemic in many tropical and subtropical countries (sub-Saharan Africa, Latin America, Southeast Asia, and Northern Australia), foci of low endemicity are also reported in temperate climates, such as the Mediterranean coast, Eastern Europe, and the southeastern United States. The asexual biological cycle of the human parasite *Strongyloides stercoralis* in the body of the human host leads to continuous auto-infestation lasting for decades or even for the whole life. In case of immune depression, the auto-infestation cycle accelerates with the production o fan increasing number of larvae mainly within the intestinal or respiratory tracts (hyper-infestation). The clinical onset of *Strongyloides* hyper-infestation may be acute or sub-acute, with malaise and asthenia. In case of fever, bacterial co-infection should be suspected. Gastrointestinal symptoms are exacerbated and may be complicated by intestinal ulcerations with melena and presence of rabditoides and filarioides larvae in the ulcers and in the stools. Protein-dispersing enteropathy may arise with edemas, ascytis, hypo-kaliemia.
Respiratory symptoms: Alveolar or interstitial hemorrhagic pneumonia may occur, as well as pleuritis and lung abscesses. Pre-existing COPD may exacerbate. Rabditoides and filariod larvae are present in the sputum, in bronco-alveolar fluid and in lung biopsies. Mortality due to acute lung failure is high (50–79%).
When also other body districts are involved, *Strongyloides* dissemination is present.
Considering the high case-fatality rate, it has been proposed that *Strongyloides* infestation should be excluded (and preliminary treated with ivermectine if detected) in those patients who are expected to undergo immunosuppressive therapy, in particular if steroid-based, as it is the case for many rheumatologic diseases.

Antimicrobial peptides – update for infectious disease physicians

S15 Antimicrobial peptides in the lung

P. Hiemstra (Haarlem, NL)*

The lung is exposed to large numbers of inhaled pathogens on a daily basis due to the large volumes of air that are inhaled. An efficient innate and adaptive immune system that is operative in the lung prevents the development of respiratory infections. Antimicrobial peptides and proteins (AMPs) are an important element of the innate immune system that protects the mucosal surface of the lung against infection. AMPs are a structurally diverse family of molecules with broad-spectrum antimicrobial activities, and are often characterized by cationic, amphipathic properties. A large variety of AMPs are present in human airway secretions, including α- and β-defensins, the cathelicidin hCAP18/LL-37, lactoferrin, lysozyme and the cationic antimicrobial serine protease inhibitors secretory leukocyte proteinase inhibitor (SLPI) and elafin. In this overview, the focus will be on the peptides in the AMPs family: the defensins and hCAP18/LL-37.
Whereas the main cellular source of the α-defensins and hCAP18/LL-37 in the lung is the neutrophil, airway epithelial cells produce β-defensins and smaller amounts of hCAP18/LL-37. Expression of β-defensins by airway epithelial cells is mainly regulated by microbial exposure and inflammation, whereas vitamin D is an important regulator of epithelial expression of hCAP18/LL-37. AMPs are an attractive candidate for the treatment of respiratory (bacterial) infections, in part because they are active against bacteria that are resistant to conventional antibiotics. Currently two strategies are employed to bring AMPs to the clinic: (i) use of (synthetic) AMPs; and (ii) enhancing their expression using e.g. vitamin D or butyrate analogues.
Interesting, AMPs not only display antimicrobial activity, but are also involved in a range of other processes, including inflammation, immunity and wound repair. These non-antimicrobial functions are thought to contribute to the in vivo antimicrobial activity of AMPs that has been demonstrated in a variety of animal models. However, excessive production of AMPs may contribute to inflammation. Current studies

indicate that not only production, but also local factors (host and pathogen-derived) that regulate the activity of AMPs are important for their role in host defence. Especially studies in cystic fibrosis have revealed a variety of mechanisms that may restrict the antimicrobial activity of AMPs, despite abundant local production.

Rare in the North, frequent in the South: infections from the Mediterranean Basin

S20 Visceral leishmaniasis

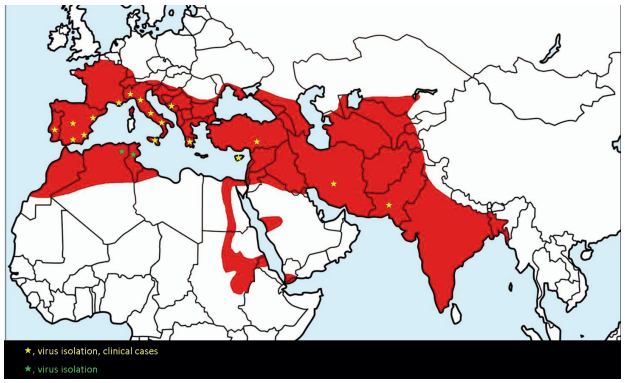
Y. Ozbel (Izmir, TR)*

Leishmaniasis is a major vector borne disease which besides being endemic in 88 countries worldwide, is the only "tropical" vector-borne disease being endemic for decades in Southern Europe. In the world, 12 millions of persons suffer from leishmaniasis and about 500,000 visceral leishmaniasis (VL) cases occur every year (more than 50,000 die). Visceral form of clinical leishmaniasis is a systemic disease and caused by protozoan parasites of the *Leishmania donovani* complex which consists of the species *L. donovani* and *L. infantum*. Transmission of *L. infantum* from dogs or infected people to people via different species of sand flies is primary route of infection.
L. donovani and *L. infantum* differ in their distribution, pathology, sand fly vectors and reservoir hosts. *L. donovani* is considered to be more "aggressive" than *L. infantum* and causes anthroponotic VL in East Africa and the Indian subcontinent. *L. infantum*/chagasi is responsible for zoonotic VL in all countries of the Mediterranean Basin and Latin America. Dogs are the main reservoir hosts for *L. infantum* and humans are considered to be accidental hosts.
VL is a serious human disease that may be fatal if untreated. In Mediterranean, VL primarily affected young children and infants, but it is also often a complication in adults infected with HIV or those receiving immunosuppressive drugs. The latter is one of the major health problems in southern Europe in last decades.
The predominant *L. infantum* zymodeme is MON-1 in all endemic areas of Europe. But recently, first autochthonous case of *L. donovani* (MON-37) was detected in Cyprus. The risk of introduction of new species from countries where non-European species are endemic should also be considered. *L. donovani* which is transmitted by different species of sand flies outside Europe might be hosted by most European sand flies.
The direct, serological and molecular tools are available for the diagnosis of VL and pentavalent antimonials are being used for treatment. They are toxic and have to be used parenterally for prolonged period. But, the lipid formulations of amphotericin B are an important advance in therapy even the cost is high. An oral drug, Miltefosine, has been found to be highly active against VL.
This presentation critically addresses the specifications of VL as a *Phlebotomus*-borne disease and currently available diagnostic, treatment and control regimens for VL.

S21 Toscana virus infections and other sandfly-transmitted phleboviruses in the Mediterranean

R.N. Charrel (Marseille, FR)*

A significant number of sandflies associated phleboviruses have been shown to infect humans and cause various diseases from mild self-resolutive febrile illness to meningitis and severe encephalitis, sometimes with a fatal outcome. Among these sandfly-transmitted viruses, Toscana virus is the most prominent one, probably due to its capacity to cause neuro-invasive infections as demonstrated first in Italy at the end of the 1990's, more recently in Spain, France, Turkey, Portugal, Croatia. In France, Toscana virus is in the top 3 viruses (with enteroviruses and herpesviruses) causing meningitis during summertime. Intriguingly, there is still little data on the medical impact of Toscana virus in countries located south of the Mediterranean.
There are accumulating evidence that other sandfly-transmitted phleboviruses (viruses within the species Sandfly fever Naples virus [SFNV] and Sicilian virus [SFSV], or related to but distinct from SFNV and SFSV) circulate in sandflies and cause infections in humans in France and Spain, but also in Algeria and Tunisia. Recent evidence suggests that there remain many other phleboviruses, not recognized at this time, circulating in the Mediterranean area and possibly involved in human infections. To address these neglected issues research programs based on multidisciplinary approaches combining virology, infectious diseases, entomology, epidemiology, social sciences must urgently be developed and promoted for funding.



New models for microbiology lab services

S27 Core labs and point-of-care microbiology labs

M. Drancourt (Marseille, FR)*

In microbiology, the availability of automats performing great series of tests for the direct diagnosis by culture and molecular biology and the indirect diagnosis by serology requires the concentration of the laboratories. The economic context also goes in this direction. These are the reasons why we are witnessing everywhere in Europe, including European countries where microbiology has been broken up into small laboratories as in France, the trend of concentration of the microbiological diagnosis activities on large platforms and "core-laboratories". Simultaneously to this trend of laboratories concentration, has raised the need for performing the microbiological diagnosis within the time of care – i.e. in less than 3 hours, which is asked more and more frequently by clinicians. We have set up the Point-of-Care laboratories which perform a definite number of microbiological analyses in less than 3 hours which result must modify the medical management of patients in terms of hospitalization, isolation and prescription of specific anti-infectious drugs. In conclusion, our laboratories must advance in two complementary directions: first is fusing into large core-laboratories in order to make cost-effective routine diagnosis in large series, to set up new diagnostic protocols, to ensure the staff training and to give a place for innovation and development of new tools and techniques; the second is the spread-out of Point-of-Care laboratories including the Remote Point-of-Care laboratories remaining under the responsibility and the supervision of the Core-Laboratory staff by using the new communication technologies.

Molecular virology

O30 Cytokine gene polymorphism and its association with Epstein-Barr virus infection outcome in paediatric liver transplant recipients

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Objectives: Paediatric liver transplant (LTx) patients (pts) are at particular risk of developing EBV associated complications, including PTLD (posttransplant lymphoproliferative disorder). The risk of PTLD

is higher in pts with high viremia. However, some pts with persistently high EBV DNA load do not develop lymphoproliferations, so there is a need for new prognostic markers to define pts at risk of serious complications. Polymorphism in cytokine genes influence susceptibility to, and progression of the disease. The aim of the study was to assess cytokine gene polymorphism that may be involved in pathogenesis of EBV infection, in relation to EBV DNA load and PTLD development in children after LTx.

Methods: One hundred seventy three children after LTx were included in the study (mean age at LTx 4.4±8.6 years, and mean follow up 32.4±24 months). EBV DNA positive pts (N = 108) were stratified into: chronically high viral load (>4000 copies/ug DNA for at least 6 months, CHVL; N = 33), high viral load (>2000 copies, HVL; N = 33), low viral load (<2000 copies, LVL; N = 26) and PTLD (N = 9) group. EBV DNA negative pts (N = 65) served as a control group. Polymorphism of IFNG +874, CCR5del32, TNFA-308, IL-10 −1117, MCP1+1543, IL-1β −511 and IL-1RN VNTR, were determined in all pts and related to EBV DNA load, and PTLD development.

Results: Increased frequency of IL-1RN VNTR allele 2 was found in PTLD pts when compared to non-PTLD pts (44% vs. 19%, $p < 0.01$, OR = 4.28, (95% CI: 1.56–11.75)). Interestingly, the frequency of this allele was significantly decreased in CHVL pts when compared to PTLD (5% vs 45%, $p < 0.001$) as well as to HVL (22%, $p < 0.01$), LVL (18%, $p < 0.05$) and controls (22%, $p = 0.001$). Moreover, CHVL pts had lower frequency of IL-1B −511 T allele then pts with PTLD (17% vs 39%, $p < 0.05$) or controls (34%, $p < 0.01$). Mean EBV DNA load during 1st year after LTx was significantly higher in: (i) pt with IL-1RN VNTR allele 1 then allele 2 ($p < 0.05$), (ii) pt with IL-10 −1117 C/C then C/T genotype ($p < 0.005$). There was no association between TNFA −308, CCR5del32, MCP1+1543 and IFNG +874 polymorphism, and PTLD development or EBV DNA load in pts after LTx.

Conclusions: Presence of IL-1RN VNTR allele 2 may be a risk factor for PTLD. Polymorphism in IL-1RN and IL-1B genes may be a useful prognostic marker of CHVL. EBV DNA load in children after LTx may be influenced by IL-10 −1117 and IL-1RN VNTR polymorphism.

O31 Cytokine gene polymorphism and congenital cytomegalovirus infection

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Objective: Cytomegalovirus (CMV) is one of the most common viral cause of congenital infections, which are associated with neurological squeal or hearing loss in children. Primary infected women have high risk for viral transmission to their foetus. Polymorphism in cytokine genes may influence the susceptibility to infection. The aim of this study was to assess the cytokine genes polymorphism that may be involved in pathogenesis of CMV infection in newborns and their mothers.

Material: Three hundred twenty seven newborns (mean age 12 days) with suspicion of congenital CMV infection and 209 mothers were included in this study. Congenital CMV infection (defined as CMV DNA detection in blood/urine and/or positive anty-CMV IgM within first 3 weeks of life) was confirmed in 34 children. Sixteen out of 209 mothers were CMV-seronegative during pregnancy. Polymorphism of CCR5del32, TNFA-308, IL-10 −1117, MCP1+1543, IL-1β −511 and IL-1RN VNTR, were determined in all newborns and mothers, and related to congenital CMV infection and CMV serological status in mothers.

Results: Significantly decreased frequency of MCP1 +1543 C genotype was found in CMV-seronegative mothers when compared to CMV seropositive mothers (16.67% vs 54.76%, $p = 0.03$). In children with congenital CMV infection a tendency for decreased frequency of TNF-A "high producer" genotype was observed when compared to non-infected newborns (11% vs 37%. $p = 0.1$). There was no association between CCR5del32, IL-10 −1117, IL-1β −511 and IL-1RN VNTR polymorphism and congenital CMV infection, seronegativity in mothers as well as no association between mother-child's cytokine genotype and risk for congenital CMV infection.

Conclusion: Genetic polymorphism in MCP1 +1543 may contribute to CMV seronegativity in mothers and thus indirectly influence the risk for CMV primary infection during pregnancy and virus transmission to the foetus.

O32 Quantitation of human herpesvirus-6 and -7 DNAs in different blood fractions from blood donors

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Objectives: The diagnosis of human herpesvirus-6 (HHV-6) or -7 (HHV-7) infections is based on the quantitation of viral genomic DNA in blood. The aim of our study was to define usual values of HHV-6 and HHV-7 loads in different blood fractions (whole blood [WB], mononuclear cells [PBMCs], polymorphonuclear cells [PMNLs]) of blood donors.

Methods: For 200 blood donors, 10 mL of blood were sampled on EDTA. After putting aside aliquots of WB, PBMCs and PMNLs were separated using Ficoll and dextran gradients, respectively. Nucleic acids were extracted using QIAamp® DNA blood mini kit. Quantitation of HHV-6 and HHV-7 DNAs was carried out using real-time PCR assays and viral loads were expressed as the number of viral genomic equivalent copies per million of cells (EqCop/M).

Results: HHV-6 DNA was detected in 8% of WB, 10.5% of PMNLs and 16.5% of PBMCs samples, whereas HHV-7 DNA was detected 51.5% of WB and PMNLs and 62% of PBMCs samples. Median loads were 81 EqCop/M in WB, 34.5 EqCop/M in PMNLs and 62 EqCop/M in PBMCs for HHV-6, and 129 EqCop/M in WB, 62 EqCop/M in PMNLs and 225 EqCop/M in PBMCs for HHV-7. One subject had chromosomally integrated HHV-6 with high viral loads: 2.23×10^6 EqCop/M in WB, 2.55×10^6 EqCop/M in PMNLs, 2.84×10^6 EqCop/M in PBMCs, and 3.21×10^6 in plasma.

Conclusion: These results confirm that PBMCs are the main compartment for HHV-6 and -7 in blood, the presence of viral genome in PMNL probably corresponding to phagocytosis phenomenon. These results allow us to define usual median viral load values in WB, 100 EqCop/M for HHV-6, 150 EqCop/M for HHV-7, commonly encountered in immunocompetent patients who control the viral infection. Finally, the prevalence of chromosomally integrated HHV-6 in France is 0.5%.

O33 Simultaneous detection of influenza A and its subtypes (H1, H3, 2009 H1N1), influenza B, and RSV A and B in respiratory specimens on an automated, random access, molecular platform

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Introduction: Multiplexed molecular detection of influenza A and B and RSV is becoming increasingly available with a number of commercial assays on the market. However, there are no 'sample-to-result' platforms that detect these viruses along with influenza A and RSV subtypes. Numerous studies have demonstrated increased sensitivity of these techniques compared to culture and antigen detection methods. This study evaluated the sensitivity and specificity of Nanosphere's Verigene System, with the Processor SP, for the detection of these respiratory viruses.

Methods: Nasopharyngeal specimens were collected at multiple sites during the 2008–9 and 2009–10 respiratory seasons. The presence of influenza A/B and RSV was determined using culture/DFA. Influenza A and RSV subtypes were determined using bi-directional sequencing. Discrepant results were resolved using bi-directional sequencing. The culture/DFA and bi-directional sequencing results for the samples were blinded to the investigators until the conclusion of the study.

Results: Of the 323 specimens enrolled in the clinical trial, 121 were culture/DFA positive for Influenza A, 14 were positive for influenza B,

33 were positive for RSV, and 155 specimens were culture/DFA negative. In comparison to culture/DFA for influenza A, the RV+ assay was 100% sensitive and 97.0% specific. For influenza B, the RV+ assay was 100% sensitive and 100% specific. For RSV, the RV+ assay was 97.0% sensitive and 99.3% specific. There were 9 discrepant specimens between RV+ and culture/DFA. Bidirectional sequencing revealed that the RV+ assay resulted in 8 additional true positives which were otherwise negative by culture/DFA, and 1 additional true negative which was resulted as positive by culture/DFA. All positive influenza A samples were subtyped as 2009 H1N1 by sequencing (n = 127). The RV+ assay performance for this subtype was 99.2% sensitivity and 100% specificity. For the H1 and H3 subtypes, the specificities ranged from 99.7–100%. For the RSV A and B subtypes, the sensitivities and specificities were 100% and 100%, respectively, for both subtypes.

Conclusion: The RV+ assay for detection and subtyping of respiratory viruses offers a rapid result time of ~2.5 hours with sensitivity and specificity equal to or greater than traditional culture-based detection methods. Additionally, the fully automated platform allows simple, on-demand assay set-up which permits workflow flexibility and requires minimal hands on time.

O34 Detection of drug-resistant hepatitis B virus mutant strains using ultradeep pyrosequencing in patients with chronic hepatitis B

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Objectives: Current methods of detecting hepatitis B virus (HBV) drug-resistance mutations are mainly direct sequencing and reverse hybridization, but haplotype assessment is not allowed by both methods. Therefore mutations detected cannot be confirmed whether they exist in the same virus strain. Massively parallel ultradeep pyrosequencing (UDPS) by Roche GS-FLX 454 Genome Sequencer was used to detect drug-resistance mutations in patients with chronic hepatitis B. We developed a sequence analysis program exclusive for analyzing haplotypes of HBV drug-resistance mutations.

Methods: Samples from 30 chronic hepatitis B patients were obtained; 25 antiviral drug-treated and 5 drug-naïve. HBV DNA titers were greater than 1.0×10^4 IU/mL in all samples. 8 primer pairs were used to obtain partially overlapping amplicons covering most of the HBV genome. Frequencies of drug-resistance mutations were estimated using the sequence analysis program and the results were compared with that of direct sequencing. Haplotypes of drug-resistance mutations in each sample were identified and their clinical relevance was investigated.

Results: A total of 369,603 clonally amplified fragments (reads) were obtained from UDPS on 30 samples and the average number of reads covering drug-resistance mutation region per sample was 1735 (451–4526). Almost whole genome of HBV except between nt1582-nt1736 was sequenced. UDPS detected additional drug-resistance mutations that were not detected by direct sequencing in 19 samples which frequencies were between 1.1% and 23.8%. Of the 25 patients with history of entecavir treatment, 20 patients were found to have haplotypes of entecavir-resistance mutations. Haplotypes of entecavir-resistance mutations were identified in all patients who experienced virologic breakthrough during entecavir treatment or who had reduced sensitivity to entecavir treatment.

Conclusion: Drug-resistance mutation testing in chronic hepatitis B patients by UDPS was sensitive and accurate, detecting mutations with frequency as low as 1.1% and estimating the mutation frequency by analyzing an average of 1735 reads per sample. Identification of haplotypes of HBV drug-resistance mutations using the sequence analysis program developed in this study will be useful in interpreting virologic breakthrough and reduced sensitivity to treatment.

O35 Real-time Epstein-Barr virus PCR for the diagnosis of primary EBV infection

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Objectives: Specific viral laboratory diagnosis of primary Epstein-Barr Virus (EBV) infection is usually based on antibody-detection assays. Nonetheless, despite the availability of several different serological markers EBV status of some patients is not easily resolved. Thus EBV PCR has been added as a diagnostic tool. The aim of the present study was to investigate the diagnostic utility of EBV DNA detection in primary EBV infection in comparison with serological diagnosis and to determine the plasma level of EBV DNA.

Methods: Sera from patients referred for suspected primary EBV infection, were tested for heterophile antibodies (Monospot test), IgM antibodies against viral capsid antigen (VCA IgM) and IgG against nuclear antigen (EBNA IgG). A quantitative real time EBV PCR assay (Light Cycler EBV Quant kit) was simultaneously performed in plasma of 118 patients, aged 1–47 years, with negative IgG antibobies for EBNA. In eighteen EBV DNA positive patients follow-up samples in the course of disease were obtained and investigated.

Results: Forty three out of 46 patients (93.5%) with serologically confirmed primary infection as shown by the presence of VCA IgM and heterophile antibody, were EBV DNA positive and the measured viral loads ranged from 4.56×10^2 to 7.6×10^4 copies/ml. The three samples found negative by PCR were collected 13 to 15 days after onset of symptoms. By performing real time RCR in the remaining 72 samples, twenty four extra cases with viral load ranging of 2.05×10^2 to 3.0×10^4 copies/ml were diagnosed: in 20 of them, mainly children, with detectable VCA IgM, heterophilic antibodies could not be detected and in the other four, VCA IgM antibodies were absent. EBV DNA load was detectable in all samples withdrawn until day 12 after onset of symptoms. Higher viral load levels were detected in younger patients.

Conclusion: According to our results, use of EBV PCR assay resulted in an increase in definitive diagnosis of primary EBV infection, enhanced overall diagnostic efficacy by 20.3%. Real time PCR is a reliable tool for diagnosis of primary EBV infection early in the course of disease and may especially serve as a useful diagnostic supplement in serologically unclear cases of EBV infection.

O36 Nucleotide variation of Dengue virus serotype 2 during viral persistence after acute infection in adults

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Objectives: Nucleotide variations during long-term virus infection are reported in flavivirus infections: hepatitis C and West Nile viruses. Dengue is also a flavivirus, regarded as the most important and widespread arthropod-borne disease. In recent ECCMIDs, our group's oral presentations demonstrated extended presence, after acute infection, of live and culturable dengue virus in several specimens including urine. Here we performed a pilot study to see if nucleotide changes were present during the time frame.

Methods: Blood (plasma and peripheral blood mononuclear cells (PBMCs)) and non-blood (urine, saliva and buccal cells) samples were collected during acute, convalescent and late convalescent periods from 5 adult patients infected with dengue serotype 2. Reverse transcription (RT) nested-PCR using primers specific to dengue envelope (E) gene, per a published protocol, was performed using RNA from the processed samples. The expected PCR products of 434 bps were purified and sequenced. Nucleotide sequences were translated into amino acid sequences using ExPASy tools. Both nucleotide and amino acid sequence variations were analyzed by CLUSTALX2.

Results: RT nested-PCR could detect dengue virus during acute, convalescent and late convalescent periods of infection in certain samples. The virus was detected in PBMCs as late as 21 and 27 days

after the onset of illness, respectively. In the other 3 patients, the virus was detected in urine as late as 14, 15, and 23 days after onset of illness, respectively. Nucleotide sequence variations were present in late convalescent PBMCs samples of both patients when compared with their own sequences in acute and convalescent samples. The differences are at 1.80 and 0.77 percents, respectively. However, the sequences amplified from urine samples of the other 3 patients did not show variation among the periods of infection. There were no amino acid changes in all 5 patients when compared among different periods of infection.

Conclusion: Survival of dengue virus in different cell types may be correlated with genetic variations. PBMCs is one of the principal sites for dengue replication. The viruses may actively replicate in such compartment which is highly exposed to and receives an impact from immune pressure. This may contribute to more diversity in dengue virus population. Future studies are warranted to delineate such phenomena.

O37 Genetic diversity of Crimean-Congo haemorrhagic fever viruses in Turkey

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Objectives: The aim of this study was to investigate the genetic diversity of Crimean-Congo hemorrhagic fever viruses (CCHFVs) by exploiting both partial S and M RNA segments. In particular, a part of the Gn region of M segment RNA that was subjected in our study harbours virus neutralising epitopes and it is also important for involving in virus pathogenecity. The analysis of Gn region of M segment would lead us the determine the critical nucleotide and aminoacid variations among virus isolates that could also facilitate the appropriate selection of vaccine candidate virus.

Methods: The present study was focused mainly in CCHFVs isolated from a total of 48 patients sera from the several provinces within the Kelkit Valley of Turkey between 2009 and 2010 years. A one step RT-PCR was used to amplify a 536 bp region of partial 'S' and a 890 bp region of partial M segments of CCHFV isolates that were then subjected for sequencing. The phylogenetic analysis were generated using the Neighbor-joining (NJ) method. Phylogenetic analyses were conducted in MEGA4.

Results: The phylogenetic analysis based on both partial 'S' and M segments revealed that the distribution of CCHFVs on phylogenetic trees reflected their continental origin and all CCHFV isolates subjected for this study were shown to place within the European lineage (Europa I). In addition, It was observed from the both S and M segments analysis that CCHFVs circulating in Turkey were classified in two main clusters (I and II) that together with representatives of viruses including eastern European viral strains (Drostov, Kashmanow) and western European viruses (Hoti, Kosovo). In addition, the nucleotide variations observed in Gn region of CCHFV isolates resulted in several amino acid variations between 638 and 763 amino acids.

Conclusions: The phylogenetic analysis of partial S and M segments of CCHFV isolates derived from infected humans have updated and the detailed the genetic status of viruses circulating in Turkey. Our results highlights the genetic stability of CCHFVs circulating in Turkey. This could be an advantage for selecting appropriate vaccine strain and enhance the success for the protective efficacy of vaccines under development. In addition, we could also suggest that CCHFVs are tend to be region or continent specific.

O38 Characterisation of HIV-1 genetic diversity in established and recent infections among intravenous drug users from Lisbon, Portugal

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Objectives: The increasing number of new human immunodeficiency virus type 1 (HIV-1) genetic forms has drawn attention to their potential differences in transmissibility and virulence, and has raised questions concerning the possible impact of molecular changes in epidemiology and spread of infection. The aim of this study was to characterize the genetic diversity of HIV-1 strains in circulation among intravenous drug users (IDU) who have acquired the infection in two specific periods of time: one group before 1998 (Established Infections, EI), and the other group between 2004 and 2006 (Recent Infections, RI).

Methods: Blood samples were collected from 46 HIV-1-infected IDU living in the Lisbon area, Portugal, including 25 cases of EI and 21 cases of RI. Viral sequences were amplified by nested PCR from proviral DNA and four genomic regions were characterized: env C2V3C3 and gp41 ectodomain, partial gag and full-length nef genes. The assignment of a viral subtype was achieved by phylogenetic inference and bootscanning analysis. Putative amino acid sequences were determined and the conservation/disruption of functional domains in Env and Nef target regions was analyzed.

Results: Combined genetic data analysis showed a significant difference between groups ($p < 0{,}001$). A high proportion of subtype B strains (60%, $n = 15$) was found in EI group, while the majority of RI genetic forms was identified as non-B subtypes (90%, $n = 19$), namely, subtype A, recombinant form AG and the mosaic genomes structures A/BJ/G/CRF02_AG/CRF14_BG, B/G/CRF14_BG, and A/G/CRF02_AG/CRF14_BG. Subtype G and BG recombinant forms were present in both groups.

The analysis of Env and Nef functional motifs revealed a high degree of conservation and no significant differences were found between both groups. However, the presence of several signatures associated with B and non-B subtypes was observed, including specific polymorphisms for subtype G, AG and BG viral forms.

Conclusions: The genetic analysis of HIV-1 strains revealed a significant change in the molecular epidemiology of infection, regarding B and non-B subtypes, with the introduction of new viral forms in this IDU population between 1998 and 2004. HIV-1 genetic diversity and the differences between the viral subtypes require further investigation in order to evaluate the potential implications in treatment and progression of infection.

O39 New microfluidic platforms for the simultaneous detection of pathogens using TaqMan® probe-based real-time PCR

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Over the past decade, nucleic acid amplification tests have been developed for a broad range of pathogens and increasingly replaced traditional methods. The key drivers for this shift of technology are the high sensitivity of TaqMan® probe based Real-time PCR Assays and also the ability to multiplex several assays in one reaction vessel. Here we introduce microfluidic tools (branded TaqMan® Array Cards) that allow the simultaneous detection of up to 384 pathogens in one sample. The validation of one application of this technology, the "TaqMan Array Human Viral Screening Card", will be documented in this study. We first tested a panel of 'real-time' quantitative PCR (qPCR) reagents for their specificity and sensitivity using 96well plates. Single tube TaqMan® Assays were designed against conserved regions of BKV, JCV, HIV-1, HIV-2, HTLV-I, HTLV-II, HCV, HBV, HPV-16, SV40, HHV7, CMV, Adenovirus C, HSV-1, HSV-2 and HHV4 genomes.

All assays successfully and specifically detected their respective synthetic target sequence oligonucleotide ("artificial template") down to the single copy level. Sensitivity and specificity were also tested in dilution series of nucleic acid extracted from cell cultures infected with those viruses. In summary, all tested assays showed excellent linearity and efficiency with a very high degree of specificity for their targets. No cross-reactivity was detected between assays of viruses belonging to the same family.

TaqMan Assays for 15 pathogen targets plus one internal positive control are pre-loaded on the TaqMan Array Human Viral Screening Cards allowing the simultaneous assessment of these targets in triplicate amplifications from 8 samples in parallel. We tested sensitivity and specificity using both artificial templates and nucleic acid extracted from cell cultures infected with those viruses on these cards. Again,

all TaqMan Assays showed excellent linearity and efficiency and a very high degree of specificity for their targets, even in such a small reaction volume (2 µl). In conclusion, the TaqMan Array Human Viral Screening Cards will be used as invaluable tools in ensuring that for example human ES cell cultures, particularly from novel sources, are pathogen-free. Moreover, additional TaqMan Array Cards for the screening & detection of viruses, bacteria and parasites are currently under development and will be made available in the coming year.

Hospital antibiotic use: causing or coping with multi-drug resistance?

O40 Clinical impact of unsolicited post-prescription antibiotic review in surgical and medical wards: a randomised controlled trial

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Objectives: To describe the clinical impact of an early review of antibiotic prescriptions in hospital using unsolicited infectious disease physician counselling (IDPC).
Methods: Patients hospitalized in surgical or medical wards and receiving any of 15 selected antibiotics were randomized between day 3 and 5 to a control group (C), where IDPC was provided only when requested by ward physicians, and an intervention (I) group, where all prescriptions were systematically evaluated by the IDP. Improved antibiotic use was sought by withdrawing or de-escalating therapy, promoting oral switch or reducing duration of therapy whenever appropriate. Clinical outcomes of patients were compared between the 2 groups using the following criteria: hospital length of stay (HLOS), in hospital mortality or at 60 days after randomization, intensive care admission (ICU) within 7 days after randomization, new course of antibiotic therapy 7 days after completing the first course and rate of readmission with antibiotic therapy within 60 days after randomization.
Results: 753 patients were included (C = 377, I = 376), of which 32.4% had hospital-acquired infection. Microbiological documentation of infection was obtained for 46.7%. Solicited IDP advices were requested for only 11.9% of patients in the (C) group; while 83.8% of antibiotic prescriptions in the (I) group prompted IDPC, including shortening the duration of therapy (27.4%) stopping (26.1%) or de-escalating (25.0%) therapy and encouraging an oral switch (15.2%), or other (17.1%) recommendation (e.g., changing the dosing regimen or increasing the duration). Most (83.2%) IDP recommendations were adhered to by physicians. The overall duration (median; [interquartile range]) of therapy was lower in the (I) group than in the (C) group (7 [5–10] vs. 8 [6–11] days; $p < 0.001$). There was no difference in clinical outcomes of patients between the (I) and (C) groups, whether in HLOS (15 [9–25] vs. 15 [9–27] days; $p = 0.95$), or rates of ICU admission (1.9% vs. 1.6%; $p = 0.78$), new antibiotic courses (4.5% vs. 6.6%; $p = 0.21$), readmission with antibiotics (7.7% vs. 10.6%; $p = 0.34$), and deaths in the hospital (6.9% vs. 6.6%; $p = 0.88$) or at 60 days (9.8% vs. 10.1%; $p = 0.91$).
Conclusions: Unsolicited antibiotic review of antibiotic prescriptions by IDP was associated with high rates of counselling and physicians' compliance, resulting in shorter courses of antibiotic therapy, with no evidence of a detrimental effect on clinical outcomes of patients.

O41 Amikacin and tobramycin for *Pseudomonas aeruginosa* and *Acinetobacter baumannii* complex according to PK/PD and microbiological breakpoints: is there a difference to revise antibiotic policies?

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Objectives: To compare amikacin (AMK) and tobramycin (TOB) susceptibility against *Pseudomonas aeruginosa* (PA) and *Acinetobacter baumannii* complex (ACN) considering the microbiological and pharmacokinetics/pharmacodynamics (PK/PD) breakpoints (BP).
Methods: Consecutive non duplicate PA and ACN isolates collected from a teaching hospital, after the species identification by Vitek® (BioMerieux), were tested by Kirby-Bauer method for AMK and TOB using disk diffusion and e-test for MIC determination according to CLSI 2009. Only the susceptible bacteria detected by disk diffusion had MIC determined by e-test. Susceptibility rates were recalculated by proposed PK/PD BP of 4 µg/mL for AMK and <2 µg/mL for TOB.
Results: A total of 237 bacteria were obtained from September 2008 to May 2010, including 164 PA isolates from 64 blood cultures, 66 respiratory tract secretions and 34 urine, and 73 ACN isolates from 37 blood cultures, 27 respiratory tract secretions, 8 urine and 1 peritoneal fluid were tested. The great majority of samples was obtained after 48h of patient admission. PA was susceptible to AMK in 89% and to TOB in 84% ($p = 0.1$), while ACN was susceptible to AMK in 70% and to TOB in 63% ($p = 0.4$) by disk diffusion method. However, analyzing the data with lower BP showed an opposite trend, PA with MIC <4 µg/mL for AMK and MIC <2 µg/mL for TOB were 57% and 76% susceptible ($p = 0.05$), respectively; as ACN were only 40% and 46% susceptible ($p = 0.4$), respectively. When MIC distributions were analyzed, the modal MIC of TOB was 1 µg/mL for both species and for AMK were 3–4 µg/mL and 3 µg/mL for PA and ACN, respectively. PA MIC_{90} was 2 µg/mL for TOB and 8 µg/mL for AMK.
Conclusion: The decrease of susceptibility was greater to amikacin when lower breakpoints were applied and MIC distributions analyzed. Nonetheless, recommendations of higher doses of AMK (25–30mg/kg/day) or TOB (7mg/kg/day) use based on PA MIC_{90} should be updated in this institution. Current BP (CLSI and EUCAST) for aminoglycosides seem to be overvalued when compared to more realistic PK/PD BP (TOB 1–2 µg/mL and AMK 2–4 µg/mL). To indicate the best choice of drug and dosing for the empiric therapy of PA and ACN infections that would provide adequate drug exposure and enhanced bacterial killing, MIC distributions interpretation and new reliable breakpoints for AMK and TOB should be available.

O42 The ECDC pilot Point-Prevalence Survey of healthcare-associated infections and antimicrobial use: antimicrobial use analysis

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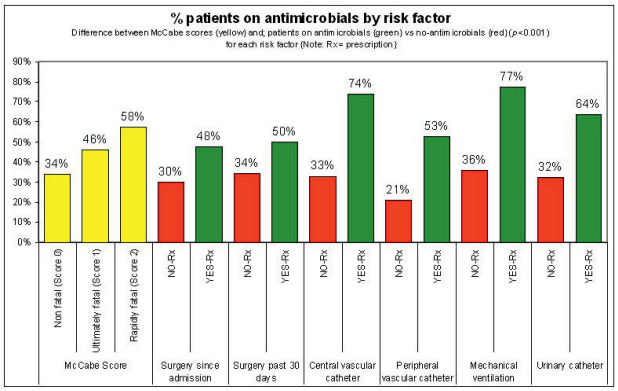
Introduction: The ECDC pilot Point Prevalence Survey of healthcare associated infections (HAI) and antimicrobial use (AU) in European acute care hospitals (ECDC-pPPS) was coordinated by ECDC and outsourced to a consortium led by the University of Antwerp (UA) in collaboration with the French Institute for Public Health Surveillance (InVS) and the Belgian Scientific Institute of Public Health (WIV-ISP).
Objectives: The objectives of the ECDC-pPPS were to test and finalize a protocol to describe and estimate the prevalence of HAI and AU in participating hospitals stratified by patients' characteristics or invasive procedures and to provide a standardized tool for hospitals to identify targets for quality improvement.
Methods: All patients on ward at 8:00am and not discharged at the time of survey were included. Each department was surveyed in one day. A patient-based (PB) and a unit-based (UB) protocol were provided. The UB protocol aggregated denominator data at ward level without information on invasive procedures or severity. Most participants used a web-based tool provided by UA for data collection and validation. This study analyzed AU by McCabe score and invasive procedures (vascular catheter, urinary catheter, mechanical ventilation (MV) or surgery) as collected in the PB protocol.
Results: The prevalence of AU in the 66 participating hospitals was 35%. Patient specialty significantly correlated to proportion of treated patients: intensive care [61%]; surgery [41%]; medicine [39%]; paediatrics [30%] ($p < 0.001$). Penicillins and enzyme inhibitor (J01CR) was the most used

class overall (18%), in treatment intent of community infections (20%) and hospital infections (18%).
Fifty hospitals used the PB protocol and included 14 329 patients, 5 201 of whom received 7 359 antimicrobials. Patients with an invasive procedure or with high McCabe score were prescribed more antimicrobials ($p < 0.001$ see figure). With increasing McCabe score, the top 5 drugs cumulative proportion decreased (42, 33 and 29%) while wide-spectrum drugs ranked higher (e.g., meropenem [5th in McCabe score 3]).
Conclusion: The PB protocol can help future surveys in analyzing trends in AU since risk factors collected can be used to adjust comparisons between hospitals with similar case-mix, helping local policy makers in identifying targets for quality improvement.



O43 Initial antibiotic treatment patterns and failure rates among hospitalised patients with complicated skin and skin structure infections: a 4-year review within an integrated US healthcare system

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Objectives: In 2005, the Infectious Diseases Society of America published practice guidelines for the treatment of complicated skin and skin structure infection (cSSSI). Changes in treatment patterns and outcomes for hospitalised patients with cSSSI following implementation of these guidelines are not well documented. This study assessed changes in empiric cSSSI therapy and the associated outcomes following publication of these guidelines.
Methods: The study was conducted at Saint Barnabas Health Care System, a 3500-bed US healthcare system with 6 acute-care facilities. Trendstar clinical billing and Cerner laboratory databases were used to identify patients hospitalised for cSSSI and stratify them into 3 infection cohorts: acute, chronic/ulcerative, and surgical-site infections. Data were collected on patients admitted between 1/1/2006 and 12/31/2009 who received parenteral antibiotic therapy for $\geqslant$48 hours beginning within 24 hours of admission. We studied patterns of initial (ie, within 24 hours of admission) antibiotic therapy and failure rates for each treatment. Failure was defined as receipt of alternate antibiotic therapy providing broader/different antimicrobial coverage not initiated within the first 24 hours of admission, drainage/debridement/amputation >72 hours from admission, or transfer to an ICU.
Results: A total of 8162 patients (6254 acute, 615 chronic/ulcerative, 1293 surgical-site) were included in this analysis. From 2006 to 2009, the top 5 regimens used empirically as monotherapy or part of a multidrug regimen changed accordingly: vancomycin from 17.9% to 50.2%, levofloxacin from 21.4% to 31.8%, piperacillin/tazobactam from 17% to 34.8%, ampicillin/sulbactam from 17.2% to 19.5%, and cefazolin from 27.7% to 26.9%.
From 2006 to 2009, failure rates for each of the regimens increased: vancomycin from 10.5% to 15.7%, levofloxacin from 10.1% to 16.0%, piperacillin/tazobactam from 9.4% to 13.2%, ampicillin/sulbactam from 5.8% to 10.1%, and cefazolin from 13.0% to 25.1%. Overall failure rates for all regimens increased significantly (chi-squared tests; $p < 0.01$).
Conclusion: Following implementation of the 2005 practice guidelines, use of top regimens (containing vancomycin, levofloxacin, piperacillin/tazobactam, ampicillin/sulbactam) increased considerably. Failure rates associated with all regimens rose significantly. These results underscore the need for new treatment alternatives to minimize the emergence of drug-resistant bacteria.

O44 Development of national prescribing indicators for antimicrobials to support reduction in *Clostridium difficile* infection

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Objective: The Scottish Antimicrobial Prescribing Group (SAPG) is a national multidisciplinary clinical forum formed to implement national improvement initiatives via a network of NHS Board Antimicrobial Management Teams (AMTs).
Reduction of *Clostridium difficile* infection (CDI) is a priority in Scotland and there is a national target of 50% reduction by March 2011. SAPG were asked to develop measures related to antibiotic use to support achievement of this target.
Method: A. Development of prescribing indicators. SAPG had issued guidance to AMTs in July 2008 advising that antibiotics associated with high risk of CDI should be restricted within local antibiotic policies and this was used as the basis for development of prescribing indicators. Following consultation with SAPG members and AMTs, three indicators were agreed and announced in Chief Executive Letter from Scottish Government in April 2009. Indicators and targets:
1. Indication recorded in notes and empirical antibiotic choice compliant with local policy. Target 95% Compliance
2. Duration of surgical prophylaxis <24 hours and compliant with local policy.
   Target 95% Compliance
3. Seasonal variation in quinolone use in winter months compared with summer months is <5%.

B. Data management. The Institute of Healthcare Improvement methodology and data management system were chosen for the hospital prescribing indictors and the primary care indicator was developed as a standard report within the Prescribing Information System for Scotland.
C. Reporting. SAPG produces reports on national compliance with the hospital indicators 3-monthly and for the primary care indicator annually. AMTs can access their own 'real time' NHS Board data.
Results: 1. In acute admission units, national compliance with empirical prescribing policy is currently 78% and compliance with indication documented in notes is 91%.
2. In a sample of surgical specialties national compliance with surgical prophylaxis policy is currently 95% and duration <24 hours is 100%.
3. Primary care seasonal variation of quinolones – 9 out of 14 NHS Boards achieved the target of <5% for winter 2009–10 compared with summer 2009.
Conclusion: National engagement and broad consultation has allowed development of national prescribing indicators to support reduction in CDI.
Use of quality improvement methodology and regular feedback of results to prescribers has driven increasing compliance with the indicators.

O45 Nephrotoxicity of continuous infusion of vancomycin in critically ill patients

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Objective: To determine the incidence and associated risk factors of nephrotoxicity during continuous vancomycin treatment in critically ill patients with Gram-positive pneumonia and/or bacteraemia.
Methods: Retrospective, observational, two-centre, cohort study. Patients with microbiologically documented Gram-positive pneumonia and/or bacteraemia and normal baseline renal function were included. Vancomycin was given as a 15mg/kg loading dose, followed by a

continuous infusion of 30mg/kg/day. Vancomycin dose was adjusted daily aiming at plateau concentrations of 15–25mg/L. Nephrotoxicity was defined according to the Acute Kidney Injury Network (AKI) classification as an increase in serum creatinine of 0.3 mg/dL or a 1.5 to 2 times increase from baseline on at least two consecutive days following initiation of vancomycin.

Results: 129 patients were studied of whom 38 (29.5%) developed AKI. Patients with AKI had a higher lean body weight (77.3±15.0 vs. 70.5±15.2 kg; $p = 0.02$), had more diabetes (79% vs. 54%; $p = 0.01$) and vasopressor need (87% vs. 59%; $p = 0.002$) and received vancomycin for a longer time period (14.9±9.8 vs. 9.2±4.9 days; $p = 0.05$). Independent variables contributing to nephrotoxicity were, in order of importance, serum vancomycin levels, body weight, and SAPS 3 score. The incidence of nephrotoxicity rose substantially when vancomycin levels exceeded the target range. (<25mg/L (n = 3) vs. 25–30mg/L (n = 9); odds ratio 9.75; confidence interval 2.41–39.52; $p < 0.0001$ and <30mg/L (n = 12) vs. >30mg/L (n = 26); odds ratio 30.69; confidence interval 10.49–89.83; $p < 0.0001$).

Conclusion: Vancomycin concentrations exceeding 25mg/L during continuous infusion are associated with increased nephrotoxicity. Nephrotoxicity is more often seen in conditions that cause either acute (shock) or chronic (diabetes) kidney injury. This study challenges the concept that continuous vancomycin infusion might better reconcile higher therapeutic efficacy with less nephrotoxicity.

O46 Continuous versus intermittent infusion of vancomycin: the eternal diatribe

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Objectives: International guidelines recommend the administration of vancomycin by intermittent infusion (InI). Nevertheless, many clinicians are used to administer it by continuous infusion (CI). Published studies comparing the effectiveness and adverse effects of CI versus InI showed inconclusive results. The main aim of this systematic review was to summarise available evidence on the effect of CI of vancomycin compared to the InI in patients with infections due to Gram-positive bacteria.

Methods: MEDLINE and Cochrane databases were searched to identify published studies (1956-November 2010) that compared the effect of CI and InI of vancomycin on mortality, clinical cure, toxicity rates and mean vancomycin serum concentration. Systematic review was conducted combining and analyzing the relative risk (RR) and computing a summary RR of the effects with 95% confidence interval (CI). The standardised mean difference (SDM) was calculated for continuous outcomes. The I2 test was calculated to assess heterogeneity across studies (significant value considered for I2 > 50%). Included studies were appraised for methodological quality independently by two authors (RCT for adequacy of randomization, allocation concealment, blinding, follow-up, and use of intention to treat analyses; observational studies through the Newcastle-Ottawa scale).

Results: One RCT and 5 observational studies were included in the analysis with a total study population of 443 patients. The quality of included studies was fairly good. Compared with InI, CI of vancomycin significantly reduced the risk of nephrotoxicity (RR 0.6, 95% CI 0.4–0.9, P = 0.02; I2 = 0) and was associated with a significantly higher vancomycin serum concentration (SDM 1.07, 95% CI 0.5–1.6, $P < 0.001$; I2 = 83%). Overall mortality was not different in the two groups (RR 1.03, 95% CI 0.7–1.6, P = 0.9; I2 = 0). The effect of CI on clinical cure and adverse effects rates was not assessed through a statistical approach due to the lack of data. The Begg's funnel plot and the Egger test indicated no evidence of publication bias.

Conclusion: Our meta-analysis suggests that continuous vancomycin infusion allows to reach an higher serum vancomycin concentration with significantly lower risk of nephrotoxicity. Evidence of a benefit on clinical success rates and mortality needs to be further explored through RCT with adequate sample size, standardized methods of vancomycin concentration measures and reliable definitions of outcomes.

O47 Are surgeons the only common denominator between *Clostridium difficile* infections, surgical site infections and poor compliance to antibiotic prophylaxis in surgery guidance?

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Background: Surgical site infections are associated with significant complications and an increase in mortality and length of stay. Literature and guidelines suggest optimal prophylactic antibiotics decrease the risk of post operative infections. Blackpool Victoria Hospital operates a successful *Clostridium difficile* infections (CDI) programme with high emphasis on antibiotic stewardship and root cause analysis[RCA] of CDIs. Inappropriate use of antibiotics has emerged as the key risk factor undermining the program. We present findings from a comprehensive multidisciplinary audit of compliance with trust antibiotic prophylaxis in surgery guidelines – conducted by trainee doctors; associated key findings from CDI-RCAs [joint microbiologist-infection control team database] and remedial actions.

Methods: Prospective case review of 182 adult surgical in-patient prescriptions including cardio-thoracic, general surgery, obstetrics & gynaecology, orthopaedics, urology and vascular cases between September to November 2010.

Review of CDI root cause analysis database.

Results: Key findings from 182 surgical case prescriptions: Mean age is 58y with 41% (74/182) females. 76% (107/132) of cases had antibiotics prescribed appropriately. Compliance markedly varied (37–100%) between specialities. The trust guidelines provide prophylactic antibiotic recommendations in only 77% (139/182) of procedures. Antibiotic prophylaxis guidance for cardiac and thoracic surgery is absent. Antibiotic at induction time was appropriate in 29% (53/182). Missing documentation in 40% (72/182); 11% (16/142) received post-operative antibiotic treatment. 13% (18/141) cases had documented antibiotic allergies.

CDI-RCAs: 113 cases of CDI were analysed. Inappropriate antibiotic prescribing and non compliance to trust antibiotic guidance was 57%[64/113] each. Key findings – delayed sampling; prolonged/repeated courses; poor documentation.

Discussion: Poor compliance to trust antibiotic guidelines, poor documentation, gaps in trust antibiotic guidance, inappropriate use of high risk antibiotics [co-amoxiclav, cephalosporins] are key findings from this audit and CDI-RCAs. These were used to inform action plan and strategy to address the CDI and HAI program. Some key steps include – revised antibiotic guidance, joint ward rounds with microbiologists, joint pharmacy-microbiologist antibiotic compliance audits, mandatory infection prevention road shows, raising awareness teaching programme, posters, performance management indicators, etc.

O48 Procalcitonin-guided antibiotic stewardship in lower respiratory tract infections. A real-life international multicentre quality surveillance (ProREAL)

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Objectives: Procalcitonin (PCT) safely and effectively reduced antibiotic use in patients with lower respiratory tract infections (LRTIs) in controlled study conditions, yet experience outside of study conditions is limited. We report the results of an observational quality surveillance including centers in Switzerland (n = 10), France (n = 3) and the USA (n = 1).
Methods: Consecutive adults with clinical suspicion of LRTI presenting to the emergency departments or outpatient offices were enrolled and registered in a central website, which provided antibiotic treatment guidelines according to a previously published PCT-guided algorithm. PCT was measured at the local sites using sensitive assays (Kryptor®, BRAHMS or Vidas®, BioMerieux). The primary endpoint was duration of antibiotic therapy.
Results: Of 1562 enrolled patients (mean age 67.8±18.5y; 44.4% female), 1391 (89.1%) had an LRTI (community-acquired pneumonia (CAP) 62.3%, acute exacerbation of chronic obstructive pulmonary disease (AECOPD) 20.4%, acute bronchitis 16.0%). Algorithm compliance overall was 69.0% with significant differences between diagnoses (bronchitis 80.6%, AECOPD 71.8%, CAP 62.6%; $p < 0.001$) and between outpatients (65.7%) and inpatients (86.6%; $p < 0.001$). Algorithm-experienced centers had higher compliance (82.5%) than algorithm-naïve centers (57.9%; $p < 0.001$). Geographical differences were detected with high compliance in the Swiss centers (75.8%), intermediate compliance in the French centers (66.0%) and low compliance in the US center (33.2%; $p < 0.001$). On admission, the most common pre-specified criteria for prescribing antibiotics despite low PCT were high clinical severity (19.3%) and respiratory instability (11.5%); no pre-specified reason was given in 58.0%. Mean antibiotic duration was 6.3±5.3 days with significant differences between the diagnoses (bronchitis: 2.8d, AECOPD: 3.5d, CAP: 8.2d; p for each comparison $\leqslant 0.001$). In a Cox proportional hazards model, non-compliance with the algorithm, inpatient treatment, diagnosis of CAP (vs bronchitis), and being algorithm-naïve were independently associated with longer antibiotic courses.
Conclusion: Cultural differences in antibiotic prescribing apparently affect compliance with antibiotic stewardship efforts. In general, compliance with the algorithm is feasible outside study conditions but has to be reinforced to achieve maximum benefit in reducing antibiotic use.

O49 Hospital antimicrobial consumption pattern in the era of multidrug-resistant bacteria

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Objectives: To study antimicrobial (ABC) consumption pattern in a general tertiary care hospital with high level of resistance mainly among Gram-negative bacteria.
Methods: Annual study of ABC consumption rates in 15 adult hospital departments (7 internal medicine and subspecialties, 7 surgical and subspecialties including solid organ transplant unit and mixed ICU) from 2005 to 2009. Antibiotic consumption calculator version 3.1 (WHO) was used and annual ABC consumptions were expressed as DDDs/100 bed-days (DDDHBD).
Results: Mean ABC consumption of different hospital departments increased from 120 DDDHBD in 2005 to 136 DDDHBD in 2009 ($p = 0.02$). Annual ABC pattern analysis at hospital level (all departments) showed that penicillins were the most commonly used ABC (33% of total ABC consumption), followed by cephalosporins (19%), fluoroquinolones (11%), colistin (7.7%) and carbapenems (7.4%). Less commonly used ABC were aminoglycosides (6.8%), macrolides (5%) and glycopeptides (2%).
Among different departments ABC consumption showed approximately the same pattern with the hospital level except for ICU. In ICU colistin was the 2nd most common ABC (mean annual consumption rate 98 DDDHBD with a constant increase from 70 in 2005 to 141 DDDHBD in 2008, $p = 0.009$) followed by carbapenems (mean consumption rate of 73 DDDHBD) and aminoglycosides (mean consumption rate of 39 DDDHBD). In ICU cephalosporin consumption constituted only 3% of total ABC consumption.
Longitudinal ABC analysis at hospital level between 2005 and 2009 demonstrated an increase in mean departmental consumption of fluoroquinolones (from 11 to 16 DDDHBD, $p = 0.004$); colistin (from 7 to 14 DDDHBD, $p = 0.02$); and linezolid (from 1 to 3.5 DDDHBD, $p = 0.008$). During the same period no significant variations were found in annual consumption rates of β-lactams. More specific, annual consumption rates of penicillins ranged between 39 and 42DDDHBDN ($p = 0.16$), and of carbapenems between 8.7 and 9.7 DDDHBD ($p = 0.09$). A non-significant trend of decrease was found in annual consumption rates of cephalosporins (from 27 to 24DDDHBD). Consumptions of glycopeptides and macrolides were relatively low (<7 DDDHBD) and did not significantly change.
Conclusions: During study period an increase of total antimicrobial consumption rate was found at hospital level. Significant consumption increase of non-β lactam antimicrobial classes including polymyxins, fluoroquinolones and oxazolidinones is of great concern.

Infection control without borders

S57 The EuRegio experience of successful cross-border infection control (NL–D)

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In a Europe without healthcare borders, differences in prevalence of multiresistant microorganisms become a crucial burden for patients seeking healthcare across the border. Infection prevention has become a keystone in reducing the spread of hypertransmittable multiresistant microorganisms and in consequence maintaining the effectiveness of antibiotic treatment. In this sense, infection prevention includes by far more than measures to avoid transmission, but also the rational use of antibiotics. Independently from the species involved, it is about facultative pathogenic bacteria of different virulence and different epidemic power that are colonizing patients and spreading in hospitals or the community. Molecular epidemiology showed that most of the MRSA infections are caused by a few epidemic clonal lineages. Although transmission dynamics is not fully understood, today 3 major transmission ways are accepted, reflecting the transmission dynamics of hospital-, community- and livestock-MRSA. As the ha-MRSA seem to follow their carriers, analysis of patient movements showed that MRSA spread inter-institutionally within a healthcare region. This becomes even more evident in border regions, where some MRSA seem not to cross the border other, but ha-MRSA seems not to do so. The difference of MRSA-prevalence between the adjacent regions in Germany and the Netherlands find here one possible explanation. A Dutch–German quality network (www.mrsa-net.eu) was set up in 2005 comprising hospitals, GPs, labs, public health services and patient interest groups. Its main objective is the MRSA prevention in the border area. In 2007, screening of risk patients become part of an external quality assurance for the hospitals supervised by the public health authorities. In parallel, a 12-month MRSA decolonisation-management was introduced for all GPs in order to reduce MRSA carriership in the regional population. Since 2009, the activities are being extended to the whole Dutch–German border region (www.eursafety).
Enhanced screening activity resulted in a higher prevalence of MRSA-carriership. In 2009, 3250 carriers, in 2010, 2080 carriers were reported. At the same time, the number of MRSAB-isolates reported was 45 in 2009 and 23 in 2010, respectively. Looking at the German part of the EUREGIO, the MRSAB-incidence per 100.000 inhabitants was there

in 2010 the lowest (3,4) within all North Rhine-Westphalia (mean 4,8; range 3,4–6,3).
The EUREGIO activities show that the implementation of regional infection control is possible and can be synchronized at the borders.
Since patient mobility across the borders will increase, tomorrow's Europe will face new challenges. Multiresistance will become crucial for the difference of healthcare quality in a Europe without healthcare borders. Preventive microbiology will foster regional and euregional infection prevention throughout Europe.

S58 The WHO experience: worldwide "clean care is safer care"

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Background: The WHO "Clean Care is Safer Care" (CCiSC) programme, launched in October 2005, aims at reducing healthcare-associated infections (HAI) worldwide. To achieve its aim CCiSC have set 3 objectives: (1) to raise global awareness about the importance of HAI; (2) to galvanize political commitment by Member States; (3) to promote best practices at the point of care.
Methods: To achieve these objectives, implemented actions were: (1) networking and collaborations with international experts and stakeholders; scientific publications and communications; educational activities at national and international levels; (2) invitations to Ministries of Health to sign a formal statement as a pledge of their commitment to tackle HAI; (3) development and dissemination of guidelines and technical tools. Promotion of hand hygiene (HH) in healthcare settings as the most effective measure for HAI prevention has been the cornerstone of CCiSC technical work. The feasibility of the WHO Guidelines on HH and the effectiveness of its implementation strategies and tools were demonstrated through testing in 8 pilot sites and in over 250 healthcare settings worldwide. We evaluated the impact of CCiSC based on outputs corresponding to the 3 main objectives.
Results and Conclusions: Following the WHO call for collaboration, key national and international infection control (IC) institutions have strongly supported the work of CCiSC. Country commitment has been reflected in the pledge signature to reduce HAI by 124 Member States and in the launch of 42 national/sub-national HH campaigns, allocation of financial and human resources to IC and development of new policies. To raise awareness about the importance of HAI, systematic reviews were conducted to establish the epidemiological burden of endemic HAI and a meta-analysis on the situation in developing countries was published. Following implementation of the WHO recommendations and strategies, significant increase of HH compliance was observed across all pilot sites, as well as improvement in infrastructure for HH and healthcare workers' perception and knowledge about the importance of HAI and HH. The local production of the WHO alcohol-based handrub formulations was shown to be feasible and at low cost (ranging from US$ 0.30 to 0.50 per 100 ml). To support long-term HH improvement worldwide, the new initiative "SAVE LIVES: Clean Your Hands", was launched on 5 May 2009. As of December 2010, 12 152 hospitals from 144 countries registered to be part of this initiative. Within the space of 5 years, CCiSC has generated unprecedented global momentum by mobilizing countries, stakeholders, patient organizations and technical experts to support its objectives and its ultimate aim. Multimodal strategies have been identified as the most effective strategies to improve HH and IC at the point of care, with important lessons learned about adaptation and feasibility in settings with limited resources.

Emergence of virulent and antibiotic-resistant bacterial forms in the marine environment: a public health concern

S61 Mobile genetic elements transferring virulence and antibiotic resistance genes among marine bacteria

M.L. Lemos, M. Balado, A.J. Rivas, A. Rodríguez-Blanco, C.R. Osorio (Santiago de Compostela, ES)*

Marine bacteria are receiving increasing interest from the scientific community. In addition to their ecological importance, it is now clear that many marine bacteria are themselves potential human pathogens, and that these bacteria are also a huge reservoir of genes encoding virulence factors and antibiotic resistance. In this review we show that some virulence factors of marine bacteria, as toxins, hemolysins and siderophore biosynthesis are encoded by mobile DNA elements and that some of them can be horizontally transmitted, not only to marine bacteria but also to other animal and human pathogens. As a model we have been studying *Photobacterium damselae*, a Vibrionaceae member widespread in the marine environment, that includes two subspecies, subsp. *piscicida* and subsp. *damselae*. The subsp. *piscicida* is the causative agent of fish pasteurellosis. Subsp. *damselae* affects a variety of marine animals and is also an opportunistic human pathogen, being reported clinical cases with fatal outcome. We have described in these bacteria several mobile genetic elements that encode virulence factors and antibiotic resistance. In this regard, we have demonstrated that siderophore biosynthesis in subsp. *piscicida* is encoded by a gene cluster which is part of a pathogenicity island (PAI) closely similar to a *Yersinia enterocolitica* PAI, that has also homologues in *V. cholerae* strains. This cluster encodes the biosynthesis, transport and utilization of a siderophore that has a relevant role in the pathogenicity of subsp. *piscicida*. This PAI is part of a 70 kb mobilizable plasmid (pPHDP70). Moreover, we showed that some strains of subsp. *piscicida* possess an ICE element of the SXT family, first reported in *V. cholerae*, that encodes tetracycline resistance and that can promote the mobilization of other genetic elements such as the virulence plasmids pPHDP10 and pPHDP70. We found that similar ICE elements are also present in a significant proportion in other marine vibrios. Besides antibiotic resistance, some ICE elements also encode heavy metals resistance. Other virulence factor encoded within a mobile element in *P. damselae* is the synthesis of hemolysins. A conjugative plasmid of 150 kb encodes two hemolysins, damselysin and HlyA, which greatly contribute to the virulence of this bacterium to fish and mice. We were able to demonstrate that some of these elements can be horizontally transmitted by conjugation to other animal and human bacterial pathogens.

S62 Pathogenic effects of marine *Vibrio* strains on human cells

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V. parahaemolyticus is an inhabitant of estuarine and marine environments that causes seafood-borne gastroenteritis worldwide and more rarely wound infections and septicemia. Recently, a type 3 secretion system (T3SS2) able to secrete and translocate virulence factors into the eukaryotic cell has been identified in a pathogenicity island (VP-PAI) located on the smaller chromosome. These virulence-related genes have previously been detected only in clinical strains. Classical virulence genes for this species (tdh/trh) are rarely detected in environmental strains which are usually considered to lack virulence potential. However, during screening of a collection of environmental *V. parahaemolyticus* isolates obtained in the North Adriatic Sea in Italy, a number of marine strains carrying virulence-related genes, including genes involved in the T3SS2, were detected.
In this study we investigated the pathogenic potential of these marine *V. parahaemolyticus* strains by studying their adherence ability, their cytotoxicity, their effect on zonula occludin protein1 (ZO-1) of the tight junctions and their effect on transepithelial resistance (TER) in infected

Caco-2 cells. By performing a reverse transcription-PCR we also tested the expression of the T3SS2 genes vopT and vopB2 encoding an effector and a translocon protein, respectively.
Our results indicate that, similarly to clinical strains, marine *V. parahaemolyticus* strains carrying vopT, vopB2 and other genes included in the VP-PAI are capable of adhering to human cells and of causing cytoskeletal disruption and loss of membrane integrity in infected cells. On the basis of data here presented, environmental *V. parahaemolyticus* strains should be included in coastal water surveillance plans as they may represent a risk for human health.

S63 Fish farms: potential role as reservoirs of antibiotic resistant bacteria and resistance genes

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Aquaculture now supplies more than half the fish and shellfish that are eaten globally. As with other farmed animals, they are affected by a range of bacterial diseases. These can have severe impacts on both productivity and their welfare. Despite the development in recent years of improved vaccines that effectively control some important diseases, such as furunculosis in Atlantic salmon, antimicrobials are still used to control many conditions. These are typically applied either in feed or direct to the culture water, with varying levels of controls in place to control their useage in different countries worldwide. This use of antimicrobials has very likely driven the development of resistance in the bacteria associated with farmed fish and shellfish. As well as posing a threat to the sustainability of the use of these important chemotherapeutants to treat infected fish, there is also concern that much of this resistance is encoded by so-called 'mobile' genetic elements that transfer the genes from otherwise harmless types of bacteria. Together with the resistance genes, come a whole host of genes of unknown function which may confer an entirely different selectable phenotype. The reservoir of genes which transfer under the selective pressure of antibiotic usage in the aquatic environment is largely unknown. A large body of scientific evidence shows that bacteria harbouring these mobile AMR genes are widely disseminated, and often at high prevalence, in all environments studied to date (e.g. soil, mining waste, livestock production sites, water treatment effluent, marine and freshwater water column and sediment samples). The potential threat that these environmental 'reservoirs' of mobile AMR genes pose to human and animal (including fish) health has not yet been ascertained, but is likely to be considerable. For this presentation we will discuss the extent to which aquaculture practices are affected by, as well as may contribute to, these significant AMR issues.

S64 Monitoring and modeling the microbiological quality of water and shellfish

M. Pommepuy (Plouzane, FR)*

Microbiological contamination in the marine environment can occur in all marine biota anytime sewage from human or animal origin is discharged to coastal waters. Bacteria and viruses from humans and animals can affect seawater quality and can accumulate in filter feeding shellfish. Microbiological contamination often results from urban wastewater discharges or non-point source pollution and dilution as well. To limit sanitary risk, European regulations have proposed standards for the classification of shellfish waters (EC/113/2006) and shellfish growing areas (EC/854/2004 modified by regulation EC/1666/2006). However, despite the legal controls shellfish outbreaks are currently reported. The European regulation on microbiological criteria for shellfish quality stipulates legal control based on the traditional bacterial indicators (*Escherichia coli*). However *Escherichia coli* indicator does not perform well for viruses, the main micro-organism involved in shellfish associated outbreaks in Europe. Gastroenteritis and Hepatitis A are the most important microbial diseases transmitted to humans through shellfish. Thus many outbreaks, even recently have been associated with shellfish fully compliant with legal bacteriological standards. In some outbreaks, multiple strains of a single virus such as NoV can be detected indicating or faecal contamination. Analysis of shellfish events leading to shellfish-related outbreaks has confirmed this hypothesis sewage, and when environmental data are available, sewage-related contamination is often demonstrated.
Microbiological contamination can cause bathing zones to be closed or shellfish sales prohibited. Both have direct effects on the coastal economy (tourist and shellfish industries). Modelling approach allows to put in place a tool for risk assessment in coastal zone and anticipate the degradation of water quality and thus, the zones closures. An example of application using the integrated SWAT and MARS models is presented. These nested models allow to estimate the impact of catchments' agricultural practices and wastewater discharges on the quality of the aquatic environment in estuaries devoted to shellfish culture. This association of models has the ability to simulate *E. coli* concentrations and virus from the catchment, via streams and watershed outlet, and into the estuary. These models consider the dynamic processes, and incorporate daily and variable pathogen fluxes into the hydrodynamic model. Models have the capability of predicting the special pattern of various hydrological factors and contaminant outflows within a watershed, and are thus widely used for simulating microbial fate and transport in watersheds. They also propose management scenario to improve the quality of water of shellfish growing areas.

The role of PK/PD in the treatment of severe infections

S65 MRSA, enterococci, *Pseudomonas aeruginosa* and *Acinetobacter* spp: where are we going with resistance patterns?

S. Stefani (Catania, IT)*

Infections caused by MDR pathogens continue to challenge physicians and to threaten patient's lives. Despite some new drugs recently licensed for treating infections sustained by Gram positive pathogens such as MRSA, a growing problem in MDR enterococci, but, above all, in Gram-negative bacteria is now emerging, not paralleled by the development of novel antimicrobials.
MRSA is still a leading cause of severe infections: it is an extremely flexible pathogens in acquiring antibiotic resistance and virulence traits. Recent results has demonstrated the isolation of hVISA and VISA strains in which empirical glycopeptide therapy is proving to be less effective or inappropriate. Even if, in this case, there is the possibility to use alternative regimens, this is not always for MDR enterococci, that, together with a diffuse natural unsusceptibility to many families of drugs, have acquired resistance to many drugs. The evolution of antibiotic resistance is reaching the most extreme level in Gram negative pathogens. MDR or PDR microorganisms were found among Enterobacteria (*Klebsiella* spp and *E. coli*) but also in non-fermentative opportunistic pathogens such as *P. aeruginosa* and, above all, *A. baumannii*.
Data from the NNISS report resistance rates among *P. aeruginosa* isolates to imipenem and quinolones as approximately 21% and 30% respectively; in ICUs the respective rates are higher (up to 51% for ciprofloxacin, 31% for piperacillin/tazobactam, 38% imipenem, and 23% ceftazidime). Relevant figures are emerging from Europe (EarsNet), in some cases showing a worsening trend.
Multicenter surveillance studies on *A. baumannii* strains have reported that resistance to carbapenems now accounts for more than 50% of isolates in ICUs worldwide, with peaks in some south-European countries as reported by the EarsNet.
We all know that antibiotic use leads to a change in the epidemiology and in resistance patterns; at the same time, in this genetic age, we are beginning to see and understand how bacteria defend themselves through mutations, acquisition and development of new strategies against antibiotic therapy. Together with this, there is also the bacterial potential for dissemination of new mechanisms (bacterial tourism), such as the recent example of the new Delhi MBL. Unless we have a global policy

for antibiotic use and support research into drug development and studies on mechanisms of resistance, we will not begin to control this dangerous phenomenon.

ISF Symposium and Presentation of the International Sepsis Forum Award

S69 Mapping QTL associated with host susceptibility to *Klebsiella pneumoniae* infection in the Collaborative Cross mouse resource population

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Objectives: Infectious diseases of the respiratory tract are the most common causes of deaths worldwide in humans. *Klebsiella pneumoniae* (Kp) is a common pulmonary pathogen causing severe pneumonia often associated with sepsis. With the rise of antibiotic resistance in bacteria, there is a need for alternative, effective and affordable control methods. For this purpose we initiated studies aimed at mapping and subsequently identifying the host susceptibility genes to *Klebsiella pneumoniae* infection in a novel and high genetically diverse mouse resource population, the Collaborative cross (CC).
Methods: In total, 434 mice of 73 CC lines were challenged by intraperitoneally (IP) with 104 CFU of *Klebsiella pneumoniae* strain K2 (KP-2) and variety of traits, including mean survival time post infection, body weight at different time points during the challenge and compared with the initial body weight, were monitored for 15 days duration of the challenge. High molecular genomic DNA of the CC lines was genotyped with 620K single nucleotide polymorphic (SNP) of mouse diversity array, and subsequently a QTL mapping was conducted using HAPPY software.
Results: Survival analysis has shown that the different CC lines differed significantly ($P < 0.05$) with spectrum of mean survival time between 1 to 12 days post infection. "Broad sense" heritability (including epistatic, but not dominance effects) of this trait in the CC mouse population was high as of 0.45. Permutation test analysis determined the 50%, 90% and 95% threshold levels and found to be 6.2, 8.1 and 8.8, respectively. Two significant QTL were mapped on chromosome 1 and 2 with logP 13.2 and 10.3, respectively at 95% threshold level, with genomic regions of less than 1Mb. Additional significant QTL was mapped with logP 8.5 on chromosome 7. Three suggestive QTL were also mapped on chromosomes 3, 9 and 11 with logP of 7.5, 7.2 and 7.1, respectively. A number of candidate genes underlying the QTL are suggested.
Conclusion: These results has strongly confirmed that host susceptibility to *Klebsiella pneumoniae* is a complex trait and controlled by multiple genetic factors and the CC mouse population is a powerful tool for dissecting this trait and can be used of studying other infectious diseases.

S70 Pathophysiology: damage associated molecular patterns

T. van der Poll (Amsterdam, NL)*

Although inflammation is important for the eradication of invading pathogens, uncontrolled or chronic inflammation accompanying infection can be detrimental to the host. The mechanisms by which pathogens initiate inflammation have been well studied. Several classes of receptors are important for sensing microorganisms and for the subsequent induction of pro-inflammatory responses; these receptors have been collectively termed pattern recognition receptors (PRRs).
It is now evident that PRRs also recognize non-infectious material released during cellular injury during infection. These endogenous molecules have been termed damage-associated molecular patterns (DAMPs). A common feature of DAMPs is that they are endogenous factors that are normally sequestered intracellularly and are therefore hidden from recognition by the immune system under normal physiological conditions. However, under conditions of cellular stress or injury, these molecules can be released into the extracellular environment (e.g. HMGB1, DNA, RNA). In addition to DAMPs from an intracellular source, there are also extracellularly located DAMPs, released by extracellular matrix degradation during tissue injury (e.g. hyaluronan, heparan sulphate and biglycan).
Several DAMPs have been implicated in the pathogenesis of sepsis. This lecture discusses the role of DAMPs in sepsis and the host receptors involved in their recognition.

S73 Is there a rationale to use immunostimulation to counteract the altered immune status of sepsis and SIRS patients?

J.M. Cavaillon (Paris, FR)*

Due to persistent frustrations following experimental approaches aimed to target inflammatory mediators in sepsis, it is now proposed that the anti-inflammatory phase should be targeted with drugs able to boost immunity. It is certainly appealing, but might still consist in treating a consequence, or a symptom of the syndrome, rather than the cause. Immunodepression, prior to the septic episode, is clearly a risk factor for mortality, as determined by many epidemiological studies performed during sepsis in humans. However, whether the modification of the immune status following sepsis or non-infectious systemic inflammatory response syndrome (SIRS), known as "compensatory anti-inflammatory response syndrome" (CARS), has serious consequences in terms of survival, remains debatable. Of course, the increased frequency of nosocomial infections in intensive care units (ICU), may have led to some deaths, but those late deaths cannot explain the initial high mortality of septic shock. In animal models, preventing apoptosis of immune cells or boosting immune cells have been shown to be protective. But before dying from anergy and apoptosis of their immune cells, don't patients rather die from shock, organ failure and ultimately from nosocomial infections or withdrawal from intensive care? In addition, alteration of immune cells has been mainly demonstrated in human blood and murine lymphoid organs, but immune cells within the other tissues might not be deactivated. For example, lung neutrophils are activated in murine hemorrhagic shock or endotoxinemia, whereas this is not the case for blood neutrophils. Or, in contrast to any other mononuclear phagocytes, murine alveolar macrophages cannot be rendered tolerant to endotoxin, while endotoxin tolerance has often been suggested to partially mimic the state of hyporeactivity of circulating immune cells. Finally, even for circulating cells, the alteration of their reactivity is not a global defect, but rather a reprogramming that maintains certain functions intact while down-regulating others, particularly, those involved in inflammation. Indeed, all organs are affected by the systemic inflammatory process, and behave specifically to the insult. In sepsis and SIRS there is not a pro-inflammatory response followed by an anti-inflammatory one, but rather both events are concomitant and compartmentalized. This rendered the therapeutic approach quite challenging, and should therefore be taken into consideration.

New diagnostic methods for invasive fungal infections

S74 Non-culture based diagnosis of invasive aspergillosis

C. Lass-Flörl (Innsbruck, AT)*

Invasive aspergillosis (IA) caused by the fungus *Aspergillus* species is a frequent and life-threatening complication of chemotherapy and bone marrow transplantation with high rates of mortality and morbidity. The most common species of *Aspergillus* causing human disease are *Aspergillus fumigatus*, *A. flavus*, *A. niger*, and *A. terreus*. There is no "gold standard" test for the diagnosis of IA, and detection currently requires data from clinical and radiological sources and from mycology and histopathology where feasible. Diagnosis is complex and problematic and can only be confirmed by identification of the fungus in biopsy samples. Capturing tissue for diagnosis is in itself hazardous, and because of this many patients receive empirical antifungal treatment

rather than undergo biopsy. Attempts have been made to develop specific and sensitive diagnostic tests that can be used to track the early onset of infection. Detection of one such signature molecule, galactomannan (and associated galactomannoprotein molecules), forms the basis of the commercial Platelia enzyme immunoassay (EIA), an assay that has found widespread use in IA diagnosis. Specificity of the GM test ranges from 66% to 100% and sensitivity from 57% to 100%. The reasons for variability in performance likely encompass both biological and epidemiological factors; including prophylactic and empirical antifungal therapies that compromise the sensitivity of the GM immunoassay: alternative strategies to diagnosis have been sought including detection of the fungal cell wall component (1,3)-b-D-glucan and polymerase chain reaction (PCR). Detection of BG relies on its ability to activate factor G of the horseshoe crab coagulation cascade. While these tests display sufficient sensitivity, they lack sufficient specificity or suffer from interference under certain conditions. The kinetics of GM, BG, and DNA release are less easily established in vivo, but comparisons of the diagnostic potential of PCR, the Platelia GM immunoassay and BG tests have been undertaken in patients with hematological disorders, with contrasting results. The "one assay-fits-all" approach to IA diagnostics is unrealistic and accurate diagnosis will necessarily rely on complementary diagnostic approaches.

S75 Latest developments in molecular detection of outbreaks

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Confirmation of suspected outbreaks of fungal infections require high resolution molecular genotyping methods. A large variety of methods have been reported in literature that differ in complexity, discriminatory power, costs, turn-around-time, reproducibility and stability. In recent years there is a growing tendency to use repeated DNA elements based or sequence based typing methods since these allow generation of typing data that is easily stored and exchanged between laboratories. However, these methods are not readily available to laboratories with no access to the specialized equipment needed for these kinds of analyses (usually a capillary DNA analyzer) or with no or only little experience in molecular typing. As a result, such laboratories often refrain from performing such typing analyses.

In an attempt to develop a typing method that is more accessible to non-specialized laboratories as well we chose to develop an entirely new genotyping approach. In this approach we choose not to try to determine if isolates may be identical or clonally related, but instead we aim to show in a definitive manner that they are not. The choice of typing targets allows easy and unambiguous genotyping. The real-time PCR format without the use of expensive fluorescent labeled probes makes this approach far more accessible than methods currently in use. Furthermore, compared to existing genotyping methods, the new approach is extremely simple, fast and economical. The diversity index evaluated on a collection of >200 isolates of various origin was 0.985. This means that there is a chance of only 1.5% that any two randomly tested isolates yield the same genotype. This approach thus provides a rapid simple and accessible screening method to evaluate potential outbreak situations.

S76 Rapid detection of *Candida* species and resistance from blood cultures

J. Guinea Ortega (Madrid, ES)*

Despite the current expanded armamentarium of antifungal agents, mortality due to candidemia remains extremely high. In patients with candidemia, early initiation of antifungal treatment is desirable, because it is associated with better outcome. The nonspecific clinical presentation of candidemia and the increasing number of antifungal-resistant isolates may lead to a delay in the initiation of adequate therapy. Therefore, efforts to improve the diagnosis of candidemia and to reduce the time to detecting antifungal-resistant *Candida* isolates are warranted.

Widespread use of sensitive and specific strategies (eg, PCR-based procedures) able to decrease time to diagnosis of candidemia is still hampered by the lack of standardization. Real-time PCR to detect *Candida* DNA in blood samples from high-risk patients is promising due to its high analytical sensitivity and low inter-sample cross-contamination. In addition, few species of *Candida* have been included in the panel of detectable microorganisms of some marketed real-time PCR systems. *Candida* DNA can also be detected in blood culture bottles that are positive for yeasts, thus anticipating the results of the conventional identification. Use of specific probes helps to avoid further sequencing of amplicons and can reduce time to identification.

Molecular detection of *Candida* isolates with antifungal resistance to fluconazole or echinocandins is based on two strategies. First, specific mutations can be investigated in genes encoding the drug targets. The technique can be performed on DNA extracted from clinical samples, in blood culture bottles, or on cultured isolates. Second, application of fluorescence in situ hybridization (FISH) assays in blood culture bottles in which yeasts can be observed in the Gram stain make it possible to detect some species of *Candida* with intrinsic decreased of the antifungal susceptibility. Finally, the Etest can be applied directly in blood culture bottles containing yeasts, and antifungal susceptibility of the isolates can be available within 24 hours.

In this presentation, I will review the above-mentioned procedures and the clinical impact of their implementation in the microbiology laboratory.

Antimicrobial pharmacology: from bench to bedside

O78 Comparative analysis of the potential of polymyxin B and gentamicin to cause apoptosis and necrosis in cultured renal LLC-PK1 cells: concentration-dependent studies with incubated and electroporated cells

M.-P. Mingeot-Leclercq, P. Tulkens (Brussels, BE)*

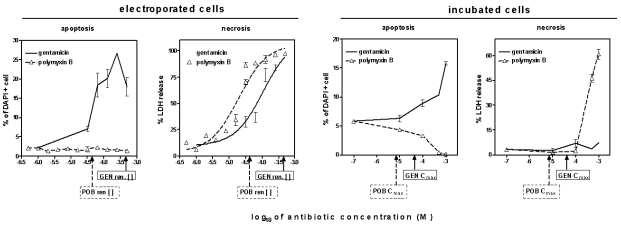
Objectives: Polymyxins and aminoglycoisdes are both known to be associated with nephrotoxicity. In previous studies, we showed that gentamicin causes concentration-dependent apoptosis and necrosis in renal LCC-PK1 cells upon incubation at large concentrations (typically above 0.5 and 1.5 g/L, respectively; Servais et al. Tox. Appl. Pharmacol. 2005, 206:321–33). Delivery of the antibiotic into cells by electroporation increases about 100-fold their susceptibility for both alterations (Servais et al. AAC 2006, 50:1213–21). Our aim has been to compare polymyxins to gentamicin in these models.

Methods: Polymyxin B was chosen as its clinical nephrotoxicity was shown recently to be similar to that of colistin (polymyxin E; Oliveira et al. Diagn Microbiol Infect Dis. 2009, 65:431–4). Gentamicin was the clinical product complying with the European Pharmacopoeia. LLC-PK1 cells were from ATCC. Incubation: cells were continuously exposed to drugs for 48 hours. Electroporation: cells were electroporated in the presence of the drug, left for 15 min, and transferred to drug-free-medium for 24 h at 37ºC. Necrosis was assessed by the release of lactate dehydrogenase (a cytosolic enzyme), and apoptosis by microscopic enumeration of condensed and fragmented nuclei after staining with 4',6'-diamidine-2'-phenylindole.

Results: Concentration-response curves are shown in the Figure with reference to the known renal (tissue) and serum concentrations for electroporated and incubated cells, respectively. While gentamicin caused apoptosis and necrosis in electroporated cells at concentrations pertinent of the expected tissue concentrations, polymyxin B caused only necrosis. For incubated cells, gentamicin caused apoptosis when the concentration reached the human Cmax but no necrosis in the range of concentrations investigated. Polymyxin B caused no apoptosis, but necrosis became massive when it concentrations exceeded 10-fold the human Cmax.

Conclusions: Polymyxin B and gentamicin markedly differ in their ability to cause apoptosis, but both agents cause necrosis when delivered into the cytosol. With incubated cells, polymyxin B appears safe as long

as it concentration remains low, which call for caution against undue increase of dosages. Extrapolation of these data to the human situation and interpretation in terms of clinical toxic potential will need to be further studied.



O79 Time-kill effect of levofloxacin against multidrug-resistant *Pseudomonas aeruginosa* isolated from patients with ventilator-associated pneumonia

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Objectives: Former results of our group in a limited number of isolates have shown that when used in vitro at concentrations within the range to those achieved in the epithelial lining fluid (ELF), levofloxacin (LVF) may be active against multidrug-resistant *Pseudomonas aeruginosa* (MDRPA) causing ventilator-associated pneumonia (VAP) (Safarika A, et al. ECCMID 2009; abstr. P1661). Validity of these results was further investigated against a larger number of isolates.
Methods: Twenty-three MDRPA isolates from patients with VAP were tested. All were genetically distinct as defined after pulse field electrophoresis of their DNA. All were isolated at a density greater than $1x10^6$/ml from tracheobronchial secretions. A log-phase inoculum of 1×10^6 cfu/ml of pathogens was exposed over time to 7.5, 11 and 25 microg/ml of LVF; this represents the range of concentrations achieved in ELF. Isolates were also exposed to 16 microg/ml of meropenem (MER), to 5 microg/ml of colistin (COL) and to their interactions with LVF. The latter are concentrations equal to their mean serum levels. Time-kill effect was defined as any more than 3 log10 decrease of viable cell counts. Synergy between antimicrobials was defined as any more than 2log10 decrease of bacterial growth compared with the most active single agent.
Results: A time-kill effect was shown by 7.5, 11 and 25 microg/ml concentrations of LVF against 13 (56.5%), 15 (65.2%) and 15 (65.2%) isolates respectively; this was mainly observed at 4, 6 and 24 hours of growth. A time-kill effect of MER was observed against five isolates (21.7%) and of COL against seven isolates (30.4%). Synergy between LVF and MER was found against 16 isolates (69.5%) notably at 6 hours of growth; synergy was achieved at all studied concentrations of LVF. Synergy between LVF and COL was found against all tested isolates (100%) notably at 4, 6 and 24 hours of growth and at all studied concentrations of LVF equal to 11 and 25 microg/ml.
Conclusions: LVF presents significant in vitro time-kill effect against MDRPA pathogens of VAP when applied at concentrations within the range of those achieved in ELF. LVF may also enhance the weak time-kill effect of COL so that a considerable synergy is produced. These results render promising the management of VAP by MDRPA with the administration of LVF at regimens delivering the studied concentrations.

O80 Staphylococcal resistance studies using susceptible organisms supplemented with their resistant mutants: linezolid and rifampin combinations simulated in an in vitro dynamic model

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Objective: Recently the clinically attainable ratio of the area under concentration-time curve (AUC) of linezolid (LZD) to the MIC (120 h) did not prevent *S. aureus* resistance in an in vitro model. To explore if combinations of LZD with rifampin (RIF) are better able to restrict *S. aureus* resistance, the selection of LZD- and RIF-resistant *S. aureus* was studied by simulating single and combined treatments with LZD and RIF at sub-therapeutic AUC/MICs.
Methods: A clinical isolate of *S. aureus* (8 log CFU/ml; MIC and MPC of LZD 2 and 14 mg/L, MIC and MPC of RIF 0.016 and 1024 mg/L, respectively) supplemented with its LZD-resistant mutant (2 log CFU/ml; MIC of LZD 8 mg/L) was exposed to twice-daily LZD and once-daily RIF, alone and in combination, over 5 days. With both LZD and RIF, the simulated AUC/MIC ratio was about half of clinically attainable values: 60 and 1850 h, respectively. Antibiotic effects on susceptible and resistant sub-populations were expressed by areas under the bacterial or bacterial mutant concentration-time curves (AUBC and AUBCm, respectively) calculated from the beginning of the simulated treatment to 120 hours.
Results: The effects of LZD + RIF combinations on susceptible sub-populations were respectively 1.4- and 1.6-fold greater than those of LZD and RIF alone. With LZD and RIF given alone, mutants resistant to 2× and 4×MIC of LZD and to 2×, 4×, 8× and 16×MIC of RIF were intensively enriched, with resistant mutants completely replacing susceptible organisms. Enrichment of LZD-resistant mutants did not occur with the LZD + RIF combinations. Moreover, the combination restricted amplification of the RIF-resistant mutants for the entire treatment period. The AUBCm's for mutants resistant to 2×, 4×, 8× and 16×MIC of RIF were respectively 2.8, 3.7, 7.1 and 7.3 times smaller with the combination than with RIF alone.
Conclusions: These findings suggest that combined treatment with LZD and RIF is effective in preventing and/or inhibiting selection of LZD-and RIF-resistant *S. aureus* in an in vitro model.

O81 Evaluation of ceftaroline activity versus daptomycin (DAP) against DAP non-susceptible methicillin-resistant *Staphylococcus aureus* strains in an in-vitro pharmacokinetic/pharmacodynamic model

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Objectives: Investigate the potential role of CPT as a therapeutic option for the treatment of DAP non-susceptible (DNS) MRSA infections. CPT is the active form of the prodrug ceftaroline fosamil, a new parenteral, broad-spectrum cephalosporin with activity against MRSA.
Methods: Four clinical DNS MRSA strains, R5717, R5563, R5996 (hVISA) and R5995 (VISA) were run in a two-compartment hollow fiber in-vitro PK/PD model at a starting inoculum of 10^7 CFU/mL for 96 hours. Simulated regimens were CPT 600 mg q 12 h (free Cmax=15.2 μg/mL, T1/2=2.3 h), DAP 6 mg/kg q 24 h (free Cmax = 7.9 μg/mL, T1/2=8 h), and DAP 10 mg/kg q 24 h (free Cmax = 15.2 μg/mL, T1/2=8 h). Model PK were verified with bioassay. Experiments were performed in duplicate to ensure reproducibility, and differences in CFU/mL between 24 and 96 h was evaluated with a Turkey's Post-Hoc test. Bactericidal activity (99.9% kill) was defined as a ⩾3-log10 CFU/mL decrease in colony count from the initial inoculum. Development of resistance was evaluated between 24 and 96 hours by plating samples on agar plates containing DAP or CPT at 3 times the MIC.
Results: CPT MIC values were 0.25, 0.5, 0.5, and 0.5 μg/mL and DAP MICs were 2, 2, 4, and 4 μg/mL for R5717, R5563, R5996, and R5995, respectively. Pharmacokinetic parameters were within 12% and 11% for CPT and DAP, respectively. CPT displayed sustained bactericidal activity [R5717 (–3.1 Log10 CFU/mL), R5563 (–2.5 Log10 CFU/mL), R5996 (–5.77 Log10 CFU/mL) and R5995 (–6.38 Log10 CFU/mL)] against 3 of the 4 strains. Re-growth occurred during the DAP 6 mg/kg q 24 h regimen for all 4 strains and during the DAP 10 mg/kg q 24 h regimen for 3 of 4 strains. At 96 h, CPT 600 mg q 12 h was significantly better than DAP 6 mg/kg q 24 h against all 4 strains ($p < 0.001$) and DAP 10 mg/kg q 24 h against 3 strains ($p < 0.01$). Isolates with increased MIC values were recovered for DAP (all 4 strains), but not for CPT.
Conclusion: CPT was the most effective regimen overall against the 4 isolates tested. This novel cephalosporin may provide a clinical option to treat DNS MRSA infections.

O82 Pharmakocinetcs of daptomycin in patients with Gram-positive infections receiving different dosage regimens

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Background: Daptomycin is a concentration-dependent antibiotic displaying bactericidal activity against Gram-positive bacteria. The manufacturer recommends a 4–6-mg/kg dose administered every 24 hrs. However, a higher dosage may be requested in some cases, and daptomycin pharmacokinetics has not been extensively evaluated.
Aim: to evaluate pharmacokinetics of daptomycin in hospitalized patients with severe Gram positive infections.
Methods: Blood collections were performed at 30 minutes, 1 h, 2 h, 4 h, 8 h, 12 h and 24 h following administration of the first dose, up to 96 hours. Daptomycin was measured using an isocratic HPLC technique.
Results: Overall, 46 patient were included in the study, 24 with bacteremia/endocarditis, 15 with complicated skin-soft tissue infection, 3 with prosthetic-joint infection, and 3 with chronic osteomyelitis. The pharmacokinetics of daptomycin was examined in two groups of patients, those receiving 4–6 mg/kg (n = 30) and 8–10 mg/kg (n = 16), respectively. Three patients underwent continuous veno-venous hemodialysis (CVVHD), four patients underwent continuous veno-venous hemodiafiltration (CVVHDF) and one hemodialysis. Only one patient treated with 8 mg/kg had an increase of creatine kinase levels. The mean area under the blood concentration-time curve (AUC) within 24 hours was significantly lower in patients receiving 4–6 mg/kg than in patients receiving 8–10 mg/kg (352.5 vs 539.3, $p < 0.05$). On the same hand, patients receiving 4–6 mg/kg had lower mean Cmax values (47.4 vs 77.9, $p < 0.05$). Patients undergoing CVVHD and receiving daptomycin every 48 hours had AUC and Cmax values similar to patients with normal renal functiom; instead patients undergoing CVVHDF had significantly lower plasma levels, especially those using "high cut-off" hemofilters.
Conclusions: Increasing doses of daptomycin may enhance the AUC and improve the pharmacokinetic profile of this drug. An increased dose seems necessary in critically ill patients undergoing CVVHDF and using high cut-off hemofilters.

O83 Clinical dose finding of sitafloxacin (DU-6859a) = pharmacokinetic/pharmacodynamic analysis in patients with community-acquired respiratory tract infections

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Objectives: Pharmacokinetic and pharmacodynamic (PK/PD) analysis is useful to select rational dosage regimens. We have conducted the PK/PD analysis to find the optimal dosage regimen of sitafloxacin (STFX, DU-6859a), a newly developed fluoroquinolone, in patients with community-acquired respiratory tract infections.
Methods: Serum STFX concentration data from six phase I studies (75 subjects) and one phase III study (137 patients) were pooled to conduct a population pharmacokinetic (PPK) analysis. The PPK parameters were estimated by pooling data from 212 subjects, using a nonlinear mixed-effect model, applying a one-compartment model with first-order absorption. Efficacy data were obtained from the phase III study of STFX orally administered 50 mg or 100 mg twice-daily (BID) for 7 days for the treatment of community-acquired pneumonia or acute exacerbation of chronic bronchitis. Individual PK parameters were estimated by the Bayesian method and MIC was measured for each organism. Furthermore, the PK/PD parameters for 100 mg once-daily (QD) dosage were simulated based on the obtained PPK parameters and MIC values.
Results: MICs of STFX were measured for 91 organisms identified from 74 adult patients. The microbiological cure rate (MCR) was stratified by AUC/MIC and Cmax/MIC. AUC/MIC $\geqslant$81 or Cmax/MIC $\geqslant$ 4.0 were associated with >95% of MCR against *Streptococcus pneumoniae*. Estimated PK/PD parameters including simulation of 100 mg QD implied that the efficacy was not different between 50 mg BID and 100 mg QD, and that mutant selection risk was low at either dosage regimen. Because STFX has a high potency against Gram(+) and Gram(−) bacteria, the twice daily regimen would be an effective option in treating respiratory tract infections.
Conclusions: The present PK/PD analysis supports the 50 mg BID regimen as a proper dosage of STFX for the treatment of community-acquired respiratory tract infections.

O84 Clinical dose finding of sitafloxacin (DU-6859a): pharmacokinetic/pharmacodynamic analysis from two clinical trial results for community-acquired respiratory tract infections

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Objectives: Sitafloxacin (STFX, DU-6859a), a quinolone antibacterial agent, has a potent activity. We had already examined the effect of STFX 50 mg BID in RTI by the clinical efficacy and the pharmacokinetics/pharmacodynamics (PK/PD) analysis. Subsequently, PK/PD parameters at 100 mg QD were also simulated to compare with those of 50 mg BID regimen. From these results, we speculate that the efficacy is rarely different between 50 mg BID and 100 mg QD, furthermore the mutant selection risk is low also in each regimen. To clarify the clinical dosage based on the bacteriological efficacy, PK/PD parameters against *Streptococcus pneumoniae* (*S. pneumoniae*) and safety, the additional PK/PD study was conducted.
Methods: In the additional PK/PD study, patients with RTI mainly caused by *S. pneumoniae* were randomized (STFX 50 mg BID:100 mg QD=1:2). Data from previous phase III study in patients with RTI who were treated with STFX 50 mg BID was pooled to merge with this study data. Safety, bacteriological efficacy, and PK/PD parameters were evaluated. PK parameters were analyzed by the population pharmacokinetics model which was reported by Tanigawara. MICs and MPCs of STFX against each organism were tested to calculate PK/PD parameters.
Results: Analytical subjects of safety were 264 patients (100 mg QD: 98, 50 mg BID: 166). Analytical subjects of efficacy were limited to patients caused by *S. pneumoniae*, and were 97 patients (100 mg QD: 56, 50 mg BID: 41). The eradication rate of *S. pneumoniae* was 98.2% (55/56) in 100 mg QD and 92.7% (38/41) in 50 mg BID. The eradication rate of multidrug-resistant *S. pneumoniae* was 97.7% (42/43) and 94.6% (35/37) in each group. The table shows PK/PD parameters calculated from relation between MIC, MPC of STFX against *S. pneumoniae* and pharmacokinetics of STFX. In any case, the critical difference was not admitted between both groups. The adverse drug reaction (ADR) rate of 100 mg QD and 50 mg BID was 33.7% (33/98) and 40.4% (67/166), respectively.
Conclusion: From standpoints of the bacteriological clinical efficacy, both 100 mg QD and 50 mg BID were good regimen for patients with RTI whose causative bacteria was *S. pneumoniae*. Furthermore, the mutant selection risk of each dosages was low. There was no clear difference in the adverse event risk from the standpoint of safety between both dosages. Therefore, both 100 mg QD and 50 mg BID were thought to be a useful dosage regimen.

		100 mg QD	50 mg BID
n		53	40
$AUC_{0\text{-}24h}$/MIC	mean(SD)	266.073 (149.904)	271.256 (135.245)
	min, max	8.66, 752.43	14.88, 681.67
C_{max}/MIC	mean(SD)	26.263 (14.446)	16.831 (8.037)
	min, max	0.66, 63.40	0.78, 34.60
Time in MSW(%)	median	6.30	0.00
	min, max	0.0, 30.0	0.0, 80.8
Time above MPC(%)	median	93.8	100.0
	min, max	0.0, 100.0	0.0, 100.0

O85 Outpatient and inpatient parenteral antibiotic therapy with daptomycin in a large non-interventional study: reducing total therapy costs

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Objectives: Daptomycin (DAP) has several characteristics that support OPAT, e.g. rapid bactericidal activity, rapid resolution of symptoms, once-daily dosing and since 2009 drug administration via 2-min injection. Here we compare the characteristics and outcomes of patients (pts) who received DAP as OPAT and IPAT.
Methods: Investigators at 237 institutions collected retrospectively anonymized demographic, antibiotic, microbiological and clinical data from medical records using a standardized CRF in the non-interventional European Cubicin Outcomes Registry and Experience in 3 consecutive enrollment periods (Jan 2006-Jun 2010). In the 2nd and 3rd collection periods some non-European sites were progressively included.
Results: A total of 3621 pts were enrolled in 15 countries, mostly from Europe, 115 pts from Latin America, 27 pts from Russia and 59 pts from India. 437 pts received DAP in OPAT (333 as OPAT following IPAT [OPAT/IPAT] and 104 only OPAT). OPAT/IPAT was most commonly used in Spain (n = 122), Italy (n = 86) and UK (n = 81); OPAT only in Venezuela (n = 26), UK (n = 24) and Spain (n = 23). The use of OPAT increased over the 3 collection periods from 2.6 to 4.5%. After regulatory approval the 2-min injection was used in 12 of 58 IPAT/OPAT pts. The median duration of DAP therapy for OPAT, OPAT/IPAT and IPAT was 14 (1–85), 10 (1–109) and 10 days (1–246) respectively. The most commonly treated infections were cSSTI and osteomyelitis in OPAT, cSSTI and endocarditis in OPAT/IPAT and cSSTI and bacteraemia in IPAT. *S. aureus* (41 of 104) was the most frequent pathogen in OPAT (MRSA rate 56%). Overall, success rates were high for all treated infections with a trend to be higher in pts at least partially treated in OPAT, except in cSSTI (Table 1). DAP doses used in OPAT or IPAT were generally similar (mean 5.5 mg/kg). AEs were reported in 19.2% of pts on OPAT vs 13.7% on IPAT vs 14.4% on OPAT/IPAT with low rates of discontinuation due to AEs in all settings.
Conclusion: Reducing hospitalization has significant impact on overall health care costs. It was previously observed that DAP was associated with shorter duration therapy in cSSSI. Here we show that appropriately selected pts receiving DAP once daily including 2-min injection may also be successfully managed either entirely in the OPAT setting or following initial hospitalization. DAP is an attractive and cost-effective therapeutic option for complicated Gram-positive infections in the outpatient setting.

Table 1: Clinical outcomes: Success with daptomycin against different infections

Infection type	OPAT only n/N (%)	IPAT only n/N (%)	Both OPAT/IPAT n/N (%)
cSSTI	28/35 (80%)	790/931 (85%)	74/83 (89%)
Bacteraemia	7/7 (100%)	541/752 (72%)	46/49 (94%)
Osteomyelitis	20/25 (80%)	137/183 (75%)	39/46 (85%)
uSSTI	9/10 (90%)	272/309 (88%)	29/35 (83%)
Endocarditis	8/8 (100%)	250/317 (79%)	56/60 (93%)
Other	16/19 (84%)	406/552 (74%)	54/60 (90.0%)

O86 Colistin minimum plasma concentration is an independent risk factor for nephrotoxicity

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Objectives: Data regarding the most efficacious and less toxic schedule of colistin are scarce.
The aim of this study was to determine the prevalence of colistimethate sodium (CMS)-associated nephrotoxicity in patients with MDR-GBN infections by using a standardised definition of acute renal failure (RIFLE criteria), and to determine the potential risk factors for nephrotoxicity.
Methods: Prospective review of patients who received intravenous CMS for >72 hours from November 2010 to November 2011. CMS doses ranged from 1–2 million U (80–160 mg colistimethate) every 8–12 hours. Collected data: demographic characteristics, Acute Physiology and Chronic Health Evaluation II (APACHE II), Charlson score, concomitant use of nephrotoxic, vasopressors and/or diuretics, cumulative CMS dose (MU), and treatment duration. Blood samples were collected on the third day of treatment (after steady-state achieved), immediately before and 30 minutes after infusion in order to asses minimum (Cmim) and maximal (Cmax) colistin concentration. Glomerular filtration rate (GFR) was calculated by the Cockcroft-Gault formula at the beginning or treatment and monitored at the discretion of the team. The RIFLE criteria were used to assess the presence of nephrotoxicity at the end of treatment.
Plasma colistin concentrations were assayed using high-performance liquid chromatography (HPLC).
In univariate analysis the differences between patients with and without nephrotoxicity at the end of the treatment were assessed. In multivariate analysis, risk factors for the presence of nephrotoxicity were identified through a multiple regression analysis backwards, stepwise variable selection.
Results: 36 patients were included. Baseline characteristics of are shown in table 1. Fourteen (38.8%) had some degree of renal dysfunction. Patients who developed nephrotoxicity were older, had a higher Charlson score, were more likely treated with diuretic drugs, had a higher cumulative doses of colistin, and had higher Cmax and Cmim values. However, only Cmim (OR, 3,888; 95% CI, 1,383–10,935; p = 0,010) was independently related with nephrotoxicity.
Conclusions:
1. Using the RIFLE criteria, 38.8% of patients developed nephrotoxicity.
2. Cmim is and independent risk factor for nephrotoxicity.
3. Colistin plasmatic levels could be useful to assess nephrotoxicity, mainly by monitoring Cmin.

Characteristics	Patients with nephrotoxicity (n=14)	Patients without nephrotoxicity (n=22)	p
Age	73.7 ± 7	60.7 ± 17.1	0.005
Male sex	12 (85.7)	18 (81.8)	1
Total CMS dose (MU)	97.36 ± 78.5	58.4 ± 41.2	0.059
Baseline GFR > 80	8 (57.1)	5 (22.7)	0,07
APACHE II score	10.29 ± 4.9	9 ± 4.4	0.42
Charlson score	5.5 ± 2.1	3.2 ± 2.5	0,009
Concomitant aminoglycoside use	4 (28.5)	6 (27.3)	1
Concomitant vancomycin use	1 (7.1)	3 (16.6)	1
Concomitant antifungal drugs	6 (42.8)	3 (16.6)	0.41
Concomitant diuretic use	9 (64.2)	5 (22.7)	0.018
Concomitant NSAIDs use	2 (14.2)	1 (4.5)	0.5
Concomitant vasopressor requeriment	1 (7.1)	1 (4.54)	1
C_{max}	1.6 ± 0.8	0.9 ± 0.6	0.01
C_{min}	1.6 ± 0.8	0.8 ± 0.6	0.007

O87 Factors influencing pharmacokinetics of prophylactic posaconazole oral solution in patients with acute myeloid leukaemia or myelodysplastic syndrome

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Objectives: To estimate pharmacokinetic properties of posaconazole in patients with acute myeloid leukemia (AML) or myelodysplastic syndrome (MDS) undergoing chemotherapy in a clinical setting.
Methods: Posaconazole concentrations in patients with AML/MDS receiving prophylactic posaconazole were determined by high-performance liquid chromatography. A population pharmacokinetic model with

nonlinear mixed effect modeling was developed. The list of tested covariates included age, weight, height, gender, posaconazole dose, ethnicity, co-administration of antineoplastic chemotherapy, ranitidine or pantoprazole, coincident fever, diarrhea, leukocyte counts and γGT plasma activity.
Results: A total of 643 serum concentrations of posaconazole from 84 patients were obtained. A one-compartment model with first order absorption and elimination as the basic structural model appropriately described the data, with an apparent clearance of 56.8 L/h (95% CI 52.8–60.8 L/h) and an apparent volume of distribution of 2,130 L (95% CI 1646–2614 L). Significant effects on apparent clearance were found for presence of diarrhea and for co-medication with proton-pump inhibitors (1.5-fold and 1.6-fold increase in CL/F, respectively), weight (33.4 L larger apparent volume of distribution per kilogram), and co-administration of chemotherapy (0.6-fold lower apparent volume of distribution).
Conclusion: We developed a prediction basis for mean posaconazole concentrations in AML/MDS patients. Patient weight, presence of diarrhea and concomitant medication (chemotherapy and pantoprazole) showed significant effects on posaconazole exposure. Corresponding adjustments of the starting dose according to presence of diarrhea and to age appear justified before TDM results are available. Further investigation of the interaction between different chemotherapeutic regimens and posaconazole is warranted.

New antimicrobial drugs in the pipeline

O88 Development of bacteriophage cocktails for the management of nosocomial and community-acquired methicillin-resistant *Staphylococcus aureus*

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Antimicrobial resistance is one of the greatest threats to human health. The human and economic costs of treating infections caused by bacteria such as nosocomial MRSA (EMRSA) continue to rise and have been compounded by the spread of this pathogen into the community (CA-MRSA). Of particular concern are the reports of development of resistance to antibiotics recently developed such as linezolid and daptomycin. This has prompted a search for novel classes of antibiotics and also for radically different approaches to combat *S. aureus* infections. One consequence of this is the recent revival of interest in phage therapy. Phages are naturally occurring viruses that infect and kill bacteria with high efficiency and specificity. Recent animal and human trials have reconfirmed the potential of phage therapy as an effective alternative and/or complementary option for the treatment of bacterial infections.
Objectives: To develop therapeutic phage combinations with a broad-spectrum of activity against a reference collection consisting of 21 clones of EMRSA and CA-MRSA of international importance (Table 1).
Methods: Lytic phages were isolated from environmental sources in Australia and screened against the reference collection. Combinations of these phages were then tested for their activity against 200 clinical isolates in Australia and the UK.
Results: Fifty-eight lytic phages were isolated from environmental sources. Nine of these phages showed a broad-spectrum range of activity against the reference collection and were consequently selected for further characterization. Combinations of these phages were then developed and their activity tested against 200 clinical isolates in Australia and the UK. The therapeutic phage combinations showed a broad host range infecting over 95% of the isolates tested.
Conclusions: Therapeutic phages were combined and tested against EMRSA and CA-MRSA clinical isolates from two geographical areas. The phage combinations reacted with over 95% of the isolates tested. The use of phage combinations extended their spectrum of activity and minimised the emergence of phage resistant bacteria. Phage combinations were shown to be endotoxin free, non-cytotoxic and free of undesirable genes. This study showed that as the antibiotic resistance crisis deepens and the number of treatment options narrows, the potential of phages as therapeutic tools continues to be a viable option for the treatment of infections caused by these difficult pathogens.

Table 1 Characteristics of *Staphylococcus aureus* reference strains

Strain	SCC*mec*	ST	Clone	Source
544	-	-	Japan- Vancomycin resistant	ATCC*
545	IV	8	USA 300 CA-MRSA, ǂPVL +	ATCC*
546	IV	-	USA 300 EMRSA ǂPVL +	ATCC*
547	II	-	USA 600, ǂPVL -	ATCC*
548	V	-	Non USA, ǂPVL +	ATCC*
621	I	250	Archaic	Westmead Hospital
622	I	~1	-	Westmead Hospital
623	II	5	NY/Japan MRSA	Westmead Hospital
624	II	36	UK EMRSA-16	Westmead Hospital
625	IIIA	239	AUS-3 EMRSA	Westmead Hospital
626	III	239	AUS-2 EMRSA	Westmead Hospital
627	IIIB	239	Brazilian	Westmead Hospital
628	IV	1	WA-MRSA-1	Westmead Hospital
629	IV	59	WA MRSA 15	Westmead Hospital
630	IV	78	WA MRSA-2	Westmead Hospital
631	V	573	WA MRSA-10	Westmead Hospital
632	V	5	WA MRSA-11	Westmead Hospital
633	Novel	1	WA MRSA-40	Westmead Hospital
634	Novel	78	WA MRSA-42	Westmead Hospital
745	IV	22	UK EMRSA-15	Westmead Hospital
746	IV	93	Queensland CA-MRSA	Westmead Hospital

Molecular characteristics of reference strains used for testing representing the major clones in the clinical setting around the world. Molecular elements include: SCC*mec*: (*Staphylococcal* cassette chromosome *mec*) and ST (sequence type or alleic profile). ǂPVL: Panton-Valentine leukocidin *ATCC: American Type Culture collection. (-): Unknown. WA: Western Australia. AUS: Australia.

O89 A novel silver colloid: assessment of antibacterial activity

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Objectives: Silver in various forms has been employed in the last few decades for the reduction of pathogen load particularly in Healthcare Associated Infections. The purpose of this study was to determine the preliminary antibacterial effects of a recently developed highly stable novel silver colloid that exhibits exceptional fabrication characteristics and has a narrow particle size distribution. Many previous studies on the effects of silver nanoparticles against microbes have not addressed the importance of the size of the nanoparticles in considering antibacterial effects.
Methods: The antibacterial activities of the silver colloid AC 1 compared to an appropriate silver control were assessed in 'large volume' MIC determination both in broth and agar using conventional methodology with a range of inocula. Plastic vessels were used throughout to prevent silver interaction with glass. The activity of AC1 against a MRSA reference strain was tested against a range of concentrations from 16 to 0.2 μg/ml incorporated into the agar. The surface was inoculated with appropriate numbers of MRSA. In addition, silver solution was applied to 'antibiotic' discs sequentially with drying. Agar plates were inoculated with MRSA and spread. Disks containing silver AC1, and controls were placed on the surface and incubated at either 30 or 37C for 18h.
Results: The activity of AC1 against Gram-positive and negative organisms was equivalent. The results were not consistent with previous work in terms of either spectrum or degree of potency. Determinations of AC1 in broth produced only moderate activity against ATCC strains *Escherichia coli* or *Staphylococcus aureus* (MIC/MBC = 50/100 μg/ml). AC1 in agar test systems produced results remarkably different to those in liquid broth. With lower inocula (105cfu/ml) agar MIC equalled 10 μg/ml – at least 5x more active when tested in agar rather than liquid. The number of colonies however exposed to AC1 show significant reduction at concentrations of 5.5 μg/ml and partial reduction at 0.6 μg/ml. The activity of AC1 against a single strain of MRSA was confirmed by bioassay producing a significant zone of clearing.
Conclusion: A negatively charged silver phosphate nanoparticle (NP) has been developed by a highly repeatable process that shows interesting activity against a range of pathogen including MRSA.

O90 Effect of novel antibacterial GSK2251052 on mammalian mitochondrial protein synthesis

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Objectives: GSK2251052 is the first in a new oxaborole class of antibiotics with robust Gram-negative coverage against drug-resistant En-

terobacteriaceae and *Pseudomonas aeruginosa*. GSK2251052 uniquely inhibits the editing active site of leucyl-tRNA synthetase (Leu-tRS), an essential constituent of the bacterial protein synthesis machinery. Some inhibitors of bacterial protein synthesis also target mitochondrial translation due to structural similarity between some components and this affect has been potentially linked to safety concerns with such drugs in the clinic. Since human mitochondria also contain a Leu-tRS, this study investigates the effect of GSK2251052 on mammalian mitochondrial protein synthesis.

Methods: A medium-throughput, mitochondrial protein synthesis inhibition assay was developed that measures levels of the mitochondrial encoded protein cytochrome oxidase subunit III (Cox III). HEK293 cells were treated with GSK2251052 for 96 h at 37ºC. Cells were lysed and protein was immobilised on a MSD electrode microplate coated with Cox III capture antibody, probed with Cox III specific antibodies and detected by electro-chemiluminescence. As control, levels of the cytoplasmically expressed outer membrane protein Tom 20 were measured.

Results: Chloramphenicol, tetracycline and linezolid are potent mitochondrial translation inhibitors with IC50s <4 uM in this study. In contrast, azithromycin is negative in the assay with an IC50 of >100 uM. GSK2251052 gave an IC50 of >100 uM and hence does not have an inhibitory effect on mitochondrial protein synthesis.

Conclusion: Unlike a number of bacterial protein synthesis inhibitors, GSK2251052 does not inhibit mammalian mitochondrial protein synthesis in vitro indicating that this clinical candidate has an excellent selectivity profile. This finding was further substantiated by the divergent alignment of the editing domains of bacterial and human mitochondrial Leu-tRSs.

Compound	Mitochondrial Cox III IC_{50} (uM)	Cytoplasmic Tom 20 IC_{50} (uM)	Inhibitor Class
tetracycline	1.5	> 100	known mitochondrial protein synthesis inhibitors
chloramphenicol	2.0	~ 100	
linezolid	3.5	> 100	
cycloheximide	20	0.5	human protein synthesis inhibitor
azithromycin	> 100	> 100	negative control
GSK2251052	> 100	> 100	novel LRS inhibitor

O91 Carbohydrate-derived fulvic acid is a novel antifungal product

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Objectives: Carbohydrate derived fulvic acid (CHD-FA) is a heat stable low molecular weight, water soluble, cationic, colloidal material with proposed therapeutic properties. The aim of this study was to evaluate the antifungal activity of CHD-FA against *Candida albicans*, and to characterise its mode of action. It was also an aim to investigate the immunomodulatory potential of CHD-FA.

Methods: A panel of *C. albicans* isolates (n = 50) derived from a range of clinical specimens were grown planktonically and as biofilms, and the minimum inhibitory concentrations (MICs) determined. The role of efflux pumps was also investigated using pump inhibitors. Scanning electron microscopy was performed to examine ultrastructural changes and different cell wall mutants were analysed to determine the possible mode of action, which included cell wall inhibitor studies. Finally, we investigated whether antifungal concentrations of CHD-FA were toxic to oral epithelial cell lines, and whether it altered the expression of IL-6 and IL-8.

Results: CHD-FA was effective against planktonic (0.125% v/v) and sessile (0.25% v/v) *C. albicans*, and was shown to be fungicidal. Efflux activity was detected in *C. albicans* isolates, however, when these efflux pumps where inhibited there was no reduction of inhibitory concentration. Cell wall mutants displayed similar sensitivity profiles to WT strains, and inhibition of hsp90 did not impact the sensitivity. CHD-FA was not toxic to epithelial cells after a short incubation (2 min), and when this compound was used on zymosan stimulated cells there was apparent down-regulation of IL-6 and IL-8.

Conclusions: This study has shown that the natural antimicrobial CHD-FA is non-toxic and has cidal activity against a range of *C. albicans* clinical isolates. The ability to kill biofilms is a beneficial characteristic not shown in conventional antifungal compounds. Therefore, CHD-FA may offer a cheap and natural alternative to these agents. There is limited scientific understanding of CHD-FA, however, given that no differences were observed from cell wall and efflux studies, the likely mode of action is the cell membrane. CHD-FA may offer potential in a number of clinical applications, including within the oral cavity (mouthwashes), skin and wounds, and the ITU (catheter lock). Further studies, both in vitro and in vivo are required, but with an increasing incidence of fungal infections then CHD-FA may be an attractive option.

O92 A novel chimeric lysin shows superiority to mupirocin for skin decolonisation of antibiotic-resistant (MRSA) and sensitive (MSSA) *Staphylococcus aureus*

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Objectives: Lysins are bacterial cell wall hydrolases generated during the infection cycle of double-stranded DNA phage. When applied exogenously, lysins (purified apart from phage) cleave essential bonds in the cell wall peptidoglycan, resulting in immediate bacterial cell lysis and death. ClyS (chimeric lysin for staphylococci) is a unique lysin specific to staphylococcus, made recombinantly in our laboratory. ClyS is bioengineered to contain a staphylococcus-specific catalytic domain fused to a unique cell wall targeting domain. Our objective was to test the ability of a topical formulation of ClyS to disinfect abraded skin infected with methicillin susceptible (MSSA) or resistant *S. aureus* (MRSA) in vivo.

Methods: ClyS solution is lyophilized and then incorporated into commercially available Aquaphor ointment. We applied topical ClyS to the skin of mice that were infected with *S. aureus* to determine its in vivo efficacy. Groups of mice were treated for one day with either Aquaphor alone (placebo), topical ClyS, or commercially available mupirocin ointment (comparator). For testing if there were differences in colony counts among those groups, both ANOVA and Kruskal-wallis were used. A P value of <0.01 was considered significant. We also tested the ability of staphylococci to develop in vitro resistance to ClyS in comparison with mupirocin when exposed to sub-inhibitory concentrations of the respective agent. Because antibodies towards ClyS may affect enzyme function, we tested antibodies that developed in mice after repeated ClyS exposure for effect on ClyS killing ability.

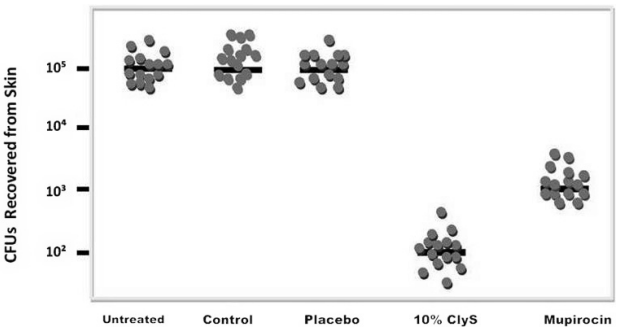

Figure 1. *In vivo* activity of ClyS ointment vs. placebo or mupirocin on tape-stripped mice infected with *S. aureus* 8325-4 or MRSA MW2. Topical treatrnents included Aquaphor® (control), 10% ClyS binding domain in Aquaphor® (placebo), 10% wt/wt ClyS in Aquaphor®, and 2% mupirocin. The median value of the data for each group is shown as a horizontal bar; each sphere represents one mouse. No statistical significance was seen between untreated, control, and placebo groups. Statistical significance ($p < 0.0001$) was noted between untreated/control/placebo and ClyS groups with a three log drop in bacteria recovered (CFUs). Statistical difference ($p = 0.001$) was noted between ClyS and mupirocin treated groups, with a one log drop difference between them. Figure is representative of experiments using *S. aureus* strain 8325-4 or MRSA strain MW2.

Results: Topical ClyS eradicated a statistically significantly greater number of MSSA and MRSA when compared with mupirocin

(p = 0.001), a 3 log as opposed to a 2 log reduction in our model (Figure 1). ClyS also demonstrated decreased potential for development of resistance by MRSA and MSSA organisms in vitro (no change in minimum inhibitory concentration for ClyS as opposed to development of low level resistance for mupirocin). Our results showed no inhibition of ClyS activity at various antibody titers.
Conclusions: Topical ClyS: i) specifically targets staphylococci, ii) has low probability for resistance, iii) does not lose activity in the presence of antibodies, and iv) was found to be more effective than a comparator drug (mupirocin). Our data substantiates topical ClyS as a plausible therapeutic to be developed for human use.

O93 Completely novel protein synthesis inhibitors demonstrate efficacy in three mouse models of infection

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Objectives: Exemplars from three completely novel classes of protein synthesis inhibitors with potent in vitro activity against a range of bacterial pathogens and good ADME properties were evaluated for efficacy in mouse infection models of *Escherichia coli* peritonitis, *Klebsiella pneumoniae* skin and soft tissue and *Staphylococcus aureus* MRSA kidney abscess.
Methods: Murine peritonitis was initiated by injecting *E. coli* into the peritoneal cavity; therapy was administered at 30 minutes, 3.5 and 6.5 hours post-infection and efficacy was measured by monitoring colony-forming units (cfu) burden in peritoneal wash fluid samples and spleen homogenates at 24 hours. Murine thigh infections were established in neutropenic mice by injecting *K. pneumoniae* into both caudal thigh muscles. Mice were dosed at 2 and 12 hr and the bacterial burden in thigh tissues was determined 24 hours following bacterial challenge. Kidney abscess infections were initiated via tail vein injection of MRSA and therapy was administered at 4, 24 and 32 hours; kidneys were harvested for bacterial quantitation at 48 hours post-infection. In some studies, plasma and tissue concentrations of drugs were obtained at various time points up to 6 hours post drug administration using an LC/MS/MS assay.
Results: Several novel compounds from the lead scaffold were efficacious in the *E. coli* peritonitis model, reducing bacterial loads to the limit of detection by 24 hours. Exemplar novel compounds from a second showed comparable efficacy, and representative novel compounds from a third showed static effects in this model. Many lead scaffold compounds also demonstrated efficacy in the *K. pneumoniae* skin and soft tissue infection (SSTI) model, resulting in 1–1.5 log10 delta-cfu reduction in bacterial burden and in the MRSA kidney abscess infection, resulting in 2–2.5 log10 delta-cfu reduction. Furthermore, one of these was efficacious in all three mouse infection models. This compound has good broad-spectrum activity, moderate PK and low protein binding.
Conclusions: Compounds from three completely novel classes of antibiotics have demonstrated efficacy in mouse infection models. A number of these were efficacious in two models, and bacterial burden reduction in all three models was exhibited in at least one case. The characteristics of this exemplar molecule suggest that a combination of activity, PK properties and lower protein binding contribute to mouse efficacy.

O94 Efficacy of the novel metallo-enzyme inhibitor VT-1129 in combination with amphotericin B for cryptococcal meningitis

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Objective: Cryptococcal meningitis is a significant cause of morbidity and mortality in immunocompromised patients. Combination therapy involving amphotericin B is often initiated in patients against this infection. VT-1129 is an investigational metalloenzyme inhibitor with potent in vitro activity against *Cryptococcus neoformans*. Our objective was to evaluate the in vivo efficacy of VT-1129 in combination with amphotericin B in an established murine model of cryptococcal meningitis.
Methods: ICR mice received intracranial inoculation with *C. neoformans* at 3.15–3.51 log cells/mouse. Treatment with VT-1129 at 5 or 10 mg/kg PO BID alone or in combination with a single intraperitoneal dose of amphotericin B deoxycholate 3 mg/kg began 1 day post-inoculation. In survival studies (N = 10/group) treatment continued until day 10 and mice were monitored off therapy until day 30. In fungal burden studies (N = 10/group), treatment continued through day 7, brains were collected on day 8, and fungal burden was assessed by colony-forming units (CFU).
Results: The VT-1129 10 mg/kg dose, both alone and in combination with single-dose amphotericin B, significantly improved the median survival and percentage of animals surviving to the study endpoint compared to untreated controls (Table). Median survival was also prolonged with VT-1129 at 5 mg/kg in combination with amphotericin B. The improvements in survival were also associated with reductions in fungal burden as the number of CFU/g of tissue in each group that resulted in a survival benefit was significantly lower than that observed within the brains of untreated mice. The combination of VT-1129 at 10 mg/kg and amphotericin B resulted in a >4 log CFU/g reduction in fungal burden.
Conclusions: The metalloenzyme inhibitor VT-1129 in combination with single dose amphotericin B demonstrated potent efficacy in this murine model of cryptococcal meningitis. Both doses of VT-1129 when combined with amphotericin B resulted in significant improvements in survival and reductions in tissue burden with the greatest benefits observed with the higher dose of this investigational agent. These data demonstrate the potential utility of VT-1129 in the treatment of cryptococcal meningitis.

Treatment Groups (BID for VT-1129; p-values v. control)	Median Survival (days)	Percent Survival	Mean Log CFU/g ± SD
Control	16	10%	5.73 ± 0.57
VT-1129 5 mg/kg	25	10%	4.81 ± 1.06
VT-1129 10 mg/kg	>30 (p = 0.002)	80% (p = 0.0055)	3.97 + 0.79 (p < 0.01)
AMBd 3 mg/kg x 1	16	0%	5.11 ± 1.10
VT-1129 5 mg/kg + AMBd 3 mg/kg	28 (p = 0.0176)	40%	3.41 ± 1.05 (p < 0.001)
VT-1129 10 mg/kg + AMBd 3 mg/kg	>30 (p < 0.0001)	100% (p = 0.0001)	1.26 ± 1.40 (p < 0.001)

O95 A phase 1 trial to evaluate the safety and pharmacokinetics of single doses of intravenous solithromycin (CEM-101) in healthy adult subjects

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Objectives: To determine the safety, pharmacokinetics (PK), dose limiting toxicity and maximum tolerated dose of single escalating doses of IV solithromycin. Solithromycin is the first and only fluoroketolide with an oral (phase 2) and IV formulation (phase 1) in development for the treatment of community acquired bacterial pneumonia (CABP) and other infections.
Methods: This was a Phase 1 single-center, randomized, double-blind, placebo-controlled, single IV dose-escalation study in healthy adults. Each cohort consisted of 7 subjects, randomized 5:2 (CEM-101:placebo). Subjects were given IV CEM-101 or vehicle placebo over 1.5 h and remained in the Clinical Research Unit for 48 hours post-dose for safety monitoring and PK assessments, returning on Day 6 for safety assessment. Physical examinations, vital signs, ECGs, clinical laboratory tests, and adverse events (AEs) were monitored. Dose escalation was based on stringent safety criteria. PK was assessed pre-dose, during the infusion and up to 48 hours post-dose.
Results: A total of 42 subjects (six cohorts of 7 subjects) were enrolled in the single ascending dose portion of this ongoing study. 30 subjects received CEM-101 doses of 25, 50, 100, 200, 400, and 800 mg and

10 received vehicle placebo. There were no serious AEs, clinically significant systemic adverse events, QT prolongation or clinically significant laboratory abnormalities. PK (Cmax and AUCinf) appeared linear up to 200 mg and slightly more than dose proportional at higher doses. Clearance decreased with dose. Volume of distribution was large and relatively constant over the dose range.
Conclusions: Solithromycin, the first broad spectrum fluoroketolide, was systemically well tolerated and showed favorable PK in single doses up to 800 mg when given IV, achieving clinically relevant plasma concentrations (~4 mcg/mL). IV exposure was 1.3–3 fold higher than what was observed with equivalent oral doses. Multiple ascending doses (7 days) are under investigation as well as determination of absolute bioavailability. Due to favorable findings in this single dose escalation study, further development is warranted.

Mean Parameter		Dose of Solithromycin [mg] (CV%)					
		25	50	100	200	400	800
C_{MAX}	(ng/mL)	95.7 (12.3)	215 (20.7)	436 (22.8)	888 (21.0)	2260 (16.3)	3780 (22.0)
$t_{1/2}$ (median)	(h)	2.84	3.11	3.5	4.9	4.9	7.26
AUC_{LAST}	(ng·h/mL)	193	487	1285	2978	9170	25400
AUC_{INF}	(ng·h/mL)	260 (18.0)	577 (15.6)	1355 (22.6)	3079 (26.8)	9580 (19.6)	25900 (45.7)
Cl	(L/h)	98.9	88.2	77.3	68.5	43.0	35.2
Vz	(L)	382	474	385	445	323	343

O96 TP-2758 is a novel oral tetracycline targeting complicated urinary tract infections and pyelonephritis

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Background: The rising number of infections caused by multidrug-resistant (MDR) bacteria is a serious public health threat globally. TP-2758 is a novel, fully-synthetic tetracycline that is being developed to specifically target MDR Gram-negative pathogens, including extended-spectrum β-lactamase-producing (ESBL) and carbapenem-resistant Enterobacteriaceae.
Methods: For UTI/pyelonephritis murine models, uropathogenic *E. coli* EC200 or *Klebsiella pneumoniae* ESBL KP453 were inoculated IV and allowed to colonize the kidney. Animals received oral (PO) or IV treatment at 12 and 24 hours post-infection. 36 hours post-treatment initiation, mice were euthanized, kidneys aseptically removed, weighed, homogenized, serially diluted and plated on growth media. CFU/g kidney was calculated and efficacy was expressed as the change in CFUs from infection controls. Pharmacokinetic experiments were performed in rat and *Cynomolgus* monkey at IV and PO doses of 1 mg/kg and 10 mg/kg respectively. Samples were taken up to 24 h post-dose and the plasma concentration of the compound was quantified by LC/MS/MS.
Results: TP-2758 at 50 mg/kg/dose PO produced a 1.49±0.66 log reduction in kidney CFUs versus 1.44±0.42 log reduction for meropenem (30 mg/kg/dose, IV) when mice were challenged with KP453; 20 mg/kg/dose IV TP-2758 was superior to meropenem, producing a 3.78±0.52 log reduction in CFUs. TP-2758 at 2 mg/kg/dose PO produced a 3.07±0.73 log reduction of *E. coli* EC200 versus 3.8±0.22 and 3.1±0.54 log reductions produced by levofloxacin (2 mg/kg/dose PO) and meropenem (20 mg/kg/dose IV), respectively. TP-2758 IV (5 mg/kg/dose) and levofloxacin IV (2 mg/kg/dose) produced 4.05±0.39 and 4.88±0.3 log reductions, respectively. The 1 mg/kg IV AUCinf, Cmax, and half-life in rat/monkey were 1046/5490 ng·h/mL, 551/1343 ng/mL, and 7.5/16.6 h. The 10 mg/kg PO AUCinf, Cmax, and half-life in rat/monkey were 963/16,333 ng·h/mL, 91/751 ng/mL, and 8.4/13.5 h. Oral bioavailability was 8.6% in rat and 30.4% in monkey for TP-2758 compared to oral bioavailabilities of 14.8% and 6.7% for tetracycline in the same respective species. Tetracycline has 60–80% oral bioavailability in man.
Conclusion: TP-2758 showed promising oral bioavailability in 2 species and oral protection equivalent to IV meropenem in UTI/pyelonephritis mouse models. TP-2758 is being evaluated in further studies as a unique IV/oral therapy for treatment of ESBL and carbapenem-resistant Enterobacteriaceae.

O97 Investigation of potential mechanisms underlying transient paraesthesia associated with PMX30063 administration in human subjects

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Objectives: The most frequent adverse event noted in Phase 1 clinical studies with the novel antibiotic PMX30063 consisted of peripheral sensory effects defined as paresthesia. Two potential causes may be direct damage to peripheral neurons or disturbances in ion channel activity. To investigate these possibilities, peripheral nerve electrophysiology studies in rats and in vitro functional assays with cloned ion channels were performed with PMX30063.
Methods: To investigate potential neurotoxicity, rats were administered PMX30063 in the clinical formulation at 3 dosages (n = 7/group) once daily by 1 hour IV infusion for 5 consecutive days. This dosing regimen matched that used in the Phase 1 clinical studies and was designed to achieve blood levels of compound in the rat that were associated with a low (1mg/kg), moderate (3mg/kg) or frequent (10mg/kg) incidence of paresthesia in human subjects. Electrophysiology measures were assessed from sensory and motor neurons at baseline, Day 1, Day 5 and 30 days (recovery group). Nerve conduction velocity (NCV) and compound amplitude were recorded from the caudal nerve, and onset latency and peak amplitude of the compound muscle action potential (CMAP) were recorded from the tibial nerve. To investigate effects on ion channels, patch clamp assays were performed in the presence (0.3, 1.0 or 10uM) or absence of PMX30063 using cell lines expressing human sodium, potassium, acid-sensing or transient receptor potential ion channels.
Results: Exposure to PMX30063 at 10 mg/kg doses for 5 days is associated with a slight (8%) slowing of caudal NCV and no significant change in CMAP following tibial nerve stimulation. Normal function was regained in the 10mg/kg recovery group. No electrophysiology changes were observed at Day 1 in any dose group. Several ion channels were inhibited by PMX30063, reaching >80% inhibition at 10uM. At 1um, current inhibitions were 43.2% (ASIC1a), 56.3% (hNav1.7) and 45.8% (hKv1.6).
Conclusions: Following PMX30063 administration, no significant electrophysiological deficits were observed in motor neurons and in the high dose group after repeat dosing only small transient NCV reductions were observed in mixed sensory and motor neurons that were fully reversible. However, several different classes of ion channels were inhibited in vitro at low concentrations. These results support disturbances in channel function rather than direct neurotoxicity in the paresthesia associated with PMX30063 treatment.

ESBLs from the environment to the clinic

O98 Does the change in the EUCAST ESBL expert rules help the patient?

M. Hoeck, B. Wiedemann for the EPICENTER Network*

Objectives: ESBL producing *E. coli* strains often leave little therapeutic options especially in intensive care units. The therapeutic options seem further limited if the former EUCAST expert rule was used to automaticly convert sensitivity to β lactams to intermediate and intermediate to β lactams to resistant, if an ESBL was recorded for the respective strain. The change for 2010 is: “Cephalosporin breakpoints for Enterobacteriaceae will detect clinically important resistance mechanisms (including ESBL). Some strains that produce β-lactamases are susceptible or intermediate to 3rd or 4th generation cephalosporins with these breakpoints and should be reported as found,

i.e. the presence or absence of an ESBL does not in itself influence the categorization of susceptibility."
We analysed blood culture isolates in order to see if the change in the EUCAST expert rule opens new therapeutic options.
Methods: The laboratory participates in the network using the automated BD PHOENIX-systems measuring MICs. The BD EPICENTER Data-Management-System is used for the evaluation of the data in the laboratory and for the transfer of the data for joint analysis. For this study we analysed the MICs of ESBL producing *E. coli* blood culture isolates in a period from 2006 to 2010. The ESBL production was checked with the associated BD-expert system, as the Phoenix shows excellent performance for ESBL detection (Maurine A. Leverstein-van Hall, et al.: J Clin Microbiol. 2002 October; 40(10): 3703). Copy strains are excluded. Quality control assays are routinely performed.
Results: With the MICs of the ESBL producing strains to ceftazidime (CAZ), cefotaxime (CTX), cefepime (FMC), and aztreonam (ATM) we calculated the sensitivity, using the new EUCAST breakpoints. The table displays that none of the 3rd and 4th generation cephalosporins and azthreonam could be used as treatment in 37% of the cases. However ceftazidime could be an alternative in 60% of all strains, aztreonam in 28%, cefotaxime in 20%, and cefepime in 17% (see table).
Conclusion: There is no doubt, that the knowledge of the underlying mechanism is of great value for epidemiological and infection control reasons. However in the every day routine laboratory, and in favour of the patient the new breakpoins together with a reliable system for the detection of ESBLs is beneficial for the patient, as it opens treatment alternatives and at the same time gives a warning for the infection control.

Sensitivity of ESBL producing E.coli to 3rd and 4th generration cephalosporins and azthreonam	
no alternative	37%
CAZ	29%
ATM CAZ	11%
ATM CAZ CTX FMC	11%
ATM CAZ CTX	6%
FMC	3%
CAZ CTX FMC	3%

O99 The adjusted attributable mortality of extended-spectrum β-lactamase-producing Enterobacteriaceae bacteraemia: a meta-analysis

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Objectives: Bacteraemia caused by Enterobacteriaceae (EB) producing extended-spectrum β-lactamases (ESBL) has been associated with attributable unadjusted mortality compared to episodes caused by non-ESBL-producing EB. Yet, since such analyses depend on observational data, adjustment for confounders, e.g. through multivariate analyses, is necessary. The appropriateness of determinants included, though, depends on the research question. For instance, inadequate empirical therapy and severity of sepsis are in the causal pathway of outcome and adjustment for these parameters can be considered "less appropriate". We performed a meta-analysis of adjusted data and determined the effects of including "less appropriate" determinants in adjustment procedures.
Methods: Studies comparing mortality from bacteraemic infections caused by ESBL-EB and non-ESBL-producing EB were systematically identified in Pubmed. Unadjusted and adjusted odds ratios (ORs) were pooled, and effects of adjustment procedures on adjusted ORs were explored in subgroup analyses.
Results: Of the 139 articles retrieved, 32 (including 9658 patients) allowed calculation of unadjusted ORs for mortality. The pooled unadjusted OR was 2.35 (95% confidence interval (CI) 1.90–2.91, I^2 = 42%). The pooled adjusted OR for ESBL EB versus non-ESBL EB bacteraemia (18 multivariate analyses, including 7 imputed ORs) was 1.76 (95% CI 1.34–2.31, I^2 = 50%). 14 analyses that adjusted for underlying disease found a pooled OR of 1.66 (95% CI 1.22–2.24). 12 analyses adjusting for inadequate empirical therapy found a pooled OR of 1.37 (95% CI 1.04–1.82). Increasing the number of "less appropriate" adjustments was associated with decreasing ORs (Table). Within the set of studies adjusting for underlying disease, two studies performed no "less appropriate" adjustments, resulting in a pooled OR of 2.87 (95% CI 1.57–5.26), and five performed 1 "less appropriate" adjustment and the pooled OR remained above 1 (1.90, 95% CI 1.20–3.02).
Conclusion: In this meta-analysis, ESBL EB bacteraemia is, compared to non-ESBL EB bacteraemia, associated with a higher mortality rate. Adjustment for underlying disease, without adjustment for "less appropriate" variables (severity of sepsis, inadequate empirical therapy), did not change this conclusion. Inadequate empirical therapy is an intermediate in the causal pathway, and adjustment for this variable reduces the association between ESBL production and mortality.

Table. Effects of method of adjustment on odds ratios

	No of analyses	No of patients	OR (95% CI)	I^2	P-value[a]
All studies	18	8811[b]	1.76 (1.34-2.31)	50	
Adjustment for underlying disease					
no	4	905	2.32 (1.15-4.66)	48	0.385
yes	14	7906	1.66 (1.22-2.24)	53	
Adjustment for inadequate empirical therapy					
unclear	1	55	1.00 (0.34-2.94)	0	
no	5	1481	2.77 (2.13-3.60)	0	0.000
yes	12	7275	1.37 (1.04-1.82)	22	
Number of "less appropriate" adjustments (inadequate empirical therapy, sepsis severity)					
0	2	398	2.87 (1.57-5.26)	0	
1	6	1386	2.11 (1.41-3.16)	37	0.069
2	10	7027	1.39 (1.01-1.92)	30	
Number of "less appropriate" adjustments and whether underlying disease was adjusted for					
0, with underlying disease	2	398	2.87 (1.57-5.26)	0	
0, without underlying disease	0	0			
1, with underlying disease	5	1032	1.90 (1.20-3.02)	43	0.043
1, without underlying disease	1	354	3.57 (1.48-8.61)	0	
2, with underlying disease	7	6476	1.24 (0.91-1.69)	13	
2, without underlying disease	3	551	1.99 (0.80-4.95)	52	

[a] P-value of mixed effect analysis. [b] For 3 studies with 2 multivariate analyses, the patient numbers are counted twice. CI: confidence interval

O100 Extended-spectrum β-lactamase-producing *Escherichia coli* bloodstream infections upon hospital admission (ESBL-EC/BSI-UHA): molecular epidemiology and virulence traits

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Objectives: ESBL-EC/BSI-UHA are emerging worldwide. These infections are major concern often associated with severe outcomes. We studied the molecular epidemiology, the CTX-M genes, and the virulence traits of ESBL-EC isolates causing BSI-UHA in our hospital.
Methods: 7-years retrospective data (2003–2009) on ESBL-EC/BSI-UHA was analyzed. 41 representative isolates were subjected to molecular and virulence analysis. Antibiotic susceptibilities were determined by Vitek-2. Genotyping was performed using PFGE and MLST. CTX-M genes, phylogroups and virulence genes were identified by PCR. Virulence of EC strains (n = 20) was evaluated using the *Caenorhabditis elegans* in-vivo model by nematode-killing assays. Statistics of survival was performed using Log-rank test.
Results: Incidence rates of ESBL-EC/BSI-UHA steadily increased along the years from 2.94, to 6, to 7.87 cases/10,000 admissions in 2003, 2007 and 2009, respectively. All isolates were MDR with co-resistance to ciprofloxacin (93%), trimethoprim-sulfamethoxazole (85%) and/or gentamicin (51%). Genotyping revealed for the first time the emergence of sequence type (ST) 131 in Israel, which gradually dominated to become the most prevalent clone (from 25% to 85%), alongside 9 sporadic clones. Except a single isolate (ST2), all isolates belonged to the virulent phylogroups B2 and D. All isolates were CTX-M-producers with the following distribution: blaCTX-M-15, n = 22, (54%); blaCTX-M-14, n = 8, (19.5%); A novel blaCTX-M-2 group-blaCTX-M-97, n = 7, (17%); blaCTX-M-5, n = 1, (2%); blaCTX-M-27, n = 1, (2%). Additionally, 2 isolates produced two CTX-M genes (15 and 97 and 15 and 14).

ST131 isolates, carrying various CTX-M genes, showed higher virulence compared to non-ST131 isolates (an overall of 3.1 virulence genes compared to 1.8, $p < 0.05$).
In *C. elegans* model the median survival was shorter after exposure to ST131 isolates (n = 10) than after exposure to other ESBL-EC clones (n = 10) (5.9±0.32 days vs. 7.3±1.13 days, $p < 0.001$). Exposure to EC OP50 control strain did not cause mortality under these experiment conditions.
Conclusions: ESBL-EC-ST131 has emerged as a major source of BSI-UHA in our hospital. The increased incidence of ESBL-EC/BSI-UHA during the 7 years studied is due to clonal expansion of ST131, carrying either CTX-M-15 or CTX-M-14. ST131 isolates showed enhanced virulence compared to other ESBL-EC clones both in-vitro and in-vivo, demonstrating the major clinical and public health concern of this clone.

O101 Characterisation of extended-spectrum β-lactamase-producing *Escherichia coli* from clinical patients, chicken meat and domestic animals

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Objectives: Extended-spectrum β-lactamase (ESBL) producing *Escherichia coli* are a serious problem worldwide. ESBL producing organisms are isolated not only from hospitalized patients but also from community settings. The aim of this study was to assess the frequency and diversity of ESBL produced *E. coli* and compare them when isolated from different human and animal sources.
Methods: In 2007, *E. coli* isolates from clinical parients and the stools of healthy chickens were collected and screened for ESBLs according to Clinical and Standards Laboratory Institute guidelines. In the case of raw chicken meat, overnight broth culture was performed using in LB broth followed by streaking on the selective CHROMagar ESBL medium. ESBL typing was confirmed by PCR analysis. Multilocus sequence typing (MLST) was carried out following the scheme on the *E. coli* MLST web site (http://www.mlst.net/).
Results: Fifty-six isolates from patients 12 from chicken meat and 11 from chicken stools were confirmed to possess ESBL genes. All ESBL genes belonged to the blaCTX-M group. Predominant ESBL genes from patients, healthy chickens, and chicken meat were blaCTX-M-9 group (69.6%), blaCTX-M-1 group (72.7%) and blaCTX-M-2 group (58.3%), respectively. Interestingly, no blaCTX-M-9 group gene was found in stools of healthy chickens. No blaCTX-M-8 group was detected. Thirty-nine (69.6%) of *E. coli* isolated from patients were related with ST131, the sequence associated with CTX-M β-lactamases genes. Approximately 72% of *E. coli* ST131 from patients (28/39 isolates) were blaCTX-M-9 positive. On the other hand, the frequency of *E. coli* ST131 related isolates from healthy chickens and raw chicken meat were 27.2% (3 isolates) and 8.3% (1 isolate), respectively. Twelve isolates were not categorized in novel STs from web site. blaCTX-M-1 group positive *E. coli* ST117 was isolated from one patient and 3 stools of healthy chicken.
Conclusions: ESBL encoding genes especially blaCTX-M were detected form human patients, healthy chicken and raw chicken meets. The characterization of the ESBL sequences shows no direct evidence for transmission of ESBL producing *E. coli* between chickens and clinical patients.

O102 Prevalence of ESBL-producing Enterobacteriaceae (ESBL-E) in Raw Vegetables

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Introduction: Recent data show that ESBL-producing bacteria are found in Dutch soil samples (Knapp et al., Environ. Sci. Technol. 2010), and in food-producing animals such as broiler chickens and pork meat. We wondered whether ESBL-E are also present in raw vegetables.
Objectives: The aim of this study was to evaluate the presence of ESBL-E in raw vegetables in the region of Amsterdam.
Materials and Methods: Between October 14 and November 29, 2010, samples of 17 different types of vegetables were obtained from the market, and from organic and conventional stores in the region of Amsterdam. We focused on vegetables that grow on and in the ground. Screening for ESBL-E was performed with a selective enrichment broth and inoculation on a selective screening agar, containing cefotaxime and ceftazidime. ESBL production was confirmed with the double disc synergy test with clavulanic acid. Species identification and further antibiotic susceptibility testing were performed with the Vitek-2 system (bioMérieux). DNA-isolation was performed with the QIAamp DNA mini kit (Qiagen). ESBL genes were characterized by microarray (Check-KPC ESBL Check-Points).
Results: Out of 79 analyzed samples, four yielded ESBL-producing Enterobacteriaceae (5%). ESBL-E were found in parsnip, bean sprouts and radish; this means that three (17,6%) of the vegetables types were contaminated with ESBL-E. Of the four positive samples, three were from vegetables of organic origin. The ESBL-producing strains were *Enterobacter cloacae* (in two samples), *Citrobacter freundii* (in one sample) and *Klebsiella pneumoniae* (in one sample). Three strains were positive in the microarray for CTX-M ESBL belonging to the CTX-M-1 family and one for an SHV ESBL. PCR and sequencing are pending.
Conclusion: Our results document the presence of ESBL-producing Enterobacteriaceae in some raw vegetables obtained in Amsterdam, the Netherlands, implying that raw vegetables may be a source of resistance genes. The possible impact of our findings on human health highlights the need to further evaluate the presence of ESBL-E in raw vegetables and to explore whether colonization of the human gut from this source does occur.

O103 Prevalence and characterisation of extended-spectrum-β-lactamase producing *Escherichia coli* isolates in healthy volunteers in Tunisia

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Objective: To analyse the prevalence of ESBL among faecal *E. coli* of healthy humans and to characterize the recovered strains.
Method: 150 faecal samples of healthy volunteers were inoculated in Levine Agar plates supplemented with cefotaxime (2mg/L), and one *E. coli* per positive sample was further studied. ESBL genes and their genetic environment were characterized by PCR and sequencing. Detection of associated resistance genes, MLST and phylogroup typing were performed by PCR and sequencing, and plasmid analysis by PBRT-typing. Presence and characterization of integrons and virulence factors were performed by PCR and sequencing.
Results: ESBL positive *E. coli* isolates were detected in 11 of 150 faecal samples (7.3%) and they contained the following genes: blaCTX-M-1 (10 isolates) and blaTEM-52c (1). The ISEcp1 and orf477 sequences were found upstream and downstream of blaCTX-M-1 gene, respectively, in all 10 isolates. Seven different sequence-types (STs) were identified among CTX-M-1-producing isolates by MLST (number isolates/phylogroup): ST58 (3/B1), ST57 (2/D), ST165 (1/A), ST155 (1/B1), ST10(1/A), ST398(1/A), and ST48 (1/B1). The TEM-52-producing isolate was typed as ST219 and phylogroup B2. Thirteen plasmid replicon types were detected among the 11 ESBL-positive strains, and 9 of them carried three or more replicon types. The plasmid IncI1 was detected in all 10 CTX-M-1-positive isolates but not in TEM-52-producing isolate. Six ESBL isolates contained class 1 integrons with the gene cassettes: dfrA17-aadA5 (5 isolates), and dfrA1-aadA1 (1). Five isolates showed tetracycline resistance and they contained tet(A)±tet(B) genes. Virulence genes detected were (number isolates): fimA (10), aer (8), papG III, papC, hly, cnf, and bfp (none).
Conclusion: A relatively high rate of healthy individuals showed faecal carriage of ESBL-positive *E. coli* isolates, mostly of CTX-M-1 class. The community could be a reservoir of these resistant isolates, representing a problem in human health.

O104 High faecal carriage rates of CTX-M ESBL-producing *Escherichia coli* in the Birmingham area: implications of global origin

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Objectives: The primary objective is to determine the period prevalence of blaCTX-M carrying *Escherichia coli* in the community population of North/East Birmingham, and determine the distribution of blaCTX-M genotypes according to global origin.
The secondary objective is to assess the prevalence of the global pandemic clone 025b-ST131.
Methods: 727 GP and outpatient stool samples sent for investigation were screened for ESBL producing *E. coli* using chromogenic agar and a combination disc method. Multiplex PCR was used to screen for the presence of blaCTX-M, and the individual blaCTX-M genotypes was determined by dHPLC and full gene sequencing. The clonal relatedness of all CTX-M-15 producing isolates was determined by PFGE analysis, and 25 isolates sequence type were determined by MLST and PCR. Isolates were assigned to either 'European', 'Middle East/South Asian' (MESA), or 'Uncategorised' groups using software to determine global origin based on patient name.
Results: Prevalence of blaCTX-M carriage in the sample population was 11.3%. There was a significant difference ($p < 0.0002$) between carriage in the European (8.1%) and the MESA group (22.4%). There was also a significant difference ($p < 0.0002$) in the carriage of blaCTX-M-15 between the two populations. 025b-ST131 accounted for 14 (23.7%) of 59 CTX-M-15 producing *E. coli* isolates. By PFGE the 025b-ST131 isolates constituted one large cluster at the 65% similarity level grouping with the local epidemic strain A. A high diversity of profiles was observed with 41/59 samples displaying unique PFGE profiles at 85% similarity level.
Conclusions: Based on the high community carriage rate and the significant difference in carriage between the groups, it is suggested that antimicrobial therapy for Gram-negative sepsis presenting from the community should take ESBL-producing organisms into account, especially in community patients originating from the Middle East/Asian sub-continent. The pattern of spread of blaCTX-M-15 is a cause for concern and highlights the potential threat that this may be replicated in the future by broader β-lactamases such as NDM-1.

O105 Tracking the presence of class 1 integrons in Gram-negative bacteria collected from surfaces and biological samples in Hospital Infante D. Pedro, Aveiro, central Portugal

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Objectives: The use and misuse of antibiotics selects for multidrug resistant strains. Within the hospital bacteria can colonize the surrounding environment being able to find their way into patients. The aim of the present work was to evaluate the prevalence of class 1 integrons in Gram-negative bacteria collected from surfaces of female ward's furniture and track their dissemination to inpatients within the period of their hospitalization in the Hospital Infante D. Pedro, Aveiro.
Methods: In a female ward, sterile swabs were rubbed in inanimate surfaces, placed in rich medium (TSB), overnight at 37ºC. Serial dilutions were plated in MacConkey agar. In the same period, bacteria were isolated from biological samples (sputum, blood, urine) from inpatients hospitalized in the same ward. The clonal relationship of the isolates was evaluated by rep-PCR and analysed with the GelComparII 5.0 (Applied Maths, Kortrijk, Belgium). Identification and antibiotic susceptibilities were determined using the automatic VITEK2 system and Advanced Expert System (VITEK2 AES) (BioMérieux, Marcy L'Etoile, France). Identification to the species level was confirmed by 16S amplification. Presence and characterization of class 1 integrons and β-lactamases enzymes were performed by PCR. Nucleotide and deduced aminoacid sequences were analyzed with Blast and ClustalW programs. PFGE of S1 digested total DNA followed by Southern blotting hybridization using intI1 DIG labelled probe was performed to locate the class 1 integrons.
Results: New class 1 integrons, never described before in Portugal, were identified. The same gene arrays were found in biological samples and also in inanimate surfaces. The most prevalent species found were *E. coli*, *C. freundii*, *K. pneumoniae*, *P. aeruginosa* and *E. cloacae*. These isolates were multidrug resistant (β-lactams, aminoglycosides, trimethoprim/sulphamethoxazole and quinolones). S1 digested genomic DNA and PFGE analysis revealed the presence of plasmids in some isolates. Hybridization with intI1 DIG labelled probe was unsuccessful, thus integron location was not determined yet. β-lactamases enzymes (TEM, SHV, CTX-M, OXA and VIM) are present.
Conclusion: Dissemination of resistance determinants by horizontal gene transfer is facilitated between bacteria within the hospital environment and can therefore infect the inpatients. These results are worrisome, since all the isolates studied are multidrug resistant.

O106 Clonal group 025b-ST131 accounts for more than 90% of clinical isolates of CTX-M producing *E. coli* from residents of 26 nursing homes in one region

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Objectives: The association of CTX-M-15 with the O25b-ST131 clonal group of *E. coli* has contributed to its dispersion worldwide. The objective of this project was to examine the prevalence of this clonal group among CTX-M producing *E. coli* isolated from nursing home residents in one region of Ireland.
Methods: Forty eight isolates of CTX-M (CTX-M group 1 ($n = 43$; CTX-M group 9 ($n = 5$)) producing *E. coli* were collected from routine clinical specimens submitted from residents of 26 nursing homes in one region of Ireland between January 1st and June 30th 2010. PCR was performed on all 48 isolates using primers specific to the pabB (region specific to the 025b-ST131 clone) and trpA genes (to confirm quality and amplification of DNA) as previously described (Clermont et al., 2009, 64:274–77). Pulsed field gel electrophoresis (PFGE) was performed with XbaI by the Pulse-Net protocol.
Results: An amplicon specific to the trpA gene was identified in all isolates, and an amplicon specific to the pabB gene was identified in 46 (96%) isolates. By PFGE analysis 39 individual pulsed field profiles (PFPs) and 6 major clusters (A-F) were identified among the 48 isolates examined based on a similarity of >85%. With the exception of 2 isolates (both cluster F isolated from residents of the same nursing home) all PFPs were closely related with a similarity of >71%. These 2 isolates were negative for the region of pabB specific to the 025b-ST131 clone.
Conclusion: This data provides evidence that the 025b-ST131 clone of *E. coli* is widely disseminated in nursing homes in one region of Ireland and that this clonal group accounts for the vast majority (96%) of CTX-M *E. coli* clinical isolates from nursing homes. PFGE discriminates between members of the 025b-ST131 and can therefore be useful for sub-typing within the clonal group for epidemiological purposes. Our data suggest that members of the 025b-ST131 clonal group cluster as >70% similar by PFGE.

O107 *Klebsiella pneumoniae* sequence types originally associated with specific ESBLs might act as substrates for recently emerged metallo-β-lactamases or KPC enzymes

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Background: *Klebsiella pneumoniae* (Kp) represent an important reservoir of β-lactamases both in the hospital and in the community settings. They have been associated with several nosocomial outbreaks and global dissemination of extended-spectrum-β-lactamases (ESBLs), metallo-β-lactamases (MBLs) or KPC enzymes. Population structure studies in this specie using an MLST typing scheme are increasingly being reported. This was further analyzed in a collection of different

β-lactamase producing Kp recovered in our institution from 1989 to 2010.

Methods: 125 Kp clinical (101) and surveillance (24) isolates with different ESBLs (19 SHV-12, 34 TEM-4, 23 CTX-M-10 and 13 CTX-M-15), MBLs (22 VIM-1) and KPC (2 KPC-2 and 12 KPC-3) and clustered in 46 PFGE-XbaI patterns were further selected for MLST typing. Allele sequences and sequence types (STs) were assigned at www.pasteur.fr/recherche/genopole/PF8/mlst/.

Results: 80 Kp-ESBL representing 38 PFGE patterns were classified in 26 STs. ST37 (11 TEM-4), ST385 (11 SHV-12) and ST16 (9 CTX-M-15) grouped the highest number of isolates. Three ST clustered isolates with different ESBLs: ST13 (1990–2005; CTX-M-10, CTX-M-15 and TEM-4); ST14 (1995–2004; SHV-12, TEM-4); ST37 (2001–2005 SHV-12, TEM-4). The rest of STs were linked to single ESBL-types. Twenty-two VIM-1 producers were clustered in 3 pulsotypes corresponding to 3 STs, 18 of them belonging to ST39 which were responsible of a nosocomial outbreak whereas the remaining isolates were grouped into ST163 and ST253 associated with CC23. KPC isolates were resolved in 3 PFGE types classified in 3 ST (12 ST384 responsible of an outbreak of KPC-3 producing isolates and one each ST388 and ST20 with KPC-2). Interestingly, ST20, ST388 and ST39, originally associated with CTX-M-10 and SHV-12, were recently described among isolates producing KPC-2, KPC-3+CTX-M-10 and VIM-1+SHV-12, respectively.

Conclusions: ESBL, MBL and KPC enzymes are expressed in our institution among different *K. pneumoniae* MLST genetic lineages. Nevertheless, some ST originally associated with specific ESBL types could act as substrates for VIM-1, KPC-2 and KPC-3 enzymes that have recently emerged in our institution. This demonstrated the importance of these clones in the capture and maintenance of different β-lactamase determinants.

Pneumococcal vaccination with focus on NTHi

S112 Epidemiology and burden of disease due to *S. pneumoniae* and NTHi

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Acute respiratory tract infections (RTIs) comprise a wide spectrum of disease, including sinusitis, bronchitis, pneumonia and otitis media (OM), and confer a large burden on society. *Streptococcus pneumoniae* and non-typeable *Haemophilus influenzae* (NTHi) are major causative pathogens in RTIs, with particular dominance in acute OM (AOM). AOM caused by *S. pneumoniae* is somewhat more severe and can be distinguished by a higher body temperature, more inflamed ear drum and an increased inflammatory and white blood cell response.[1] NTHi tends to be more commonly associated with bilateral, recurrent and non-responsive AOM.[2] However the clinical picture is too indistinct to permit a differential diagnosis in the clinical setting.

It has long been thought that both *S. pneumoniae* and NTHi are involved in similar ways in AOM. However, new evidence is emerging on various stages of AOM (i.e. simple AOM, recurrent AOM, non-responsive AOM) and its bacteriology, much of which has been derived from prospective studies on AOM and vaccine studies. In a study of over 5000 cases of bacteriologically proven cases of pneumococcal AOM in Southern Israel, 40% were in fact mixed pneumococcal-NTHi infections (M-AOM) and only 60% were single-pathogen pneumococcal AOM (S-AOM). Further analysis demonstrated that M-AOM was associated with crowded populations, recurrent and non-responsive cases, older age and bilaterality, consistent with a more prolonged or complicated course. Moreover, pneumococcal serotypes appeared to differ between S-AOM and M-AOM. These findings suggest that some virulent pneumococcal strains cause more S-AOM than M-AOM cases, and that in M-AOM somewhat less virulent pneumococcal strains are found in conjunction with NTHi.

Mixed infections are more frequently found in more complex (i.e. recurrent and non-responsive) OM. One reason for this could be the presence of biofilm, which is often polymicrobial and renders bacteria non-responsive to treatment. Recent data in a chinchilla model of AOM suggesting a passive protective effect of NTHi towards *S. pneumoniae* due to biofilm and β-lactamase production support the intractable nature of such dual infections.[3] Observations made in AOM might provide insights on how these pathogens behave and interact in RTIs in general.

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S113 Current management and treatment guidelines: their implications and the status of antibiotic resistance in Europe

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Antimicrobial resistance in *Streptococcus pneumoniae* has changed over time and is the result of a complex interplay between factors. Important factors to consider are antibiotic use, population density, spread of resistant clones and, more recently, the effects of pneumococcal conjugate vaccination. Many studies have examined the links between these factors, the use of specific antibiotics and resistance to related and unrelated antibiotic classes. The insights generated by these studies have affected treatment guidelines for respiratory infections.

As a community-acquired respiratory pathogen, non-typeable Haemophilus influenzae (NTHi) is second in importance to *S. pneumoniae*. Particularly in Japan and neighbouring regions, β-lactam-resistant NTHi strains have rapidly attained high levels of prevalence,[1] leading to antibiotic treatment challenges for infection, such as recurrent or chronic otitis media. Unlike *S. pneumoniae*, these highly β-lactam-resistant NTHi will not respond to increased doses of aminopenicillins or oral cephalosporins. The observations in Japan raise the concern of spread of highly resistant and epidemic NTHi clones. Data from some European countries indeed suggest a similar development.[2],[3] However, regular NTHi surveillance is lacking in many countries, and there are conflicting data on which antibiotics are driving this increase in resistance. Should the above trends in accelerated resistance be confirmed in Europe, this would profoundly affect current treatment guidelines for respiratory infections.

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S114 Potential role of vaccination in decreasing antibiotic use

P. Marchisio (Milan, IT)*

Respiratory tract infections (RTIs) are the most commonly encountered diseases in infants and children. *Streptococcus pneumoniae* is a major bacterial pathogen, being the main cause of, among others, acute otitis media (AOM) and community-acquired pneumonia (CAP). These diseases, in particular AOM, are the main drivers for antibiotic prescriptions in young children, both in Europe and in the USA.

In clinical efficacy studies, different pneumococcal conjugate vaccine (PCV) formulations have demonstrated benefit against different RTIs. Since the introduction of the heptavalent PCV (PCV7) in the USA in 2000, significant reductions in the rates of RTI visits (in particular AOM), pneumonia admissions and RTI-related antibiotic prescriptions have been reported.[1,2] In addition, PCV7 has been associated with changes in the pathogenesis of RTIs, with proportionate increases in the importance of non-vaccine serotypes as well as other microorganisms, in particular non-typeable *Haemophilus influenzae*. Similar trends have been observed in Italy, where PCV7 has also been found to be effective in the prevention of AOM and CAP and in reducing the number of antibiotic prescriptions.[3] The potential benefits of vaccination could be taken into account in clinical guidelines for the management of RTIs, in terms of lessening the indiscriminate use of antibiotics and lessening selective pressure for resistance.[4] Similar to other European countries, various attempts have been made in Italy to define the most appropriate ways to manage RTIs. Recently this gap was filled by the Italian Society of Pediatrics, which coordinated the preparation of the national Italian Multidisciplinary guideline for AOM;[5] a similar guideline for CAP is currently underway. These guidelines are based on the broad participation of representatives of different specialties (paediatricians, otolaryngologists, pharmacologists, allergologists, microbiologists, etc.) and are aimed at translating the newly acquired knowledge on RTIs, antibiotic resistance and the consequences of old and new pneumococcal vaccines into precise recommendations for clinical practice, also taking into account the peculiarities of microbiological data in the Italian setting.

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S115 The need for improved surveillance of invasive bacterial disease

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Surveillance of invasive bacterial diseases (IBD) caused by *Neisseria meningitidis*, *Haemophilus influenzae* and *Streptococcus pneumoniae* is important to monitor epidemiological trends, circulating strains and antimicrobial resistance. Following the widespread introduction of conjugate vaccines for *H. influenzae* type b (Hib), *N. meningitidis* serogroup C and multiple-valency pneumococcal conjugate vaccines (PCVs), there has been a dramatic decrease in the occurrence of diseases caused by vaccine-preventable strains.[1,2] However, monitoring of IBD caused by these organisms to assess the impact of vaccination programmes, detect vaccine failures and to assess any geographical or temporal variations remains a necessity. It is also important to monitor circulating strains to investigate evidence of disease replacement by non-vaccine-preventable strains, including non-vaccine serotypes of pneumococcus, non-b capsulated *H. influenzae*, non-typeable *H. influenzae* (NTHi) and non-Group C meningococcus, which in many countries now constitute the majority of invasive infections caused by these three organisms. For example, in the post-Hib era in the UK, NTHi emerged as a leading cause of remaining *H. influenzae* invasive disease.[3]

The EU-IBIS surveillance network (funded by EU DG SANCO) collected data on invasive meningococcal and *H. influenzae* disease between 1999 and 2007. From 2008, laboratory surveillance and external quality assurance for meningococci, *H. influenzae* and pneumococci has been organised by IBD-labnet (funded by ECDC). The data generated by these networks indicate that IBD surveillance varies widely between countries in terms of completeness, the age of the population surveyed, the clinical presentations included, the level of identification of isolates and the geographical area covered. In addition, surveillance systems in some countries have changed over time; comparisons between and within countries should therefore be viewed with caution.

There is a need for continued large scale clinico-epidemiological surveillance to collect comparable data on IBD, particularly those that are potentially vaccine-preventable. Standardised questionnaires examining clinical presentations, underlying risk factors, clinical management and attributable death would contribute to a fuller understanding of IBD caused by these pathogens.

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Genetics and epidemiology of mobile resistance: mobilome and resistome

S124 Transposable elements in mobilisation and spread of resistance genes in Gram-negatives

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At a time when the clinical fraternity is lamenting the lack of novel, even new, worthy antimicrobials in the Pharmaceutical pipeline, MDR bacteria are now consistently reported in most countries – a situation which 15 years ago would have been unimaginable. Human travel and globalisation aside, the relentless evolving of bacteria to MDR, and some pan-drug resistance, status is a direct result of the bacteria's ability to mobilise and ultimately share DNA, a phenomenon called horizontal gene transfer which occurs considerably more frequently in Gram-negative bacteria. Intrinsic to this DNA plasticity are transposable elements; mobile sections of DNA capable of moving themselves and adjacent DNA which in this case involves antibiotic resistance genes.

The emergence of MDR in the early clinical isolates was due to the fact that genetic engineering tools and resistance mechanisms were already present at the beginning of the antibiotic era of the 1950's and 1960's. In the 1970's MDR was determined in many cases to be associated with transmissible plasmids and transposons, and in 1989 the class 1 integron was first described. The resistance genes carried by these elements have also evolved in response to increasingly broader-spectrum antibiotics e.g. TEM-1 in the 1960's to NDM-1 in 2010. However, even before the introduction of sulpha drugs and penicillin, the use of mercury in industry, agriculture and medicine probably aided and abetted the establishment of mercury resistance transposons e.g. Tn21 that are now prevalent and mobilise MDR regions.

We now appreciate the evolvement of class 1 (including 2 and 3) integrons in MDR phenotypes but have also recently witnessed the contribution of their ancestor Tn5050 which has made a renaissance and has shown to be active as a transposon. Coupled to one end of some of the classic class 1 integrons are Tn21 transposons allowing

inter-plasmid/chromosome movement. Five years ago, ISCR elements were but an academic curiosity but have now been shown to be instrumental in moving huge sections of bacterial DNA carrying many antibiotic resistance genes and pathogenicity factors. ICEs can also move DNA using one-ended transposition. This presentation will review the definition and classification of transposons and provide recent examples of antibiotic resistance gene transfer which are the most clinically relevant.

New aspects of antimicrobial treatment of anaerobic infections

S127 Antimicrobial resistance in *Clostridium difficile*

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Clostridium difficile has been identified as one of the major causative agents of antibiotic-associated diarrhoea. The antibiotic susceptibility of *C. difficile* strains, including epidemic clones, is changing. Most of the prevalent types responsible for disease in humans were resistant to clindamycin and/or erythromycin due to an ermB gene encoding for an rRNA methyltransferase, whereas, currently, we are observing an increase in the number of resistant ermB-negative *C. difficile* isolates, including the epidemic strain *C. difficile* BI/NAP1/027. Resistance to tetracycline has also been observed in both historic and recent isolates. Tetracycline resistance is commonly conferred by a tet(M) gene that encodes for a ribosomal protection protein. This gene is usually carried by a Tn5397 transposon, but recent strains can harbour elements belonging to the Tn916 family, which are widely dispersed in Gram-positive and negative bacteria. Moreover, *C. difficile* clinical isolates resistant to fluoroquinolones have recently appeared and their number is on the rise too. In particular, resistance rate to moxifloxacin has increased dramatically over the last years due to amino acid substitutions in the DNA gyrase subunits GyrA or GyrB. The current standard treatment for *C. difficile* infection is oral metronidazole or vancomycin, the latter as the antibiotic of choice for severe cases. Few *C. difficile* strains resistant to metronidazole have been described in the world, but recently an increase in minimum inhibitory concentrations and heteroresistance have been observed. The exact mechanism of reduced susceptibility to metronidazole remains to be determined since this pathogen does not show nitroimidazole genes, commonly associated with this resistance in other bacteria. Antibiotic resistance is important in infection, as it would provide *C. difficile* a competitive growth advantage in the gut of patients after antibiotic treatment. A comprehensive picture of different phenotypic and molecular characteristics of the antibiotic resistance in a very large sample of clinical isolates collected during an European survey of *C. difficile* infection will be presented.

S128 *Bacteroides*, *Prevotella* and *Porphyromonas* are becoming more resistant to antimicrobial agents

E. Nagy on behalf of the ESGAI*

The most frequent anaerobic pathogens are the members of the *Bacteroides*, *Prevotella* and *Porphyromonas* genus. Among *Bacteroides* strains, chromosomally mediated β-lactamase production and tetracyclin resistance are most prevalent world-wide. Resistance to β-lactam/β-lactamase inhibitor combinations has been increasing throughout the years both in the US and in Europe. The same is true for cefoxitin, and heteroresistance can be observed among isolates. The resistance to clindamycin is mediated by a macrolide-lincomycin-streprogramin mechanism; its frequency differs from country to country in Europe. The occurrence and spread of resistance to imipenem and metronidazole among *Bacteroides* strains, and their expression mechanisms, merit special clinical attention. The presence of the cfiA gene responsible for carbapenem resistance is much more prevalent than the expression of high-level imipenem resistance. Nim genes are responsible for the metronidazole resistance. High-level resistance can easily be induced in nim-positive strains by culturing in the presence of a subinhibitory concentration of metronidazole. Moxifloxacin resistance has proven to be increasing in all countries. The lowest rate of resistance was observed in the case of tigecycline in Europe.

Prevotella and *Porphyromonas* strains are usually considered more susceptible to anti-anaerobic drugs than *Bacteroides*, though surveillance data on the susceptibility of these organisms are rather limited. About 50% of *Prevotella* species are resistant to ampicillin, due to different β-lactamases, including those coded by the cfxA gene. The resistance of *Porphyromonas* to ampicillin is much lower (5–10%). β-lactam/β-lactamase inhibitor combinations, carbapenems and metronidazole are uniformly active against most isolates of both genera, but some *Prevotella* isolates harbor nim genes and demonstrate high level of resistance to metronidazole. Clindamycin resistance among *Prevotella* and *Porphyromonas* isolates is rare. The spread of tetracycline resistance can be discerned in both genera, mostly due to the presence of the tetQ gene. Tigecycline, which can overcome resistance mechanisms against tetracycline, is highly active and the same is true for moxifloxacin. To assess the real importance of the development of resistance in *Prevotella* and *Porphyromonas* is difficult, due to the lack of regular antibiotic sensitivity testing in routine laboratories and to the infrequency of surveillances.

Q fever: a major threat in Europe

S130 Q fever in the Netherlands: epidemiology of the largest ongoing European outbreak

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Objectives: Since 2007 the Netherlands is facing an epidemic of Q fever with a size that is unprecedented in the world. Q fever has become a major public health problem with political implications and has led to drastic veterinary measures, including the large-scale culling of pregnant goats on infected farms. The presentation will give the latest figures from the surveillance of Q fever and results from ongoing studies, primarily those focusing on risk factors for human disease.

Methods: Monitoring of the epidemic is based on mandatory notification of acute Q fever (fever, or pneumonia, or hepatitis with laboratory confirmation). To fill the remaining knowledge gaps there is an extensive ongoing research agenda, including at least 18 PhD projects, covering such fields as host-pathogen characteristics, transmission and risk factors, and chronic Q fever.

Results: Epidemiological studies show that living close (<2km) to a large dairy goat farm where an abortion wave due to *Coxiella burnetii* has occurred, is the most important risk factor for human Q fever. Seroprevalence among the general population has increased from 2.4% before the first outbreak to around 12% in the high incidence area in 2009. The number of notified acute Q fever patients went down from 2354 in 2009 to 508 in 2010. Because several veterinary measures were implemented at the same time, it is difficult to establish the relative contribution of vaccination, culling, and hygiene measures to this decline in incidence.

Conclusion: Despite the veterinary measures that were implemented, and the reduced incidence of acute Q fever in 2010, Q fever is not expected to disappear from the Netherlands, because of the long-term survival of *C. burnetii* in the environment and the occurrence of the bacterium in other animals than goats. A major challenge ahead is early detection and treatment of patients who are at risk for chronic Q fever, and developing effective treatment strategies for patients with other sequels of acute Q fever.

S131 Q fever in animals: epidemiology, diseases in animals and zoonotic risk

A. Rodolakis on behalf of the ESCMID Study Group for Coxiella, Anaplasma, Rickettsia and Bartonella (ESCAR)*

Coxiella burnetii, infects a wide range of wild and domestic animals including mammals, marsupials, birds, reptile and arthropods all around the world. Lice, mites and parasitic flies are also infected.
There is consensus among public health and veterinary professionals that most of the human Q fever outbreaks are linked to small ruminants, however, other species in the vicinity of livestock (dogs of herds, rodents, migratory birds, ticks, ...), could play a role as secondary reservoirs.
In ruminants infection is mostly asymptomatic but, especially in small ruminants, C burnetii can induce abortions, stillbirth or premature delivery. High rates of abortion have been observed only in goats. In cattle, abortions are sporadic and metritis and infertility are frequently the main clinical signs.
Infected ruminants shed C burnetii in huge quantities into parturition products, during abortion and normal delivery, and smaller numbers in faeces, urine, vaginal mucus, or milk. This shedding may persist over several months particularly in milk. However vaginal and faecal bacterial discharges have the major impact on environmental contamination.
The transmission of infection is mostly associated with abortion of sheep and goats. The infection is mainly due to inhalation of aerosolized bacteria or contaminated dust resulting from contaminated manure and desiccation of infected placenta and body fluids. Farmers, veterinarians and slaughterhouse workers may be infected through occupational exposure, but direct contact with animals is not required, handling contaminated wool, manure, or clothes contaminated with faeces are sufficient to become infected. Transhumance of infected flocks and increased urbanisation in rural areas are hypothesised as contributing factors to human outbreaks. In addition the environmental survival of C burnetii allows it to be transported by wind far away from the original source. C burnetii DNA was detected in the soil of farms but also in urban areas, brough by wind or birds.
Dogs and cats infected by aerosol inhalation, by tick bite or by feeding on infected placentas shed C burnetii in urine, milk and birth products. Ticks are considered to be a reservoir and vector of C burnetii in several countries. They transmit the agent via bite or feces to birds, rodents or ruminants. Infected domestic poultry can transmit Q fever to humans through aerosolized fomites.
Vaccination of ruminants is currently the best way to prevent human Q fever.

S132 Q fever: Prevention and treatment issues during an outbreak

A.M. Horrevorts, M.H. Nabuurs-Franssen (Nijmegen, NL)*

Since 2007, the Netherlands has experienced the largest Q fever outbreak ever. From 2007 till today more than 4000 patients have been reported. Q fever is a zoonosis caused by *Coxiella burnetii* that occurs worldwide. The most common animal reservoirs are goats, sheep and cattle. These animals, when infected, shed the bacteria in urine, feces, milk and birth products. *C. burnetii* is a highly infectious bacterium. Humans are infected by inhalation of contaminated aerosols or ingestion of contaminated milk or milk products. Diagnosis is based on serological tests and PCR's. Infection may lead to asymptomatic seroconversion (60%), acute disease (40%) or chronic infection (1–2% in NL). In the Dutch outbreak, an association with intense goat farming was suggested. One MLVA type of *C. burnetii* prevails on Q fever-positive dairy goat and diary sheep farms, indicating clonal spread. The outbreak prompted preventive regulations: Hygiene measures were instituted on farms, a ban of the spread of manure was issued, vaccination of goats was ordered, testing for *C. burnetii* in bulk milk was started, breeding on infected farms was prohibited and finally all pregnant goats and sheep on infected farms were culled. The decreasing numbers of human cases in 2010 might indicate that these measures have been effective. Special attention was paid to infected pregnant women concerning treatment during pregnancy and hygiene measures during birth. A retrospective study showed so far no significant correlation of seropositivity for Q fever during early pregnancy and adverse pregnancy outcome. In the Netherlands blood donations are not regularly tested for the presence of *C. burnetii* and/or antibodies against the bacteria. A prevalence study is ongoing.
A vaccine against *C. burnetii* has been manufactured. In the Netherlands, vaccination is now available for patients with underlying cardiac and vascular diseases without immunity against *C. burnetii*.
Acute Q fever is frequently self-limiting. When treatment is needed in acute Q fever, doxycycline is still the drug of choice as is co-trimoxazole in children and pregnant women. The newer quinolones such as moxifloxacin have also been shown to be effective against *C. burnetii*. Treatment of chronic Q fever is difficult, prolonged, requires monitoring of serum levels of the drugs used, and in special cases surgical intervention.

S133 *Coxiella burnetii*: lessons from genomics

D. Raoult (Marseille, FR)*

Coxiella burnetii is an intracellular bacterium causing Q fever. The genomic sequence carried out of *Coxiella burnetii* could make it possible to obtain significant advances. Firstly, the techniques of molecular genotyping, based on the sequence, allow defining the microbial groups showing that if all species must be associated with chronic infections, only a selected group of species appear to be causing acute Q fever. This chromosomal genotyping is coherent with the genotyping plasmid. In addition, the availability of the genome made it possible to do genomotyping which finds the same classification. This genotyping made it possible to identify *Coxiella burnetii* on the strain level and this type of genotyping was set up in order to show the clonality of the epidemic currently prevailing in the Netherlands. On the epidemiologic level, the genomotyping made it possible to highlight that a group of bacteria was specifically related to the ticks, and which found in this group all the bacteria found in the hard ticks, but also isolates obtained in animals or humans. It appears, based on this work, that the role of the ticks in the epidemiology of *Coxiella burnetii* was underestimated. Lastly, the study of the genome made it possible to do exhaustive proteomic studies and to define the best conditions allowing in vitro culture. This work made it possible to determine that *Coxiella burnetii* was microaerophilic, and that its medium had to be supplemented, which brought to the realization of axenic mediums now allowing the easier culture of *Coxiella burnetii*.

Opportunistic infections in solid organ transplant recipients

S134 Human cytomegalorvirus infection in transplant recipients

P. Grossi (Varese, IT)*

Human cytomegalovirus (CMV) was the first "opportunistic" virus described in renal transplant recipients under azathioprine and prednisone. It has been the most frequent cause of infectious complications after solid organ transplantation with all subsequent immunosuppressive regimens. In seronegative recipients, latently infected allografts and leukocyte containing blood products are documented means of virus transmission. Among pre-transplant CMV seropositive transplant recipients, CMV infection may occur after reactivation of latent infection or after reinfection. Active CMV infection occurs during the first three months after transplantation and may be accompanied by a broad spectrum of disease manifestations. The primary infection is usually more severe than reactivation or superinfection. Antiviral prophylaxis and pre-emptive therapy are similarly effective in preventing CMV disease after transplantation. However, current guidelines prefer antiviral prophylaxis over pre-emptive therapy in preventing CMV disease in high-risk SOT recipients. Antiviral prophylaxis is preferred due to the potential benefit of reducing not only the incidence of CMV disease, but also the indirect

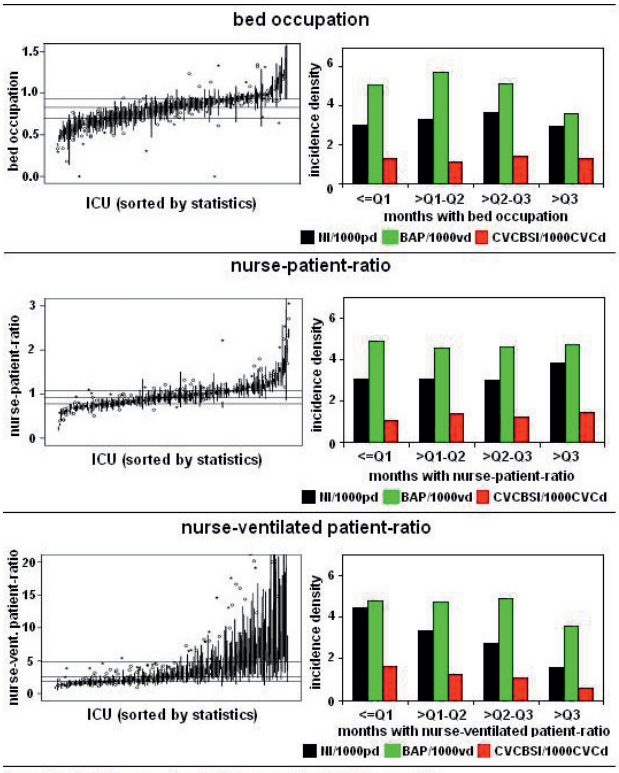
effects of CMV on allograft and patient survival. The major drawback of antiviral prophylaxis is delayed-onset CMV disease which has been significantly associated with allograft loss and mortality. Pre-emptive therapy consists of weekly laboratory monitoring to detect CMV in the blood using quantitative PCR, prior to the onset of symptoms. Once CMV is detected, pre-emptive therapy is initiated to prevent the progression of asymptomatic infection to clinical disease. The potential advantage of pre-emptive therapy is the exposure of the host's immune system to CMV, thereby allowing earlier CMV-specific T-cell reconstitution, which could potentially explain the very low rates of late-onset CMV disease with this approach. The success of pre-emptive therapy is highly dependent on the consistency of collecting weekly specimens and the predictive value of the assay used. Undetectable viraemia should be achieved prior to discontinuation of therapy in order to reduce the risk of virological relapse.
In conclusion, the current availability of powerful antiviral agents, given as prophylaxis or pre-emptive treatment, has dramatically reduced the rate of CMV disease after solid organ transplantation. However, there is a lack of consensus on the best strategy to be used.

Can we decrease hospital-acquired infections in "real" life?

O138 Understaffing, overcrowding, inappropriate nurse-to-ventilated patient ratio and nosocomial infections – which parameter reflects deficits best?

F. Schwab, P. Gastmeier, C. Geffers, E. Meyer (Berlin, DE)*

Objective: We investigated the impact of nurse to patient ratio, nurse to ventilated patient ratio and bed occupancy on nosocomial blood stream infections (BSI) and pneumonia in 182 intensive care units. In stressed and high-throughput systems, periodic overcrowding (high bed occupancy) and understaffing (low nurse to patient ratio) are widely described risk factors in infection control. We compared the impact of these well known parameters with a new parameter (nurse to ventilated patient ratio).



legende: Q1, 25% percentile; Q2, 50% percentile; Q3, 75% percentile

Methods: ICUs reported data on device use and nosocomial device-associated infections to KISS which is the German hospital surveillance system for nosocomial infections and we analyzed data from the year 2007. Information of the number of health care workers on the ward per 24 hours in 2007 and structure parameters (type and size of ICU and hospital) was obtained by questionnaires. We categorized the parameters by (a) the quartiles in each ICU and by (b) the quartiles in all ICUs and analysed association of the number of nosocomial infections per month by generalized linear regression models based on Poisson distribution.
Results: 182 ICUs provided data on all parameters. Of these KISS-ICUs 1,921 months (10.6 month per ICU) were analysed including 159,400 patients with 563,177 patient days, 1313 pneumonia cases (of them 1064 were ventilator associated) and 513 BSI cases (491of them were central venous catheter associated). Less nosocomial infections were associated with more nurses per ventilated patient. Interestingly, the nurse-patient ratio did not reveal to be a significant parameter with respect to the occurrence of nosocomial blood stream infections and pneumonia and a high bed occupation (above the 75% percentile of all ICUs) was associated with less nosocomial infections.
Conclusion: The estimation of thresholds (of parameters) above which the infection risk increases would be the paramount importance for administrators and policy makers. Formal economical evaluation is needed, which will probably demonstrate that the benefit resulting from cutting down on nursing resources is by far outweighed by the cost of nosocomial infections attributed to staff shortages.

O139 Selection of qacA carriage in MRSA clonal complex 22 but not 30 during a successful infection control programme incorporating chlorhexidine-based decolonisation

R. Batra, E. Halligan, P. Cliff, J. Otter, J. Edgeworth (London, UK)*

Objectives: A chlorhexidine (CHX) based methicillin-resistant *Staphylococcus aureus* (MRSA) decolonisation policy was introduced into our intensive care unit (ICU) in 2004. This led to a 70% reduction in transmission of endemic MRSA (predominantly clonal complex (CC)30 and CC22), which had low carriage rates of the antiseptic resistance gene qacA (<10%), but no effect on an outbreak strain (sequence type (ST)239-TW) carrying qacA (Batra R et al. Clin Infect Dis 2010;50:210–217). Around the same time a hospital-wide heightened infection control programme incorporating CHX decolonisation policy for MRSA carriers was introduced which led to a 90% reduction in MRSA bacteremias. The aim of this study was to assess whether qacA selection has occured in dominant UK MRSA clones following implementation of CHX decolonisation policies.
Methods: ICU MRSA acquisition isolates during one year before and one year after introduction of the CHX policy and 399 consecutive hospital-wide CC22 and CC30 MRSA bloodstream isolates obtained between 2001 and 2009 were analysed. Discriminatory qacA and qacB genotyping was performed using a single nucleotide polymorphism genotype assay. Clones were defined using Based Upon Repeat Pattern clustering of spa type and staphylococcal cassette chromosome mec allotype or restriction modification typing.
Results: qacA carriage by ICU-acquired CC22 and CC30 isolates increased in CC22 but not CC30 following implementation of the CHX policy (1/14 before and 3/4 after ($p = 0.028$) v. 1/20 before and 1/9 after ($p > 0.05$), respectively). qacA carriage by bloodstream CC22 and CC30 isolates in 2001 and 2002 was low (5–10%), but from 2003 there was a significantly increased carriage of qacA in CC22 (range 24–37% per annum OR 1.25 95% CI 1.07–1.45; $p = 0.003$), but not CC30 (<10%), associated temporally with the decline in MRSA bloodstream infection rates. From being the dominant cause of bloodstream infection CC30 declined more rapidly than CC22, a phenomenon observed nationally.
Conclusions: These observations provide the first evidence of qacA selection in response to intensive CHX use in some but not other MRSA clones. Selection for qacA in CC22 but not CC30 may help explain changing national epidemiology and provides further evidence that CHX use has been an important component of MRSA control programmes.

The emergence of qacA positive strains is a potential concern for effectiveness of MRSA decolonisation policies.

O140 First global survey on hand-hygiene compliance before patient contact – Results from 47 countries

B. Allegranzi, H. Sax, F. Eggimann, L. Pievaroli, H. Attar, N. Colaizzi, C. Kilpatrick, D. Pittet (Geneva, CH)*

Objectives: The WHO SAVE LIVES: Clean Your Hands (SLCYH) is a global campaign launched by the Clean Care is Safer Care programme; it supports sustained hand hygiene (HH) improvement worldwide by promoting registrations of healthcare facilities in this global movement and providing guidance and tools. Global call for action is renewed every year through the participation in the 5 May initiative, a day focused on HH in health care worldwide.
Methods: In March 2010 all SLCYH registered facilities were invited to participate in a global survey on or around 5 May 2010 by observing HH compliance with the indication "Before touching a patient" (Moment 1; J Hosp Infect 2007;67:9–21) for one or few days and to submit their data to WHO. A simple, validated form and instructions for observation were made available and facilities were recommended to collect approximately 50 opportunities in the selected wards/departments. Electronic data submission and a system for enquiries via email were made available by WHO. Data revision and analysis were performed by WHO while keeping facilities' identity confidential.
Results: Overall, 327 healthcare facilities from 47 countries submitted data collected in 1527 wards. A total of 76,803 HH opportunities were included in the analysis. The overall mean HH Moment 1 compliance was 51.4+0.07% (weighted mean adjusted by number of opportunities per facility). HH actions were performed by handrubbing in 60.7% of cases, by handwashing in 37.6% and using both methods in 1.7%. HH Moment 1 compliance was highest in European countries (64%, 22278 opportunities) and lowest in the Americas' (26%, 23183 opportunities). Compliance by department and professional category are reported in Table.
Conclusion: The high number of participating facilities and of reported opportunities allow the first estimation of HH compliance before patient contact around the world. Healthcare workers miss HH actions when this indication applies almost once in every two opportunities. Nurses show better compliance than doctors. HH practices require urgent improvement in some areas of the world. This survey helps catalyse attention on a frequently neglected HH indication aiming to protect the patient from microbial transmission through healthcare workers' hands.

Table. Moment 1 compliance by department and professional category

	% (N opportunities)
Compliance by department*	
Medical	60% (12862)
Intensive care	59% (12571)
Surgery	55% (11976)
Mixed patient population	60% (8764)
Paediatrics	56% (8483)
Obstetrics	37% (5340)
Emergency	54% (3539)
Ambulatory	72% (2950)
Other	53% (9111)
Compliance by professional category**	
Nurse	64% (33932)
Doctor	48% (17945)
Auxiliary staff	58% (11954)
Others	54% (12808)

*Missing information about department=1207 opportunities (1.6%)
**Missing information about professional category=164 opportunities (0.2%)

O141 A comparison of the microbiological efficacy of hydrogen peroxide vapour and ultraviolet light processes for room decontamination

N.L. Havill, J. Boyce (New Haven, US)*

Objectives: Since standard methods of cleaning surfaces in hospitalised patients' rooms are often suboptimal, several novel technologies have been used to decontaminate more reliably patients' rooms after discharge. We compared the microbiological efficacy of an ultraviolet light (UVC) process with a hydrogen peroxide vapour (HPV) micro condensation process for decontaminating patient rooms.
Methods: Fifteen patient rooms were selected for the study to represent a range of shapes and sizes (from 46 m^3 to 86 m^3). Each room was decontaminated once using HPV and once using UVC, separated by at least 2 months (range 11–70 weeks). Five standardised high touch sites were sampled using D/E neutralizing contact agar plates both before and after the automated decontamination process. Aerobic colony counts were determined for each site. In addition, stainless steel carrier discs inoculated with approximately 10^6 *Clostridium difficile* (CD) spores and commercially available biological indicators (BIs) containing 10^4 and 10^6 CFUs of *Geobacillus stearothermophilus* were placed in 5 sites in the patient rooms before decontamination. After decontamination, a modified ASTM 2197 method was performed to determine the log reduction of the CD spores on the carrier discs. All BIs were placed in Trypticase Soy Broth and incubated at 60 C for 5 days and results were recorded as growth or no growth.
Results: Of 75 sites sampled, 70 yielded aerobic growth in rooms before HPV decontamination and 93% (65/70) of the samples were negative after HPV. 68 sites yielded aerobic growth before UVC and 52% (35/68) were negative after UVC decontamination ($P \leqslant 0.0001$). The percentage of the five sites negative after decontamination ranged from 87–100% for HPV and 9–87% for UVC (figure). Shadowed areas were less likely to be decontaminated by UVC. HPV yielded a 6 log reduction for all of the carrier discs compared to a median of a 2 log reduction after UVC. After HPV, none of the 10^4 BIs grew and 71% grew after UVC with a range of 47–92% for the 5 sites ($p < 0.0001$). For the 10^6 BIs, none grew after HPV and all grew after UVC ($p < 0.0001$).
Conclusion: Both HPV and UVC significantly reduced the bacterial contamination in patient rooms. HPV is more effective than UVC for the eradication of bacteria, including CD spores. The number of spores on the carrier discs and BIs is greater than that present on surfaces in patient rooms, presenting a difficult challenge for decontamination processes.

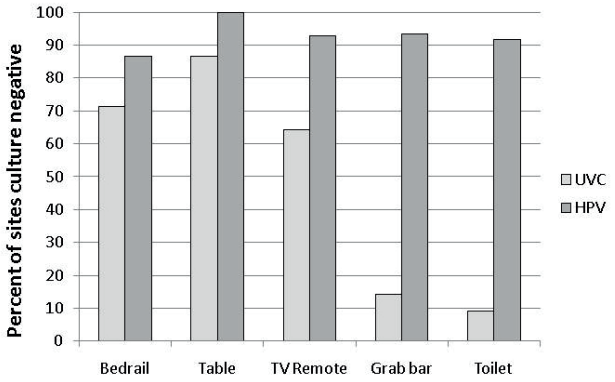


Fig. 1. Percent of cultures from the five high touch sites that were negative for aerobic growth after decontamination with the HPV and UVC process.

O142 State-wide admission prevalence screening in Saarland, Germany (MRSAarNetz)

C. Petit, B. Biechele (Homburg, DE)*

Objective: The Federal State of Saarland is located in southwest Germany, and comprises of 24 hospitals, including one University hospital and one additional tertiary care hospital. ~250.000 admissions are registered annually. While methicillin-resistant *S. aureus* (MRSA) rates are available for some institutions (typically expressed as MRSA/MSSA clinical isolate ratio), overall data on MRSA prevalence are lacking. In order to assess the burden of colonization and disease for MRSA in the context of a regional MRSA network (www.mrsaar.net), a State-wide admission prevalence screening (APS) was conducted.
Methods: APS was performed during October-November, 2010, in all Saarland hospitals. For screening, combined throat and nasal swabs were taken from patients immediately upon admission using a novel swab (ESwab, Copan, Italy) system for enhanced pathogen recovery. Samples were processed by a novel, fully automated, roboter driven specimen processor (WASP, Copan, Italy) and plated on selective biplate media for MRSA and methicillin sensitive *S. aureus* (MSSA). All analyses were performed in the State Hygiene laboratory at the University of Saarland Hospital.
Results: Of 20,007 specimen tested, 405 (392 throat/nose swabs, 13 wound swabs) were positive (prevalence, 2.0%; 11.7% of total *S. aureus*); prevalence varied between institutions (0–8,6%). These figures are higher than those previously determined in other German APS programs (Euregio MRSA-net 1.6%, Siegen 1.4%), and they also differ from those previously reported from other central European regions or institutions. spa type 003 (ST225) was most frequent among MRSA followed by spa type 504 (previously rarely reported). A detailed risk assessment was documented (analysis pending).
Conclusion: The conduct of a large regional APS study allowed for comparative determination of MRSA colonization/infection upon admission in an entire Federal State. These results are important to estimate the burden of MRSA cost and disease for the hospitals, for designing a cost-effective continuous screening program for the region, and for developing a rational approach to combat the circle of MRSA introduction from the community and care facilities into hospitals and back into the community. As a side effect, a general enhanced attention of general public, institution administrators, and policy makers towards the quality goals of MRSAarNetz was observed.

O143 Long-term maintenance of very low incidence of nosocomial multidrug-resistant pathogens in a tertiary hospital in Spain

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Objectives: Most reports on control of multidrug-resistant organisms (MDRO) refer to single organisms or units. In Spain, high incidence of MDRO are usually reported. We report the long term results of a very active MDRO control program in a teaching hospital.
Methods: Both an infection control program for MDRO and an antibiotic use program were implemented in 1997 in a 950-bed hospital in Spain, including periodic continuous educational activities, use of alcohol-based handrubs, protocolised environmental cleaning, targeted active surveillance of colonisation with MDRO, contact precautions, hygiene of colonised patients with clorhexidine, and audits plus active consultancy for antibiotic use. We previously published our successful results in the control of methicillin-resistant *Staphylococcus aureus* (MRSA) and *Acinetobacter baumannii* (AB) until 2002. The yearly incidence (new cases per 1,000 patient-days) from 2002 to 2009 for MRSA, AB, carbapenem-resistant *Pseudomonas aeruginosa* (CRPA), extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* (ESBLKP) and *Escherichia coli* (ESBLEC), carbapenemase-producing enterobacteria (CPE) and vancomycin-resistant enterococci (VRE), and antibiotic consumption are reported. Data were compared by Poisson regression.
Results: The incidence of colonization/infection caused by MRSA decreased from 0.13 cases per 1,000 patient-days in 2002 (38 cases) to 0.09 in 2009 (26 cases); and that of AB, from 0.17 (50 cases) to 0.01 (4 cases) ($p < 0.01$ for both comparison). The rates of ESBLKP, and CRPA were very low during the whole study period and without trends, ranging from 0.01 to 0.06 (2 to 17 cases per year), and 0.02 to 0.07 (6 to 22 cases per year), respectively. As regards ESBLEC, the rate increased from 0.12 to 0.35 (37 to 105 cases per year), although neither the cases were clustered nor the isolates were clonally related; many cases were colonised at admission. No cases of VRE, CPE or outbreaks caused by other MDRO were detected despite active search. No significant trends were noted in antibiotic consumption, which ranged from 46 to 49 DDDs per 100 patient-days.
Conclusions: In an area where average rates for MDRO are usually moderate to high, a very low incidence of these pathogens was maintained in our hospital from 2002 throughout 2009, in association with very active specific infection control and antibiotic use programs. Specific efforts to prevent infection in colonised patients with ESBLEC are needed.

O144 What is the optimal period for measuring hand-hygiene compliance? Are longer periods any better than 20-minute periods?

S. Stone, C. Fuller, S. Michie, A. Charlett (London, UK)*

Objective: Direct observation of hand hygiene is considered the gold standard for measurement of hand hygiene compliance. Most studies or audits use 20–30 minute periods of observation. Recent WHO guidance recommends this. However, it is not clear whether such short periods provide a representative, unbiased sample of hand hygiene behaviour. The aim of this study was to examine whether compliance over 20 minutes differed significantly from that recorded over one or four hours.
Methods: Using a robustly standardised measure (the Hand Hygiene Observation Tool). Hand hygiene observations were covertly collected, in consecutive 20-minute segments, over 53 "4 hour" observation periods, comprising 3989 hand hygiene moments, during the morning and early afternoon, on 13 Intensive Care Units (ICUs) and 36 Acute Care of the Elderly (ACE) wards in 13 hospitals, A mixed effects logistic regression model analysed compliance with hospital & ward as random effects, and with type of ward (ICU or ACE) & sequential 20-minute or 1-hourly observation periods as fixed effects.
Results: Overall compliance was 75%, being lowest in the 1st 20-minute period (69%) and in the 1st hour (71%). Analysis of sequential 20 minute observation periods showed the estimated odds ratios for compliance rose significantly in the 2nd 20-minutes (1.42, 1.02–1.96; $p = 0.04$), remaining stable thereafter (Table). For sequential hour periods, the estimated odds ratios for compliance rose significantly (1.32, 1.08–1.61; $p = 0.007$) in the 2nd hour, remaining stable thereafter. Type of ward had no significant effect.

Table. Observed compliance & estimated odds ratio (OR) for hand hygiene compliance in each sequential 20 minute and one hour periods of observation.

	No of observations	HHC(%)	O.R.	95% CI	p. value
20-minute period					
1st	417	69.1	*ref*		
2nd	391	74.4	1.42	1.02 to 1.96	0.04
3rd	404	70.8	1.30	0.94 to 1.78	0.11
4th	381	78.0	1.63	1.17 to 2.29	0.004
5th	341	75.4	1.43	1.01 to 2.01	0.04
6th	366	77.6	1.75	1.24 to 2.46	0.001
7th	333	76.6	1.67	1.17 to 2.36	0.004
8th	333	75.1	1.53	1.08 to 2.16	0.02
9th	331	77.0	1.63	1.15 to 2.30	0.006
10th	285	78.9	1.90	1.31 to 2.77	0.001
11th	251	70.1	1.26	0.88 to 1.81	0.2
12th	156	82.1	2.44	1.51 to 3.96	<0.001
Hour-period					
1st	1212	71.4	*ref*		
2nd	1088	77.0	1.32	1.08 to 1.61	0.007
3rd	997	76.2	1.32	1.07 to 1.62	0.008
4th	692	76.4	1.40	1.11 to 1.76	0.004

Conclusions: Compliance rose by a small but significant amount in the 2nd 20 minute period of observation, but remained stable thereafter. Possible explanations are a delayed but small reactivity to being observed (Hawthorn effect), improving health care worker performance as the shift progresses, or observer fatigue, especially in the latter stages of observation. This study supports the WHO recommendation of a 20-minute observation period. Frequent measurements are required to establish time trends & changes. In both clinical audit & research settings, 20-minute observation periods appear able to provide a relatively unbiased sample, provided the number of hand hygiene moments reaches observation tools' established reliability criteria. Researchers should state & explain the period & frequency of observations in both grant applications & publications.

O145 Control measures for preventing the spread of vancomycin-resistant enterococci in hospitals: where is the evidence?

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Objectives: A systematic review was performed to determine the efficacy of control measures (CM) for preventing the spread of vancomycin-resistant enterococci (VRE) in hospitalised patients. Secondary outcomes were: crude mortality, length of hospital stay, costs and CM's adverse effects.
Methods: Cochrane Wounds Group Specialised Register, The Cochrane Central Register of Controlled Trials, MEDLINE, EMBASE, and CINAHL were searched (1980-May 2010) for randomized clinical trials (RCTs), non-randomized controlled clinical trials (CCTs), interrupted time-series (ITS) and controlled before and after studies (CBA) that prospectively compared wards/hospitals applying two different intervention policies or wards/hospitals where intervention policies were used to hospitals/wards where those policies were not used. In the absence of clinical and statistical heterogeneity (significant if $I2 > 50\%$) a fixed-effect model was used to estimate the pooled relative risk (RR). The methodological quality of included studies was assessed according to the criteria developed by the EPOC group.
Results: Three CCTs and 5 ITS were included. The overall quality of the studies was low. Intervention was represented by physical barriers by health care workers (HCWs) to transmission (3 studies), environmental cleaning (2), hand-washing measures (2), and antibiotic formulary interventions (2). Implementation of hand hygiene measures was associated with a 47% decrease in the VRE acquisition rate (RR 0.53, 95% CI 0.39–0.73). Physical barriers by HCWs and environmental cleaning did not significantly reduce the VRE acquisition rate (RR 0.97, 95% CI 0.58–1.62 and 0.66, 95% CI 0.32–1.38, respectively). Slight heterogeneity was detected only among studies analysing the effectiveness of physical barriers (I2=45%). The effectiveness of antibiotic formulary was not assessed through a statistical approach since the heterogeneity of the outcomes. Implementation of CMs did not result in a significant reduction of length of stay. None of the studies analysed costs or adverse effects. One reported on crude mortality.
Conclusions: Surprisingly, review of the literature did not show any RCT on the efficacy of CM for preventing the acquisition of VRE. Our study seems to suggest a significant effect of hand-hygiene measures on VRE acquisition. However, the level of the evidence is still very poor due to study designs, insufficient study quality and lack of data to show the effect of a single intervention.

O146 Low environmental contamination of rooms in ESBL-positive patients

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Objectives: The steep rise of patients colonized with ESBL-producing enterobacteriacea creates a challenge in developing adequate transmission precautions. In many institutions, the rate of ESBL exceeds the rate for MRSA positive patients. The isolation policies for ESBL positive patients are less standardized and differ between institutions. We wanted to know if the rooms of ESBL-positive hospitalized patients are contaminated with ESBL producing bacteria.
Methods: Patients positive for ESBL and hospitalized for more than 48 hours in one room were selected. In all these patients, rectal, inguina, urine, throat and wounds were screened for ESBL. Additional swabs of the bed (frame, bed cradle), patient telephone, sink and toilet seat were taken and cultured for ESBL. Room cleaning was performed according to routine protocol. Contamination rate of the patient rooms were calculated.
Results: From June 2009 until December 2010, twenty patients were included. ESBL was found in 17/20 inguinal, in 18/20 rectal, in 13/20 in urine, in 3/20 in wounds and 3/20 in the throat. ESBL was found in 4 (2 patients), 3 (9 patients), 2 (4 patients) and 1 (2 patients) screened sites. Only in 2/20 patients, ESBL was found on surfaces of the environment. In one patient, the sink was positive, and in one patient, the telephone and the toilet seat were positive. These two patients had both 3 colonized sites (urine, rectal, inguina). No relationship between positive environment and colonisation load could be observed. The contamination of environment was calculated 10% (95% CI 0–23%) for rooms and 3.75% (95% CI 0–7.9%) for screened locations.
Conclusion: Compared to data for MRSA and VRE published in literature, we found a low contamination rate of environmental surfaces in hospitalized ESBL positive patients. Main route of transmission may not be indirect via contaminated surfaces. This might influence isolation policy.

O147 Model-based cost-effectiveness evaluation of methicillin-resistant *Staphylococcus aureus* screening and control in general medical hospital wards

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Objectives: To use dynamic transmission models to assess the cost-effectiveness of methicillin-resistant *Staphylococcus aureus* (MRSA) screening and infection control strategies in general medical (GM) wards.
Methods: A dynamic model of MRSA transmission in GM hospital wards was developed. Model parameters were derived from literature review, data analyses and expert opinion. Twenty-five intervention combinations were evaluated, involving screening all vs. high risk patients, and using conventional culture, chromogenic agars or rapid (PCR) technologies. Screening was combined with isolation (contact precautions, side rooms, isolation wards or cohorting) or decolonisation (nasal ointments and/or body-washes). Incremental costs and health benefits (measured in quality adjusted life years, QALYs) were evaluated under different assumptions about MRSA prevalence, proportion of high risk patients and ward size. Probabilistic sensitivity analyses (PSA) were conducted, incorporating full uncertainty in model parameters.
Results: All isolation strategies led to only small reductions in MRSA infection rates (less than 5%) and had cost/QALY values much higher than the usual National Health Service willingness to pay threshold (£30,000 [€35,500] per QALY). This was reflected in the PSA, which showed the baseline 'do nothing' approach to be preferred. For a slightly higher willingness to pay, a strategy of pre-emptive isolation of high risk patients (without screening) was cost-effective. Decolonisation strategies, though able to reduce MRSA infection rates by nearly 50%, were not cost-effective (at the most likely parameter values). Accounting for full parameter uncertainty, the strategy of decolonising only those identified via clinical culture gave both the highest expected net monetary benefit and had the highest probability of being cost-effective.
Conclusion: The potential for screening and control strategies to be cost-effective in GM wards is much more limited than in critical care settings. Full PSA showed that within the usual willingness to pay threshold, neither screening with isolation nor decolonisation were cost-effective. This reflects the fact fewer infections in GM wards are due to cross-transmission and, because infection and mortality rates are much lower, interventions that depend on expensive screening are much less likely to be cost-effective. However, our results are likely to underestimate

intervention benefit as they ignore QALY loss due to MRSA following ward discharge and long-term effects.

What is changing in the invasive mycosis field?

O148 Six-year prospective candidemia survey from the fungal infection network of Switzerland (FUNGINOS) – *Candida* species distribution and antifungal susceptibility according to recent EUCAST and old vs. new CLSI clinical breakpoints

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Objective: Changing *Candida* spp. distribution and emerging cross-resistance to azoles and multiresistance to azoles and echinocandins is increasingly reported in Europe and North America. To analyse the species distribution of *Candida* blood isolates prospectively collected during 2004–9 from 27 hospitals (7 university, 20 affiliated) of the FUNGINOS network and to compare the antifungal susceptibility according to the clinical breakpoints (bp) defined by EUCAST in Europe and CLSI in North America.
Methods: *Candida* isolates were identified in the FUNGINOS mycology reference lab and tested for susceptiblity to fluconazole (F), voriconazole (V) and caspofungin (C) by microtitre broth dilution method with the Sensititre® YeastOne™ test panel. Clinical bp were: (i) EUCAST 2010/ CLSI 2008 = old for F and V, (ii) CLSI 2011 = new for F, V, C.
Results: 1090 *Candida* blood isolates were tested: 675 (61.9%) *C. albicans*, 191 (17.5%) *C. glabrata*, 64 (5.9%) *C. tropicalis*, 59 (5.4%) *C. parapsilosis*, 33 (3%) *C. dubliniensis*, 22 (2%) *C. krusei* and 46 (4.2%) rare *Candida* spp. No change in *Candida* species distribution occurred during 2004–9. The table shows the percentages of susceptibility and numbers of non-susceptible isolates for each *Candida* species according to the different bp (μg/ml).
Eight isolates (4 *C. tropicalis*, 3 *C. albicans*, 1 *C. parapsilosis*) showed a cross-resistance to azoles (F and V) according to EUCAST bp vs. 2 isolates (*C. albicans*) according to old and new CLSI bp. One *C. tropicalis* isolate was tested multi-resistant according to EUCAST bp (MIC F: 2 μg/ml, V: 0.25 μg/ml, C: 16 μg/ml) vs. none according to old and new CLSI bp.
Conclusions: In Switzerland the majority of *Candida* blood isolates were *C. albicans* (61.9%), followed by *C. glabrata* (17.5%). *C. albicans* was susceptible to all antifungals independently of the applied bp, whereas the proportion of fluconazole-susceptible *C. tropicalis* and *C. parapsilosis* was lower with the EUCAST and new CLSI vs. the old CLSI bp. Applying the EUCAST bp for voriconazole lowered the proportion of susceptible *C. tropicalis* and *C. parapsilosis* isolates vs. old and new CLSI bp. As expected, all *Candida* isolates were susceptible to caspofungin, except in *C. krusei* and *C. parapsilosis*. Only 4 isolates were cross-resistant to azoles and 1 *C. tropicalis* was multiresistant to azoles and caspofungin. The impact of recent EUCAST and CLSI breakpoints for predicting clinical response to therapy remains to be investigated.

Candida species	Fluconazole			Voriconazole			Caspofungin
	old CLSI (≤ 8 ug/ml)	new CLSI (≤ 2 ug/ml)	EUCAST (≤ 2 ug/ml)	old CLSI (≤ 1 ug/ml)	new CLSI (≤ 2) ug/ml	EUCAST (≤ 0.12 ug/ml)	new CLSI (≤ 0.5 ug/ml)
C. albicans (n=675)	99.6% / 3	98.4% / 11	98.4% / 11	99.6% / 3	99.6% / 3	99.6% / 3	100% /0
C. tropicalis (n=64)	97% / 2	**89% / 7**	**89% / 7**	100% / 0	100% / 0	**78% / 14**	98% / 1
C. parapsilosis (n=59)	96.6% / 2	**84.7% / 9**	**84.7% / 9**	100% / 0	100% / 0	**93.2% / 4**	**79.7% / 12***
C. krusei (n=22)	R	R	-	95.5% / 1	100% / 0	n/a	**81.8% / 4**
	old CLSI (≤ 8 ug/ml)	new CLSI (SDD ≤ 32 ug/ml)	EUCAST	old CLSI (≤ 1 ug/ml)	new CLSI (≤ 2) ug/ml	EUCAST (≤ 0.12 ug/ml)	new CLSI (≤ 0.5 ug/ml)
C. glabrata (n=191)	**51.8% / 92**	**0% / 191**	n/a	96.3% / 7	97.9% / 4	n/a	100% / 0

*new CLSI bp for *C. parapsilosis* ≤ 4 ug/ml; R : resistant, n/a : not applicable

O149 Epidemiology of filamentous fungi in cystic fibrosis patients in the Netherlands

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Objectives: The clinical significance of fungal infections in Cystic Fibrosis (CF) are poorly understood. Infections with viruses and bacteria are the main cause of deterioration of lung function in these patients. However there is also evidence of an increasing prevalence of fungi in sputum but the reported results vary widely between countries and centers (9–56%). Very little is known about the epidemiology, genotypes and resistance patterns of filamentous fungi in Dutch patients with CF. Here we describe the first results of a recently initiated large prospective study of fungal colonization in CF patients in the Netherlands.
Methods: Standardized routine sputum samples were collected in four CF centers. All samples were cultured on mould-selective plates and incubated at two temperatures. All non-*Candida* fungi were collected and stored centrally. After initial morphologic identification, molecular confirmation was sought using AFLP and ITS + D1/D2 sequencing. All *Aspergillus fumigatus* isolates were additionally analyzed for presence of the most common mutations in the CYP51 gene.
Results: A total of 484 filamentous fungi were isolated from 231 patients in the period May 2010–November 2010. The main species found was *A. fumigatus* (58%). In addition, a variety of other *Aspergillus* species were found: *A. flavus*, *A. terreus*, *A. sydowii*, A. protuberans and *A. versicolor*. Next to these *Aspergillus* spp., *Penicillium* spp. (12%) and *Scedosporium* spp. (5%) were the most common fungi. *Exophiala dermatitidis*, an already known potential pathogen in CF, was isolated 10 times (2.1%). Interestingly we also report the first isolations of *Geosmithia argillacea* in Dutch CF patients. At least 10% of the samples showed a discrepancy between morphologic and molecular identification. A total of 5.0% of the tested *A. fumigatus* appeared to contain the TR/L98H mutation while no other mutations (G54, G138, M220) responsible for azole resistance where found.
Conclusion: These results suggest that the Dutch epidemiology of filamentous fungi in CF patients is comparable to our neighboring countries. The main fungus was *A. fumigatus*, but also other fungal species such as *Scedosporium* spp. and *Exophiala* spp. were identified including the newly described species *Geosmithia argillacea*. In addition 5% of *A. fumigatus* isolates appeared to harbour mutations reported to confer azole resistance. These are the first epidemiological data of azole resistance in *A. fumigatus* from CF patients.

O150 Improved clinical diagnosis of invasive *Candida* infections in patients undergoing cardiac surgery

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Objectives: The high mortality of invasive *Candida* infections in the intensive care unit (ICU) and the absence of rapid diagnostics require identification of risk indicators, *Candida* colonization and *Candida* presence for early diagnosis. To investigate these characteristics in long-term intensive care patients after cardiac surgery, a prospective observational study was performed at the University Hospital of Vienna.
Methods: One hundred sixty-nine consecutive cardiac surgery patients with prolonged ICU stay of at least 7 days were prospectively enrolled in a two-year period. Preoperative, perioperative and postoperative data were assessed. *Candida* colonization and *Candida* score (CS) were evaluated twice weekly. Variables associated with both proven and possible invasive candidiasis ($P < 0.05$) in the univariate analysis were considered to be candidates for building multivariate models using stepwise logistic regression analysis.
Results: Ten patients (5.9%) developed proven, 71 patients (42%) developed possible invasive candidiasis. Multi-site *Candida* colonization and a CS $\geqslant 3$ preceded proven infection in 9/10 and 10/10 patients. In 59 patients with both multi-site colonization and a CS $\geqslant 3$, the score

was positive 7.1 days (arithmetical average) earlier than the colonization index. Redo surgeries ⩾2, multi-site *Candida* colonization, persistent fever, bacteremia, and increasing or persistently high sequential organ failure assessment (SOFA) score >7 were significantly associated with both proven and possible invasive candidiasis by univariate analysis ($P < 0.05$). Only increasing or persistently high SOFA score >7 (adjusted odds ratio 6.2, $P = 0.018$) and bacteremia (adjusted odds ratio 4.6, $P = 0.043$) were independent risk factors for proven infection using stepwise logistic regression analysis.

Conclusions: Long-term ICU patients after cardiac surgery are at high risk for invasive *Candida* infections in the presence of preceding bacteremia and a persistently high or increasing SOFA score. Severe *Candida* colonization and a CS ⩾3 are of value in identification of cardiac surgery patients with present or future invasive candidiasis.

O151 Analysis of aetiology of invasive fungal infections using a panfungal PCR-based method in tissue biopsies with proven infection

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Objectives: The etiological cause of many IFIs is never revealed. PCR-based methods can detect specific DNA of fungal species in tissue biopsies, in order to know the prevalence of fungal species. Those data will help us to choose the most efficient therapeutic alternative for first line therapies of IFI.

Methods: A total of 116 paraffined and fresh tissue biopsies were sent to Spanish Reference Mycology Laboratory between 2006 and 2010 from 36 different hospitals. Biopsies came from 93 patients suffering from proven infection as microscopical examination of tissues showed invasion by fungal structures. Cultures were negative for all samples. A total of 36 (30%) biopsies came from lungs, 23 (19%) were subcutaneous or skin biopsies, 19 (16%) from gastrointestinal tract, 10 brain biopsies, 9 from nasal sinus, 7 from liver, and 18 from other tissues. Procedures of DNA extraction and amplification followed routine methods. The tissue samples were analyzed using a panfungal Real-Time PCR-based assay. It was designed to amplify the ITS regions 1 and 2 from fungal rDNA gene complex. Amplified DNA was identified by sequencing. Controls were used in each set of experiments which were done in duplicate.

Results: A total of 80% of biopsies were positive by the PCR-based technique (93/116). When data were analyzed by patient, fungal DNA was detected and identified in 77 out of 93 patients (83%). Results by patients of species identification were: 37/77 (48%) *Aspergillus fumigatus*, 4 (5%) *Aspergillus flavus*, 7 (9%) Zygomycetes, 7 (9%) other phaeohyphomycosis with 3 scedosporiosis, 6 (7.7%) hyalohyphomycosis with 3 fusariosis, and 6 (7.7%) *Candida*. A total of 10 (13%) cases of histoplasmosis were detected as well. Other 4 cases were mixed infection. By samples, main data were: *Aspergillus* DNA was detected in 76% of positive lung biopsies and 43% of brain tissues, and *Candida* DNA in 50% of gastrointestinal biopsies.

Conclusion: (i) PCR-based methods can be used for species identification in tissue biopsies as a complementary technique of cultures. (ii) Emerging fungal pathogens could be underestimated as microbiological conventional methods have a limited accuracy for species detection and identification. (iii) Further studies are warranted.

O152 Comparison of a commercial *Aspergillus* real-time PCR assay with galactomannan testing of BAL fluid for the diagnosis of invasive pulmonary aspergillosis in lung transplant recipients

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Background: Early diagnosis and treatment of invasive pulmonary aspergillosis (IPA) improves outcome.

Methods: We compared commercially-available *Aspergillus* real-time PCR and Platelia galactomannan (GM) assays performed on 150 BAL samples from lung transplant recipients (16 proven/probable IPA, 26 *Aspergillus* colonization, 11 non-*Aspergillus* mould colonization and 97 negative controls).

Results: Sensitivity and specificity of pan-*Aspergillus* PCR (optimal Cq ⩽35.0 by ROC analysis) and GM (⩾0.5) for diagnosing IPA were 100% (95% CI: 79–100%) and 88% (79–92%), and 93% (68–100%) and 89% (82–93%), respectively. Corresponding positive and negative predictive values were 50% (30–65%) and 100% (77–100%), and 48% (29–67%) and 99% (95–100%), respectively. Sensitivity and specificity of *A. fumigatus*-specific PCR were 69% (41–89%) and 96% (92–99%). Most false-positive PCR and GM were in samples from patients colonized by *Aspergillus* and negative controls, respectively. *A. fumigatus*-specific PCR had sensitivity and specificity of 69% (11/16) (41–89%) and 96% (129/134) (92–99%), respectively, for diagnosing IPA due to this species. *A. terreus*-specific real-time PCR was positive for the one patient with IPA due to this species; specificity was 99% (133/134).

Conclusions: A recently developed, commercial *Aspergillus* PCR assay and GM testing of BAL fluid may facilitate diagnosis of IPA after lung transplantation. Both tests offer the potential advantage over culture-based diagnostic methods of rapid turn-around, which may facilitate more timely initiation of antifungal therapy. In distinguishing between clinically important species, PCR may afford a further advantage over indirect markers like GM.

O153 A phase IV, open-label study evaluating efficacy and safety of intravenous anidulafungin followed by oral azole for the treatment of candidaemia/invasive candidiasis in US/Korean patients

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Objectives: To evaluate the efficacy and safety of a Rapid Switch Regimen (RSR; short course of intravenous [IV] anidulafungin [ANID], followed by an optional switch to oral azole therapy with either fluconazole [FLU] or voriconazole [VORI]), for the treatment of candidaemia/invasive candidiasis (C/IC).

Methods: A Phase IV, open-label, multicentre, non-comparative study. Patients received 200 mg IV ANID (loading dose) followed by 100 mg/day thereafter. After 5 days, patients meeting criteria could be switched to oral azole therapy (FLU 400 mg/day or VORI 200 mg BID). The primary endpoint was global response (clinical + microbiological response) at the end of all treatment (EOT) in the modified intent-to-treat (MITT) population (at least one dose of ANID + positive *Candida* within 96 h of study entry). Secondary endpoints, assessed by the investigator included: global response at end of IV treatment (EOIV), 2 weeks post treatment and end of study (EOS; 6 weeks post-therapy). Global response was also determined at EOT in predefined patient subsets. Safety and tolerability were evaluated by adverse event (AE) monitoring and clinical and laboratory assessments.

Results: 294 patients were enrolled (270 in US, 24 in Korea): 53.5% male, mean (SD) age 55.4 (16.7) years, and mean APACHE-II score 14.1. Ten patients were neutropenic when enrolled. *C. albicans* (42%) was the most common baseline pathogen isolated. The mean (SD) duration of ANID treatment was 8.6 (5.8) days; 44.3% of patients received ANID with no switch to oral azole therapy. 105 patients switched to FLU (mean [SD] duration 9.3 [5.6] days) and 54 patients switched to VORI (mean [SD] duration 11 [7.8] days). Mean (SD) duration of total antifungal therapy (IV + oral) was 14.1 (8.2) days. Global response rate at EOT in the MITT population was 83.7% (95% CI: 78.7, 88.8). Global response rates in patient subsets are shown in the table. EOT global response in neutropenic patients was 42.9% (3/7). 33 patients (11.7%) developed treatment-related AEs, with nausea and vomiting being the most frequently reported (4 cases during ANID treatment and 4 during oral azole therapy). AEs were mostly mild or moderate in severity.

Conclusions: An RSR was shown to be effective in the treatment of C/IC, with a comparable safety profile to previous studies. RSR has shown to be a tolerable and effective treatment in different subgroups. RSR should ease the burden of long-term parenteral therapy to manage these infections.

Table: Global response rates at EOT for predefined subgroups within the MITT population

	Global response rate[a] n/N (%) [95% CI]
Overall MITT population	170/203 (83.7) [78.7, 88.8]
C. albicans at baseline	80/92 (87.0) [80.1, 93.8]
C. glabrata at baseline	45/52 (86.5) [77.3, 95.8]
C. parapsilosis at baseline	26/34 (76.5) [62.2, 90.7]
C. tropicalis at baseline	15/19 (78.9) [60.6, 97.3]
C. krusei at baseline	6/10 (60.0) [29.6, 90.4]
Non-*albicans* at baseline overall	99/120 (82.5) [75.7, 89.3]
APACHE-II >20 at baseline	23/33 (69.7) [54.0, 85.4]
Aged ≥65 years at baseline	58/67 (86.6) [78.4, 94.7]
Switched to oral fluconazole	82/91 (90.1) [84.0, 96.2]
Switched to oral voriconazole	44/47 (93.6) [86.6, 100.0]

[a]Global response success: achieved when both clinical and microbiological responses are successes. Excludes missing and unknown (indeterminate) responses.

O154 Survey of antifungal combination therapy for treatment of proven or probable invasive fungal diseases in Italian haematological centres. The Seifem-Combo Study (NCT 00906633)

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Background: This prospective observational Clinical Trial (NCT 00906633) evaluated the feasibility, efficacy and toxicity of Antifungal Combination Therapy (Combo) as treatment of proven or probable Invasive Fungal Diseases (IFDs) in Hematological patients (pts).
Materials and Methods: Between Jan 2005 and Dec 2009, 84 cases of Combo were reported from 20 Hematological Centers in Italy. Median age of pts was 34 yrs (range 1–73) and 37% had less than 18 yrs. Acute Leukemia was the most common underlying hematologic disease (68/84; 81%). The status of hematologic disease was: onset 21/84 (25%), remission 18/84 (21%), refractory/relapse 45/84 (54%). The main site of fungal infection was lung with or without other sites. The etiological agents were: *Aspergillus* sp 68 cases (81%), *Candida* sp 6 cases (8%), Zygomycetes 4 cases (5%), *Fusarium* sp 4 cases (5%)and others (2 cases).
Results: The most used Combo were: Caspofungin + Voriconazole 35/84 (42%), Caspofungin+Liposomal Amphotericin B (L-AmB) 20/84 (24%), and L-AmB+Voriconazole 15/84 (18%). The median duration of Combo was 19 days (range 3–180). The Overall Response Rate (ORR) was 73% (61/84 responders) without significant differences between the Combo regimens. The most important factor that significantly influenced the response rate (in univariate and multivariate analysis) was PMN recovery during Combo ($P < 0{,}0001$). Only one patient discontinued therapy (voriconazole related neurotoxicity) and 22% experienced mild and reversible adverse events (hypokalemia, ALT/AST increase, creatinine increase) without differences between pediatric and adult pts. The IFI attributable mortality rate was only 17%.
Conclusion: This is the first multicenter, prospective, observational study exploring feasibility, efficacy and toxicity of Antifungal Combination Therapy (Combo) as treatment of proven or probable Invasive Fungal Diseases (IFDs) in Hematological patients (pts). The results of this study indicate that: 1) Combo was well tolerated in both children and adults hematologic pts. 2) The Overall Response Rate was 73% and the mortality IFDs related was only 17%. 3) The most used Combo regimens were Caspofungin+Voriconazole (ORR 80%) and Caspofungin+L-AmB (ORR 70%). 4) In univariate and multivariate analysis PMN recovery during Combo predicts a better outcome.

O155 Mortality due to invasive fungal infections is low in lung transplant recipients receiving voriconazole prophylaxis

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Background: Invasive fungal infections (IFIs) are major complications and causes of mortality in lung transplantation (LTX). The role of voriconazole prophylaxis (VOR px) in LTX pts (pts) is controversial.
Methods: We reviewed 154 consecutive pts who underwent LTX or heart-LTX from 1/05–9/06. All pts received alemtuzumab induction and VOR px ⩾3 mos.
Results: 49 received single, 94 double and 11 heart-LTX. 54% of pts had >1 episode of respiratory mould colonization, and 21% had respiratory IFI (16 episodes proven, 14 probable, 5 possible). The mean (median) time from LTX to IFI was 13 (12) mos. 66% of IFIs involved lung parenchyma, 26% anastomotic sites and 9% pleura. Parenchymal IFIs and empyemas were more likely to occur as late onset (>6 mos), and anastomotic infections as early onset (⩽6 mos) ($p = 0.02$). Parenchymal infections were by *Aspergillus* (48%), *Mucor* (13%), other moulds (13%), *Cryptococcus* (13%) and unidentified moulds (13%). 74% occurred >6 mos and 63% >12 mos after LTX; 24% of late IFI occurred during VOR px (*A. fumigatus*, *A. niger*, *Mucor*, *Penicillium*, 1 each). All 6 early onset parenchymal IFI occurred during VOR prophylaxis (2 *Cryptococcus*, 1 *Mucor*, 1 *Scedosporium*, 2 unidentified). The overall mortality was 8% for pts with parenchymal IFI. 1 died from ruptured *Scedosporium* mycotic aortic aneurysm, and 1 from lung and facial IFI due to *Mucor*. Anastomotic site IFI were due to yeasts (67%, *Candida* spp. and *S. cerevisiae*), *A. fumigatus*, *Penicillium* and *Cladosporium* (11% each). 78% occurred early during VOR px. Late infections by *A. fumigatus* and *C. albicans* (1 each) occurred after discontinuation of VOR. Empyemas were caused by *C. glabrata* and *Histoplasma* late (off VOR), and *C. parapsilosis* early (on VOR). None of the pts with anastomotic infections or empyema died.
Conclusions: Despite routine VOR px for ⩾3 mos post-LTX, the incidence of IFI remained high. VOR px was associated with delay in onset of parenchymal IFI to >1 year post-LTX, and low incidence of *Aspergillus*, an observation that raises the question if VOR selected for emergence of antifungal-resistant moulds like *Mucor* and *Scedosporium*. VOR px did not change the epidemiology of anastomotic site IFI. Mortality among pts with IFIs, in particular parenchymal infections, was much lower than previously reported. By delaying serious IFIs to periods of reduced immunosuppression, VOR px might facilitate improved survival.

O156 Impact of posaconazole prophylaxis on fungal colonisation and invasive fungal infections: a prospective study

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Background: The use of posaconazole (PZC) prophylaxis in patients with haematological conditions has increased in our hospital. Emergence of resistant azole isolates and invasive fungal infection (IFI) has not been extensively studied.
Methods: Prospective monocentric study. Patients receiving PZC prophylaxis for graft versus host disease (GVHD) after hematopoietic stem cell transplantation (HSCT) or chemotherapy for Acute Myeloid Leukemia (AML) were enrolled. Serial mouth swabs and stool samples were obtained for fungal identification: before initiation of PZC (baseline), during treatment and up to 3 months after PZC discontinuation. Clinical data with a focus on IFI and plasma PCZ concentrations were also collected during the study period.
Results: From october 2009 to november 2010, 56 patients were included, 32 for AML and 24 for GVHD. There were 37 men and 19

women, with a mean age of 52 years. Median PZC duration was 35 days (9–120 days). Patients could be colonized with multiple isolates. Ten out of 49 patients (20%) were colonized at baseline with a positive mouth or stool culture (*C. albicans* n = 7, *C. glabrata* n = 4, *C. kefyr* n = 1, *Geotrichum* sp n = 1), 20/49 (41%) colonized during prophylaxis (*C. albicans* n = 11, *C. glabrata* n = 9, *Saccharomyces* n = 4, C. Kefyr, *C. norvegensis*, *C. guilliermondii*, *C. tropicalis*, *Geotrichum* sp, n = 1 each) and 4/23 (17%) colonized three months after PZC discontinuation (*C. glabrata* n = 2, *C. albicans*, *C. tropicalis* and *C. kefyr*, n = 1 each).
An IFI was diagnosed in 4 patients under PZC prophylaxis (7%): invasive aspergillosis (n = 2), disseminated zygomycosis (n = 1), fusariosis (n = 1). Median through plasma PZC concentration was 0,27 mg/l (0,1–0,55) in 3 of these 4 patients while it was 0,53mg/l (0,1–1,15, p = 0,9) for patients without IFI. Four other patients developed an IFI a mean of 26 days after PZC discontinuation: invasive aspergillosis (n = 2), disseminated zygomycosis (n = 1) and *C. parapsilosis* candidemia (n = 1). Among patients with IFI, 6 out of 8 had a fungal colonization. Six patients (11%) died during the study period. The death was related to the IFI in 3 cases.
Conclusion: Fungal colonization is frequent in patients receiving PZC prophylaxis, particularly due to *C. albicans* and *C. glabrata*. The observed high rate of breakthrough IFI among PZC recipients requires further evaluation.

O157 Stewardship in antifungals. How much do prescribing physicians know?

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Background: The use of antifungal agents and their cost has rocketed in most hospitals. Implementation of antifungal stewardship requires training of prescribers and the use of restrictive policies. Assessing the knowledge of those who prescribe antifungal agents is a key first step to guide the design and implementation of training strategies for providers.
Objective: To assess prescribing physician's knowledge and compliance with current guidelines in the diagnosis and therapy of invasive fungal infection (IFI).
Methods: Attending physicians and residents that prescribe antifungals in our institution were invited to complete a 20-question test that was scored from 0 to 10. Questions assessed different aspects of management of invasive candidiasis and invasive aspergillosis (IA).
Results: Overall, 60.8% (200/329) of the physicians, belonging to the following departments (medical 59.5%, pediatric 19%, ICUS 16.5% and surgical 5%) completed the survey. Their mean age (±SD) was 34.9±9.8 and the mean number of years of medical practice was 10.7±10.3.
Mean score was 5.14±1.72. Scores differed among departments: medical: 5.39±1.7, ICUs: 5.27±1.64, pediatrics: 4.6±1.74 and surgical: 3.95±1.28. Attending physicians scored better than residents (5.5±1.6 vs 4.6±1.6, p=NS).
Regarding candidemia, only 54.7% of the physicians distinguished colonization from infection and 17.5% were aware of current rates of fluconazole resistance. Only 32.6% knew the indications for antifungal prophylaxis and 23.1% when to begin empiric therapy. However, most physicians knew which antifungals were recommended as empirical therapy (73.3%). Indications of amphotericin B, azoles or candins were answered correctly by 57.3%, 26.1% and 41.2%, respectively.
Regarding IA, 67.3% of the physicians could distinguish colonization from infection and only 34.6% were familiar with galactomannan use. Radiological features of IA were well recognized by 63.8%, but only 31.1% knew the first line therapy for IA and 36.1% knew the recommended length of therapy. The clinical benefit of measuring antifungal seric levels was acknowledged by 66.8%.
Conclusion: A simple test enabled us to assess the knowledge of prescribing physicians in important aspects of IFI diagnosis, prophylaxis and antifungal therapy. The results of the study have revealed the inadequate knowledge of prescribers in this area of their practice and serve as a guide to design a tailored training program for management of IFI.

The Big Question – are we staying ahead of resistance in the hospital setting?

S158-S162 The Big Question – are we staying ahead of resistance in the hospital setting?

T. Welte, M. Sánchez García, J. Picazo, M. Bassetti, J. Garau (Hannover, DE; Madrid, ES; Genua, IT; Barcelona, ES)

Serious infection is a common reason for patient admission into critical care and frequently arises as a complication during a patient's stay in the intensive care unit (ICU). Within the hospital environment, staphylococci are the most prevalent Gram-positive pathogens, with *Staphylococcus aureus* being a leading cause of infections in the ICU. Currently, the prevalence of infections with methicillin-resistant *Staphylococcus aureus* (MRSA) is unacceptably high in many parts of Europe, although changes in infection control strategies have reduced incidences in some countries. To optimise clinical outcomes in patients with serious infections, it is necessary to initiate appropriate antibacterial therapy, using antibacterial agents with efficacy against all key pathogens including multidrug-resistant strains, administered at correct doses and via correct routes. Strains of *S. aureus* that exhibit reduced glycopeptide susceptibility, such as vancomycin intermediate *S. aureus* (VISA) and heterogeneous VISA, increase the risk of poor clinical outcomes. In cases of Gram-positive infections that are non-susceptible to established antibacterials, the implications for patient care are significant due to the increased risks of treatment failure, increased duration of hospitalisation, increased mortality, and increased costs. In addition, there are worrying signs that resistance to newer anti-staphylococcal agents is emerging among MRSA and other staphylococci. Optimising antibiotic use is therefore important, and can be achieved through effective multidisciplinary cooperation between clinicians, infectious disease specialists, hospital pharmacists and medical microbiologists. Increasing attention has also been paid to antimicrobial stewardship and antibacterial heterogeneity as strategies for optimising outcomes for patients, minimising the risks of toxicity, and the emergence of resistance. Inherent within this strategy is an even greater need for a wider choice of anti-Gram-positive agents of different classes or mechanisms of action to reduce selective pressure and the risk of resistance development. To enable this and to provide physician reassurance and optimal outcomes in seriously-ill patients, novel and potent antibacterial agents are needed. Potential future options for the treatment of serious Gram-positive infections in severely ill patients may be found in new antibacterial classes, such as the lipoglycopeptides.

New challenges for the infectious disease laboratory

S163 Gram negative resistance: new β-lactamases and testing methods for detection

D.W. Wareham (London, UK)*

Resistance to antimicrobials is one of the most serious challenges facing modern healthcare. The situation is most acute with respect to Gram-negative infections, with extended-spectrum β-lactamase and carbapenemase (NDM-1) producing Enterobacteriaceae and *Acinetobacter baumannii* strains being particularly problematic. Accurate and timely detection and identification of multi-drug resistant isolates is a key role of the clinical laboratory. In this session the options currently available to diagnostic laboratories (phenotypic, molecular, automated) will be reviewed and their ability to influence treatment and infection control interventions discussed.

S164 New advances in virological monitoring of herpes viruses in solid organ transplantation

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The risk of infection in the transplant recipient is a continuous function of the interplay between the net state of immunosuppression and the epidemiologic exposures of the donor and transplant recipient. Traditionally, herpes viruses were considered the most important viral pathogen in terms of their ability to cause clinical illness after transplantation. The prevention of post-transplantation cytomegalovirus and other herpesvirus infections/diseases and the availability of oral antiviral agents have revolutionized post-transplantation care.

The vast majority of infections due to herpes virus simplex 1 and 2 (HSV1–2) and varicella zoster virus after solid organ transplantation (SOT) represent reactivation of latent virus. The first month after transplantation represents the period at highest risk of HSV1–2 reactivation. There is no specific indication for the surveillance of these viruses because the antiviral prophylaxis with acyclovir/valacyclovir has markedly reduced its incidence after transplantation.

During the intermediate post-transplantation period most opportunistic viral infections occur and viruses such as cytomegalovirus (CMV), Epstein Barr virus (EBV) and human herpesvirus 6 (HHV6) are commonly observed. Virological tests are the best means of establishing a diagnosis of the infection and monitoring the virus infection in SOT. In cases of disseminated virus infection, blood is the specimen of choice. Local organ samples (secretions or tissue biopsy specimens) are the best specimens to use in cases of organ syndromes, either separate from or associated with systemic infection. The test method of choice is quantitative measurement of viral DNA in the different pathological materials and real-time PCR assays are now the most commonly used diagnostic method.

Standardized protocols and methods for virological monitoring are mandatory for the correct surveillance of CMV and EBV infection in transplant patients receiving pre-emptive therapy. Multicenter studies conducted to examine the intra- and inter-laboratory variability in qualitative and quantitative CMV and EBV DNA assays indicated that virus DNAemia may be reliably quantified using a variety of commercial and in-house molecular protocols.

In the future, the best approach to monitoring transplant patients for CMV and EBV infections is to perform a careful virological and immunological follow-up in all patients.

S165 HBsAg mutants in routine diagnostic: impact and consequence

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Worldwide about 350 million people are chronically infected with HBV and are at risk of developing liver cirrhosis and hepatocellular carcinoma. The detection of HBsAg in the serum of patients is a corner stone in the diagnosis of HBV. Additionally antibodies against the viral HBsAg are associated with immunity towards viral infection either induced by exposure to HBV or by HBV vaccination. In recent years numerous cases of vaccination failure of newborns born to chronically HBV infected mothers have been reported. Most often mutations in the HBsAg of HBV lead to this immunoescape and could also hamper the detection of HBsAg by diagnostic screening assays. Diagnostic escape can cause severe complications, since the HBV diagnosis might be delayed and/or the risk of HBV transmission might be overseen (like the risk of mother to child transmission at birth or during surgery). Therefore it is mandatory for diagnostic assays used for HBsAg screening not only to be highly specific and sensitive but also to tolerate HBsAg variability of clinical isolates. Even though the frequency of HBsAg mutants causing vaccination and/or immunoescape is still a matter of debate, there is evidence that these mutations are not only selected in patients failing HBV vaccination. The same HBsAg mutantions are frequently observed in patients receiving aHBs antibody immunoglobulin therapy after liver transplantion. Due to the overlapping reading frame of the HBsAg and viral polymerase mutations selected by antiviral treatment with nucleot(s)ide analoga can also lead to changes in the HBsAg sequence. Moreover it was shown that mutations previously associated with immunoescape in the HBsAg might also be compensatory in the polymerase reading frame of drug resistant HBV isolates. Newer antiviral treatment regimens for HBV currently provide the opportunity of sustained viral suppression in the majority of HBV infected patients without the selection of drug resistance. However, worldwide efficient antiviral treatment of HBV is limited by HIV coinfection, the accessibility of the newer drugs and higher costs. HBsAg screening assays have to take care of the probably increasing sequence variability of clinical HBV isolates selected by the antiviral therapy and the immune system.

Prospects for the control of neglected tropical diseases

K176 Prospects for the control of neglected tropical diseases

D. Molyneux (Liverpool, UK)*

Over recent years the profile of the Neglected Tropical Diseases (NTDs) has been increased through as series of high impact publications which have highlighted the problems of these diseases in the context of the Millennium Development Goals and as major contributors to poverty – indeed it will be argued that the prevalence of NTDs are the best indicators of the poverty status of populations. The NTDs, some 17 of which are included in the first WHO Global Report of 2010 "Working to overcome the global impact of neglected tropical diseases" (www.who.int/topics/tropical_diseases/en/) are categorised as "other diseases" within MDG 6, a derisory status, given that some current estimates of the Burden of these diseases outweighs those of malaria or TB whilst the numbers of deaths attributed to NTDs is also greater than the estimated mortality attributed to maternal mortality. The recognition that these infections, which are mainly parasitic, and affect more than one billion people with more than two billion at risk, can and have been cost effectively controlled has

a. increased advocacy for their control through higher prioritisation by policy makers
b. maintained the availability of long term quality drug donations from major pharmaceutical companies
c. ensured increased resources are committed to research on those disease where tools remain inadequate and
d. recognised the cost effectiveness of the interventions allied to robust monitoring and evaluation methodologies.

These factors amongst others are driving the increased commitment from bilateral donors, Foundations, Non Governmental Development Organisations (NGDOs), civil society and most importantly national health authorities to commit more resources to their control or elimination. In addition, there is a real prospect that Guinea worm disease will be eradicated as it only remains endemic in 5 countries in Africa. NTDs are "low hanging fruit" in terms of our capacity to make a rapid impact on the health of the poorest populations with the tools currently available and the proven reach of some programmes which in the case of preventive chemotherapy for helminth infections – lymphatic filariasis, onchocerciasis, schistosomiasis and soil transmitted helminthiasises – through various delivery methods reached some 680 million people in 2009 alone. The annual per person delivery costs being often much less than US$0.50 and in some settings as low as US$0.02. NTDs are diseases, which have been largely ignored whilst the international focus has remained on HIV, TB and malaria, yet they are diseases most prevalent amongst the poorest sectors of the most vulnerable populations. Any attempt at poverty alleviation must include addressing NTDs if development targets are to be met. The address will focus on recent developments and progress towards the goals set by various World Health Assembly Resolutions in controlling and eliminating NTDs whilst highlighting the challenges posed by the need

for diverse interventions against biologically very different organisms in the different health system and geographic settings.

Amoeba as genitor of new pathogenic microorganisms

K177 Amoeba as genitor of new pathogenic microorganisms

D. Raoult (Marseille, FR)*

The amoebas are phagocytic protists that live in water, on the ground and in the mucous membranes of animals. These protists have the capacity to phagocytise all particles of size higher or equal to 100 nanometers. Phagocytosis is not specific but depends on size. Once inside the phagocytes, some micro-organisms acquired the capacity to resist to the intracellular destruction. The protists feed microorganisms, and some microorganisms worked out mechanisms which allowed them, while resisting to the phagocytic protists, to resist to the macrophages later on or to even become potentially disease-causing agents. Inside these phagocytic protists, a microbial community is seen which comprise bacteria, but also giant viruses and eukaryotes, of which fungi. This community life (sympatry) allows exchanging of genes. In fact, genomes of micro-organisms that live in the amoebas are the largest of the intracellular microorganisms and in contrast with other intracellular microorganisms, have chimerical genomes composed of genes from various inhabitants from the amoeba. The amoebas make it possible to create new repertoires and to generate new microorganisms likely to resist the phagocytic action. The first example of bacteria, selected in the amoebas, becoming pathogenic to humans is *Legionella pneumophila*, but the majority of the giant viruses, of which, Mimivirus and Marseillevirus and of many pathogenic microorganisms including mycobacteries, seem to have followed in the same way.

Pro vs Con: guideline vs. clinical practice-driven strategies in treating severe infectious diseases

S184 Nothing can replace clinical judgement

B.E. de Pauw (Nijmegen, NL)*

It has become customary to issue guidelines for the management of life-threatening diseases such as invasive fungal infections. These guidelines are supposed to offer the clinician easy access to the overwhelming amount of sometimes confusing data assuming that the assorted information will help to select the most suitable treatment for the seriously ill patient. Moreover, patients and authorities alike demand clear regulations to provide insight in the medical procedures and an objective benchmark for quality control, repectively. It is obvious that administrative people and clinician appreciate treatment guidelines in a different way. The individual patient occupies the central position in the vision of the clinician, while administrators see a group of patients with a similar disease who require a particular therapy.

Guidelines for its treatment have been published in English by working parties from various countries and as all committees had the same data set at their disposal, great congruency amongst the various documents could be expected. The differences, however, are considerable. Most discrepancies are related to a different interpretation of the clinical trials. However, the value of clinical trials for this purpose is largely overestimated, especially by unexperienced practitioners and regulatory authories. Stringent inclusion and exclusion criteria will prohibit enrollment of children, pregnant women, certain concurrent drugs and serious concomitant diseases. In daily practice there are no such exclusion criteria, which renders study populations often more suitable for the statistian than for the clinician. In addition, empirical trials and salvage studies, which are rather strategic than drug-efficacy studies are of no value at all. Therefore the published guidelines for the treatment of invasive fungal disease have to be taken into consideration but applied with prudence. They are provided for groups but there are frequently good reasons to deviate from the recommendations in individual cases.

Biofilm: from pathogenesis to preventive measures

O187 Modelling of controlled biofilm infection using rats with totally implantable port access intravenous catheter

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Objectives: Safe and easy-to-use port-access intravenous catheters (PAVCs) are integral part of daily clinical practices that may be subjected to major complications such as thrombosis and biofilm infections. The main objective of our study is to develop and validate an IN VIVO rat model of controlled biofilm infection in PAVCs that will allow the real time monitoring of the biofilm establishment and its consequences.
Methods: The PAVC was implanted into the jugular vein by a procedure close to the one used for implantation in patients with the aim to monitor, on a real time basis, biofilm formation within the PAVCs and possible associated infection using bioluminescence. PAVCs were inoculated with controlled dose of bacteria (luminescent variants of biofilm-forming pathogens ESCHERICHIA COLI, STAPHYLOCOCCUS AUREUS and PSEUDOMONAS AERUGINOSA) and monitored over a period of time for biofilm development in both the chamber and along the catheter, using Xenogen IVIS-100.
Results: Immunocompetent rats were able to clear the bacterial load from the blood within 8 days restricting the infection to PAV catheters and in addition, no bacteria were detected in the surrounding organs as determined by colony count method. Defects in the host immune system commonly promote microbial infection. Influence of animal immune system on biofilm development on PAV catheter was analysed and our results showed that immunocompromised rats were highly susceptible to the PAV catheter biofilm related infection leading to bacteremia and metastatic infectious disease that ultimately led to the death of the animal.
Conclusions: We were able to reproducibly establish and non-invasively monitor biofilm development in PAV catheterised rats by three different clinically relevant pathogens allowing us to monitor the progression of the colonisation process along the catheter as well as detection of the associated infection in the blood stream and in the organs. With the application of our in vivo model of PAVC biofilm infection to immuno-suppressed animals we clearly demonstrated that the immune system of the rats is essential to restrict biofilm infection to the device itself. This model will allow evaluating antibacterial strategies as well as anti-adhesive and anti-thrombotic catheter coatings to prevent biofilm infections. Moreover, this controlled model of catheter infection opens the way for studies evaluating the influence of host immune factors on the biofilm-associated infections.

O188 Multidrug-resistance efflux pumps are required for biofilm formation of *Salmonella enterica* serovar typhimurium

S. Baugh, M.A. Webber (Birmingham, UK)*

Objectives: Multidrug resistant (MDR) efflux pumps export a wide variety of substrates including antibiotics, detergents and dyes. As well as this export of a broad spectrum of substrates we show here that MDR pumps have a role in the formation of biofilms. Inactivation of a range of MDR pumps impacts on the ability of *Salmonella* to form a biofilm. We have investigated the mechanisms by which efflux pumps contribute to biofilm formation using the well characterised RND pump, AcrAB-TolC in *Salmonella enterica* serovar Typhimurium as a model. This study investigated three hypotheses; AcrAB-TolC exports a factor required for biofilm formation, mutants lacking components of AcrAB-TolC have

altered membrane integrity, loss of AcrAB-TolC alters expression of biofilm related genes.
Methods: The amount of biofilm produced by wildtype and mutants was quantified under various conditions. Assays used to investigate export of a 'biofilm factor' were; transwell biofilm assays, co-incubation biofilm assays and biofilm assays with addition of exogenous efflux pump inhibitor (EPI). Settle assays, hydrophobicity tests and LPS analysis were used to investigate differences to the cell surface membrane resulting from the loss of AcrAB-TolC. Expression of matrix components was also investigated in all strains.
Results: AcrB and TolC mutants both formed much less biofilm than wildtype in the simple 'crystal violet' biofilm model and mutants formed no biofilm in the more complex 'biofilm mat' model in comparison to wildtype forming an established biofilm mat on the surface of the liquid. Transwell and co-incubation assays showed no rescue of mutant biofilms when cultured with wildtype. Addition of exogenous EPI reduced biofilm formation of wildtype *Salmonella* in a dose dependent manner. Aggregation and hydrophobicity assays all showed no significant difference in the mutants' ability to aggregate compared to wildtype and LPS analysis uncovered no differences between mutant and wildtype LPS composition. Mutants lacking AcrB or TolC did however not express specific components of the biofilm matrix.
Conclusion: An intact, functional AcrAB-TolC efflux system is crucial for *Salmonella* to form a complex biofilm, inhibition of this system blocks biofilm formation, representing a therapeutically useful avenue for EPI development. The role of AcrAB-TolC in biofilm formation appears to be related to regulation of matrix formation, a model is proposed to explain this regulatory circuit.

O189 Lysogenic phages promote pneumococcal biofilm development

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Objectives: *Streptococcus pneumoniae* recovered from human infections have a high prevalence of lysogeny. As pneumococcal biofilms have been implicated both in colonization and infection, we decided to investigate the impact of lysogeny in pneumococcal biofilms. Since extracellular DNA (eDNA) is a major factor in the biofilm matrix, we reasoned that prophage spontaneous activation with the consequent bacterial lysis could provide a source of eDNA, enhancing pneumococcal biofilm development.
Methods: We monitored biofilm development of the pneumococcal isogenic strains R36A and R36AP, which differ only in the presence of a prophage (R36AP is lysogenic for phage SV1). Biofilm growth was followed by biomass quantification, viable cell counts and confocal laser scanning microscopy (CLSM). Phages released during biofilm formation were measured by the total number of PFUs at specific time points. The impact of phage-mediated lytic events within the biofilm structure was determined by comparing biofilm development of the lysogenic strain R36AP to that of the mutants for the phage lysin Svl (R36APdeltasvl), bacterial autolysin LytA (R36APdeltalytA) or both lysins (R36APdeltalytAdeltasvl). To further explore the potential role of eDNA on biofilm development, we determined the effect of the addition of external DNA and DNase I and measured the amount of eDNA released within biofilms by real-time PCR.
Results: Phage-infected bacteria are more prone to form biofilms, yielding structures with higher biomass and cell viability and with CLSM showing denser biofilms for the lysogenic strain R36AP. Phages were detected throughout biofilm growth, indicating that spontaneous phage induction is occurring continuously. Treatment with DNase I resulted in sparser and thinner biofilms while supplementation with DNA resulted in a more densely packed structure. Moreover, the addition of DNA overcomes the impairments created by the ablation of either the phage or bacterial lysins. The quantification of eDNA within pneumococcal biofilms also supports that phage-mediated lytic events are an important source of this matrix component.
Conclusion: Our data indicates that limited phage-mediated host lysis constitutes an important source of eDNA in *S. pneumoniae* biofilms favoring biofilm formation by lysogenic strains.

O190 HWP1 gene polymorphism in *Candida albicans* isolates from bloodstream infections: a possible role in biofilm formation?

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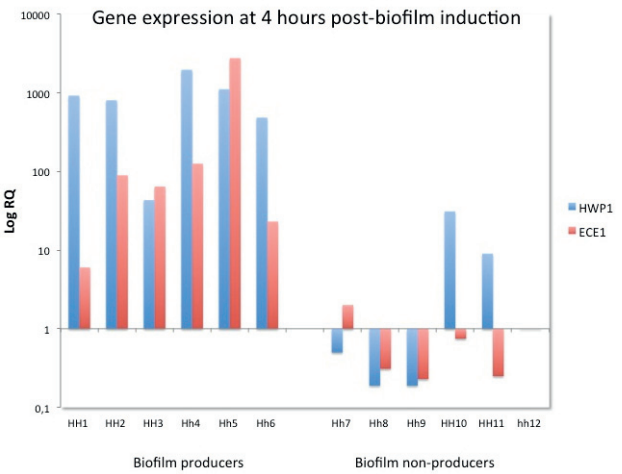
Objectives: Specific signaling pathways regulate *Candida albicans* biofilm formation, involving several gene families. A novel allele of HWP1 (Hyphal Wall Protein 1), a protein required for hyphal growth, has recently been associated to a less adherence capability. The aim of this work was to assess the biofilm formation ability of 142 *C. albicans* BSI isolates, the natural occurrence of the HWP1 polymorphism in this population, and its significance in biofilm formation.
Methods: Biofilm forming ability was assessed in a 96-wells microtitre plate system through quantification by crystal violet (CV) staining and by XTT reduction assay. The polymorphism of HWP1 locus was determined by PCR, and a semi-quantitative RT-PCR was used to discriminate HWP1 alleles expression in heterozygous strains. For gene expression experiments, *Candida* biofilms were formed on polystyrene flasks, and harvested at 4, 8 and 24 hours post biofilm induction. The relative quantifications of HWP1 and ECE1 (Extent of Cell Elongation 1b) gene expressions were made by real-time PCR in a selected group of 12 isolates (6 producers and 6 non-producers).
Results: The propensity to form biofilm was shown in 65/142 *C. albicans* isolates, 60 of them being HWP1 wild type (HH) homozygous strains. The heterozygous presence of HWP1 (Hh) was observed in 5 biofilm producers and in 6 non-producers. The homozygous type HWP1–2 (hh) was seen only in one biofilm non-producer isolate. Only one Hh strain, biofilm producer, showed a preferential expression of the H allele during the early stages of biofilm production. In the initial adhesion stage (4 h) we observed a differential gene expression in biofilm producers, either HH or Hh strains, for both the studied genes (Figure), although Hh strains showed a poor presence of hyphae in their biofilms.
Conclusions: The frequency of the HWP1–2 allelic variant in our *Candida* population was low, including the heterozygous strains. This aspect could reflect the particular setting of our isolates, all from BSI, and the pathogenic importance of HWP1 in invasion. Clinical strains of *C. albicans* vary significantly in their ability to form biofilm. HWP1 seems to play a role in biofilm production, but other genes must cooperate in the biofilm formation such as ECE1. Our results confirm the complexity of molecular mechanisms underlying biofilm production, and the need to investigate its possible pathogenic role in vivo, where other factors as the immune system interact.



O191 Characterisation of accumulation-associated protein interaction partners involved in *Staphylococcus epidermidis* biofilm formation

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Staphylococcus epidermidis is a leading cause of implant-associated infections. Surface colonization crucially depends on biofilm formation, representing the key virulence mechanism of *S. epidermidis*. The 220 kDa accumulation associated protein (Aap) mediates intercellular adhesion and biofilm formation after proteolytic processing and exposure of repetitive domain B. In order to function as an intercellular adhesin, Aap domain B must interact with surface structures on neighbouring cells. Therefore, the aim of this study was to identify Aap domain B ligands involved in intercellular adhesion. To this end we used recombinant Aap domain B to affinity purify binding partners from crude preparations of *S. epidermidis* surface proteins. Amongst other candidates mass spectrometry identified a protein with so far unknown function which we refer to as the Aap associated *Staphylococcus epidermidis* adhesin (AaStrA). AaStrA is a 20 kDa protein which possesses an N-terminal export motive, but lacks homologies to other proteins with known function. Analysis of interactions using recombinant proteins in solid phase ELISA and ligand blotting experiments validated specificity of Aap domain B–AaStrA interactions. In addition, independent t from Aap, AaStrA bound to the staphylococcal cell surface, induced bacterial aggregation and biofilm formation. These data suggest that AaStrA itself is a bona fide intercellular adhesin. aastra is widespread in clinical significant *S. epidermidis* isolates from catheter-related blood stream and prosthetic joint infections. Moreover, AaStrA-specific antibodies were detected in sera from patients with infections related to aastra-positive *S. epidermidis* isolates, indicating that AaStrA is produced in vivo. In conclusion we here identified a novel cell surface protein involved in *S. epidermidis* biofilm formation contributing to implant associated infections, giving noel evidence that protein dependent biofilm formation results from specific protein–protein interactions.

Antimicrobial activity of different antibiotics alone or in combination against MDR Gram-negative goals

O192 The effect of NaCl on carbapenem susceptibility in *Acinetobacter baumannii*

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Objectives: Carbapenem-resistance in *Acinetobacter baumannii* is mediated most frequently through carbapenem-hydrolysing oxacillinases (OXA). NaCl is reported to completely inhibit the action of most OXA and this has led some researchers to screen for OXA by performing carbapenem-MICs in the presence of 200 mM NaCl. However, data on the effectiveness of this is contradictory, and observed effects may be a combination of strain-related characteristics as well as OXA-inhibition. The aim of this study was to investigate the effect of NaCl on carbapenem-susceptibility with defined OXAs expressed in *A. baumannii* ATCC 19606 and ATCC 17978.
Methods: The genes encoding OXA-58 and OXA-164 were cloned into shuttle-plasmid pWH1266 and transformed into ATCC 19606 and ATCC 17978. Clinical plasmids carrying genes encoding OXA-23, OXA-40 and OXA-143 were isolated and transformed in ATCC 19606 and 17978 strains. Transformants (TFs) were selected on Mueller-Hinton Agar (MH) plates with ticarcillin [100 µg/ml]. Plasmid transfer was confirmed by PCR. Susceptibility of TFs and parent ATCC strains against imipenem (IPM) and meropenem (MEM) was determined by Etest using MH plates with and without 200 mM NaCl.
Results: The IPM and MEM MIC values (µg/ml) of the wild-type (wt) and OXA-transformants tested with and without NaCl are shown in the table. OXA-23 did not transfer into ATCC 19606. MICs appear to be strain-dependant; OXA-58/164 transformed ATCC 19606 recorded higher MICs than OXA-58/164 transformed ATCC 17978. Untransformed strains showed a small increase in carbapenem-susceptibility when tested in the presence of NaCl. While MICs of some TFs were reduced when tested in the presence of NaCl, this also appears to be strain-dependant; ATCC 19606 (OXA-164/40) exhibited greater susceptibility than ATCC 17978 (OXA-164/40) when tested with NaCl. OXA-58 TFs were unaffected.
Conclusions: Because carbapenem-MICs of TFs in the presence of NaCl did not reach the susceptibility of untransformed parent strains, the effect of NaCl is probably not due to complete OXA-inhibition. These data also suggest that the effect of NaCl on carbapenem-susceptibility may be dependent upon a combination of strain and OXA-type. We therefore do not recommend performing MICs in the presence of NaCl for screening for the presence of OXA in *A. baumannii*.

		ATCC 19606					ATCC 17978					
	OXA / Agar	wt	58	164	40	143	wt	58	164	143	40	23
IPM MIC [µg/ml]	MH	0.125	>32	>32	>32	>32	0.125	16	16	>32	>32	>32
	MH+NaCl	0.094	>32	6	16	16	0.094	16	16	32	>32	32
MEM MIC [µg/ml]	MH	0.125	>32	>32	>32	>32	0.125	16	32	>32	>32	>32
	MH+NaCl	0.094	>32	1	8	24	0.064	16	32	32	24	32

O193 In vitro activity of doripenem in combination with polymyxin against multidrug-resistant *Acinetobacter baumannii*

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Objective: The prevalence of pandrug resistant (PDR) *Acinetobacter baumannii* (AB), sensitive to polymyxins only is increasing in Singapore; leaving few therapeutic options available. Polymyxin B (P) monotherapy is a viable option, but heteroresistance has become a major problem. Combination therapy may be the only therapeutic option until new antimicrobial agents become available. We evaluate the in-vitro activity of doripenem (D), a new carbapenem in combination with P against PDR AB.
Methods: Multidrug-resistant (MDR) AB isolates from all public hospitals in Singapore were collected from 2006–07. Clonal relatedness was determined by multiplex PCR strain typing; with cluster analysis of banding data performed using the unweighted pair group method with arithmetic mean. MICs were determined according to CLSI broth-dilution method. Time-kill studies (TKS) were conducted with approximately 10^5 CFU/ml at baseline with maximally clinical achievable concentrations of D (13 mg/L, corresponding to 1g every 8h over 4h) and P (2 mg/L, corresponding to at least 1MU q12h), alone and in combination against PDR AB.
Results: Among 361 non-repeat MDR AB isolates screened, 31 PDR AB isolates found were mostly susceptible to P (MIC 1 to 64mg/L) and resistant to all antibiotic classes, including D (MIC >64mg/L). Molecular typing data demonstrated limited clonal clustering of the 31 isolates. In TKS, regrowth occurred in all isolates at 24h with D alone,. With P alone, regrowth occurred in 24/31 isolates and bactericidal activity ($\geqslant$3 log decrease from baseline inocula) were achieved with 7/31 isolates at 24h. This showed that heteroresistance was present in most of our PDR AB isolates. For D+P, it was synergistic (>2 log decrease when compared to its most active antibiotic) in 11/31 isolates (bactericidal in 9 isolates), bacteriostatic (<1 log difference when compared to baseline inocula) in 10/31 isolates, additive in 3/31 isolates and undetermined in 7/31 isolates (P alone is bactericidal) at 24h.
Conclusion: D+P may be a potential antibiotic combination as pre-emptive therapy for PDR AB infections even though PDR AB may remain susceptible to P. The in-vivo relevance of our results warrants further investigations.

O194 Antimicrobial activity of telavancin combined with low-dose colistin versus multidrug-resistant strains of *Acinetobacter baumannii*

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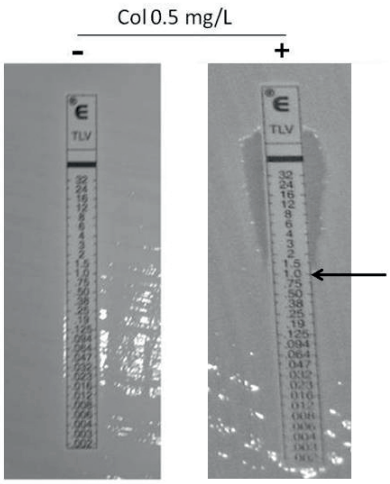
Objectives: Antimicrobial treatment of MDRAB infections remains an important therapeutic challenge. With strains now resistant to tigecycline and colistin, clinicians are increasingly forced to use unorthodox antimicrobial combinations in the hope that they may elude the myriad of resistance mechanisms present in MDRAB. We recently described synergy when the glycopeptide vancomycin was combined with low doses of colistin due to its ability to permeabilise the MDRAB outer membrane. In this study we assessed the activity of telavancin (TLV) when combined with colistin 0.5 mg/L (COL) versus a well characterised collection of MDRAB.
Methods: Forty-two MDRAB isolates were studied, including 5 belonging to the UK epidemic lineages known as 'SouthEast, OXA-23 clone 1, 2, T and Burn'. The remaining isolates were identified as *A. baumannii* by API 20NE and confirmed by species specific PCR (blaOXA-51-like). Susceptibility to β-lactams, quinolones and aminoglycosides was performed using the BSAC disc diffusion method and MICs determined by Etest (tigecycline) or agar dilution (COL). The MIC of TLV was determined by Etest (0.002–32 mg/L range) using isosensitest agar supplemented with and without COL at a fixed sub-inhibitory concentration of 0.5 mg/L.
Results: 42 isolates were studied, all of which were resistant to β-lactams, quinolones and aminoglycosides, 4 were also resistant to tigecycline (MIC >2) and 1 resistant to COL (MIC >256 mg/L). Although the MIC of TLV was >32 for every isolate tested, this was reduced in the presence of COL in 34/42 (80%) isolates. The mean fold reduction in TLV MIC was 4.5 dilutions. For 27 isolates, the TLV+COL MIC was less than the BSAC breakpoint for susceptibility to glycopeptides (2 mg/L), and for 11 isolates, it was below the FDA TLV *Staphylococcus aureus* breakpoint of ⩽1 mg/L, including 3 of the tigecycline resistant strains (Figure 1).
Conclusion: Potent synergy was observed when COL was combined with TLV versus the majority of MDRAB tested in-vitro. This effect was observed with relatively low concentrations of COL, an important consideration when considering potential toxicity. With little prospect of new agents becoming available for the treatment of MDRAB in the near future, further work to assess the relevance of the interactions observed is warranted.



O195 Activity of polymyxin B and rifampin against *Acinetobacter baumannii* in suspension and biofilm

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Objectives: Multidrug resistant (MDR) *A. baumannii* is a major nosocomial pathogen. Polymyxins are predictably active against carbapenem resistant isolates, with dose related nephrotoxicity. Bacterial adherence and biofilm production is associated with antibiotic resistance. Rifampin is able to penetrate into bacterial biofilms. We studied polymixin B and rifampin combination for synergy against carbapenem-resistant *A. baumannii* isolates in suspension and biofilm, thus potentially allowing polymixin B dose reduction, decreasing nephrotoxicity.
Methods: A total of 21 MDR *A. baumannii* isolates, from an institutional outbreak were typed by pulse field gel electrophoresis (PFGE), showing 5 clonally distinct types. All 21 isolates were tested for biofilm production by modified microtiter assay. The strongest biofilm producers of each clonal type were selected for susceptibility testing. MIC's for imipenem, polymyxin B and rifampin were determined by broth microdilution assay. Combination of polymixin B at 0.015–8 mcg/ml and rifampin at 0.125–16 mcg/ml was tested for synergy by checkerboard method. Fractional Inhibitory Concentration Index (FICI) was calculated, synergy being defined as FICI ⩽ 0.5, indifference as >0.5 to ⩽4 and antagonism as >4. The same isolates were then tested in a biofilm model. Biofilm sampling was done and the MBC's for polymyxin B and rifampin were determined. MBC's were also determined for the antibiotic combination in suspension and biofilm.
Results: Three strains were strong and 2 were weak biofilm producers. All were resistant to imipenem and susceptible to polymixin B (MIC ⩽1 mcg/ml). MIC's for rifampin were 4–16 mcg/ml. Polymixin B MBC's in suspension were ⩽1 mcg/ml for 4 isolates but higher (MBC ⩾8) for all isolates in biofilm. The combination of polymixin B and rifampin showed synergy (FICI ⩽ 0.5)against all isolates at polymyxin B concentrations ⩾0.125 mcg/ml and rifampin concentrations ⩾1 mcg/ml. Polymixin B combined with rifampin at 0.25 and 2 mcg/ml showed at least a 2 fold decrease in MBC's for 4 isolates in suspension but not in biofilm.
Conclusions: Polymyxin B and rifampin are synergistic against bacteria in suspension but not bacterial biofilms, which significantly increases antibiotic resistance even in "weak" biofilm producers. Polymyxin B dose reduction may not be feasible in biofilm-associated infections.

O196 The in vitro antimicrobial effect of colistin alone and in combination, against clinical isolates of multidrug-resistant *Pseudomonas aeruginosa*

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Introduction: The global emergence of "pan resistant" clinical strains of *Pseudomonas*. aeruginosa has led to the increasing use of colistin (CS) in the treatment of infections due to these isolates.
Objectives: The aim of this study was to ascertain the in vitro effect of CS alone and in combination on MDR clinical strains of *P. aeruginosa*.
Methods: 30 clinical strains of *P. aeruginosa* genetically characterized for their β-lactam resistance were collected. All strains harbored multiple resistance determinants (ESBLs, AmpCs, carbapenemases, loss of porins and efflux mechanisms). The MICs and the in vitro synergistic effect of colistin and 7 other antibiotics [ceftazidime (CAZ), aztreonam (ATM), meropenem (MEM), doripenem (DOR), amikacin (AKN), levofloxacin (LEV), rifampicin (RIF)] were determined by the checkerboard method. For 10 strains these associations were also evaluated by the E-test diffusion method. The bactericidal effect of the most frequently synergistic combination (RIF-CS) was assessed by killing curves.
Results: All strains were susceptible to CS (MIC = 1–2 mg/l), but presented different levels of resistance to the other antibiotics tested: MICs (mg/l): CAZ, 1–>64, ATM: 4–>64, MEM, 0,2–>64; DOR, 0,1–64; AKN, 4–>64; LEV, 0,2–>64; RIF, 8–>64. The MICs determined by E-test were in concordance. The SigmaFIC analysis showed that the combination of CS to β-lactams was primarily additive

(CS-CAZ, n = 22; CS-ATM, n = 25; CS-MEM, n = 16; CS-DOR, n = 23), regardless of the mechanism of resistance present in the strain. The combination of CS to AKN or LEV was additive or indifferent (n = 21 and 25, respectively) whilst CS-RIF was additive or synergistic (n = 28). By killing curves, preliminary results with two strains showed that the combination CS-RIF was more synergistic when CS was used at sub-inhibitory concentrations (0.5mg/L) than when used at the breakpoint concentration (2mg/l).
Conclusion: Colistin improved readily the in vitro activity of the antibiotics tested on all MDR clinical strains of P aeruginosa. No antagonism was observed. The most synergistic combination was CS-RIF. This combination of antibiotics could represent a valuable therapeutic option for the treatment of patients infected by MDR *P. aeruginosa*.

Excellence Award

K197 Vaccines, medicine and public health in the XXI century

R. Rappuoli (Siena, IT)*

During the 20th century, vaccines have eliminated most of the childhood diseases with the major exceptions of the diseases caused by meningococcus and respiratory syncytial virus (RSV). What is the role of vaccination in the 21st century? The first target will be to develop vaccines for meningococcal meningitis, which is perhaps the last disease that in a few hours can attack and kill healthy children and young people, and RSV that affects virtually every single child in the first few months of life. Fortunately, thanks to several revolutionary technologies developed during the last 30 years, including conjugation, genomics and new adjuvants, we are in the final stages to conquer meningococcal meningitis and new approaches are being tested for RSV. The second and perhaps most important target of vaccination in the 21st century will be to take care of the global health problems of this century. These include taking care of the aging population, with new vaccines targeting the diseases typical of the elderly with an aging immune system, to control emerging antibiotic resistance, to preventing cancer, taking care of the diseases present only in countries affected by poverty, and taking care of emerging diseases such as pandemic influenza. Overall, vaccines in the 21st century will have an increased safety, and will be used as an insurance to ensure health across all ages, for the entire life.

Are probiotics growth promoters in humans?

S204 Are probiotics growth promoters in humans? – No

Y. Sanz (Valencia, ES)*

It has been hypothesized that the intestinal microbiota could be causally related to obesity and associated metabolic disorders and, therefore, its intentional manipulation could be a therapeutic target. Evidence of the role microbiota plays in obesity is primarily based on comparisons between germ-free and conventional mice showing that germ-free mice have 40% less body fat, and that conventionalization with the caecum microbiota causes a 60% increase in body fat and insulin resistance. Observational studies comparing the gut microbiota of obese and lean subjects have led to establish associations between obesity and specific phyla and bacterial groups. Although, there is not full consensus, several studies have associated obesity with reduced proportions of Bacteroidetes and increased Firmicutes, and weight loss in obese human subjects with increases in Bacteroidetes or *Bacteroides* subgroups. Observational studies on the relationships between bacteria used as probiotics for humans (*Lactobacillus* and *Bifidobacterium*) and obesity have, however, yielded conflicting results or no associations. In addition, the use of probiotics as growth promoters in farm animals has led part of the scientific community to attribute to these bacteria an obesogenic role. Nevertheless, the effect of probiotics on animal weight-gain can be secondary to suppression of infections and no primary role in obesity has been proven; this is supported by the fact that probiotic administration is not accompanied by increases in body fat, which would be expected from an obesity promoter. Moreover, most interventions conducted in infants and children indicate that probiotics do not influence growth, except for a few cases of undernourished children or those suffering infections. Conversely, other scientists are exploring the possibility that probiotic bacteria help in the management of obesity and associated disorders by regulating the host's metabolic and immune functions. So far evidence from human studies is limited but in obese animal models specific *Lactobacillus* and *Bifidobacterium* strains and prebiotics have exerted protective roles, for instance reducing lipid absorption, liver steatosis, adipocyte size, intestinal permeability and inflammatory markers associated with insulin resistance. Although, the possible anti-obesogenic role of probiotics has yet to be proven by conducting appropriated human intervention studies, there is no evidence that probiotics constitute growth promotes and contribute to the obesity epidemic in humans. Most likely the answer to the question whether probiotics have a positive or negative impact on obesity cannot be addressed by a simplistic answer but would depend on the complex interplay between specific strains, the host status and the diet.

S205 Are probiotics growth promoters in humans? – Yes

D. Raoult (Marseille, FR)*

Several bacteria, of which much was used for the fermentation of milk, are used in humans as probiotics, such as various species of *Lactobacillus*, *Bifidobacterium*, or *Lactococcus*. Some are used as growth promotor factors in farm animals, such as species of *Lactobacillus*, *Lactococcus*, *Enterococcus*, and *Bifidobacterium*. Until a recent past, no comparative study between those which were used in the farm industry as growth promotors, to obtain weight gain in farm animals (pigs, calves, chickens) and those used on humans for health benefit, had been compared. We undertook an exhaustive study, species by species, of the role of these bacteria, by comparing the work completed in the farm animals, in the experimental models (mouse and rats) and the preliminary studies carried out in humans. The probiotics were tested in children, and the quantification of *Lactobacillus* in obese, compared with lean subjects were tested. The whole of these studies shows that a certain number of species seems protective against obesity, and other species marketed for human consumption seem significantly associated to weight gain in all the studies that were carried out.

Molecular bacteriology: insight in the virulence of Gram positive cocci

O210 The contribution of bacterial virulence to the clinical course of *Staphylococcus aureus* bloodstream infection

S. McNicholas, A. FeTalento, J. O'Gorman, M. Hannon, M. Lynch, A. Shore, D. Coleman, H. Humphreys, D. Fitzgerald-Hughes (Dublin, IE)*

Objectives: Bloodstream infections (BSI) caused by *S. aureus* occur in many different patient groups with a variety of outcomes. The contributing factors to these outcomes are unclear. We prospectively determined the genotypes and virulence characteristics of 58 *S. aureus* isolates recovered from patients with methicillin-resistant *S. aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA) BSI.
Methods: Demographic information and clinical details were recorded. Isolates were characterised by spa-typing and using a DNA microarray (Alere Technologies, Germany) which detects 185 *S. aureus* genes (and allelic variants) including strain affiliation and virulence-associated genes.
Results: Almost a third (32.8%) of patients were renal and in half of all the patients an infected intravenous catheter was the source. Over 20% of patients developed a complicated infection (e.g. infective endocarditis). Thirty different spa-types were represented among the 58 isolates tested. All MRSA isolates (n = 13) belonged to accessory gene regulator (agr)

type I, capsule type 5 and the ST22-MRSA-IV clone. The MSSA isolates (n = 45) belonged to a variety of agr types, capsule type 5 or 8 and a number of clonal complexes (12 in total). All isolates were lukPV negative. The enterotoxin gene cluster (egc) (seg, sei, sem, sen, seo and seu) and the staphylococcal enterotoxin genes sec, sel, were more prevalent in MRSA than MSSA ($p < 0.05$). There was no significant difference in the prevalence of virulence genes, such as toxic shock syndrome and enterotoxin genes or in clonal complexes between isolates from complicated (n = 12) and uncomplicated clinical infection (n = 46).
Conclusion: The clinical course of infection appeared independent of the clonal complex. Carriage of virulence genes, particularly the pathogenicity islands, appears to be clonal. The virulence potential of the infecting organism, in terms of carriage of virulence genes, does not appear to be a significant factor in clinical outcome. However, the increased prevalence of egc and sec/sel among the ST22-MRSA-IV clone, the predominant clone in Irish hospitals, may be a marker for increased pathogenic potential compared to MSSA clones.

O211 Correlation between bacterial DNA load and severity of disease in *S. aureus* bacteraemia

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Objectives: Bloodstream infection with *Staphylococcus aureus* is a serious infection that requires rapid and adequate treatment. Little is known about the bacterial DNA load (BDL) of *S. aureus* in different types of infection, ranging from less severe uncomplicated phlebitis to severe life threatening endocarditis. Previous studies have shown that level of BDL in infections with other pathogens corresponds to severity of disease, but this has not yet been shown for *S. aureus*. We determined the BDL in blood of patients with *S. aureus* infection and concurrent bacteraemia.
Methods: A cohort of 32 consecutive patients with culture proven *S. aureus* bacteraemia was categorized into superficial tissue infections (postoperative wound infections and phlebitis), deep tissue infections (osteomyelitis and arthritis) or endocarditis. Temporarily stored whole blood samples, which had been drawn at the time of the blood culture, were collected. Quantification of *S. aureus* specific DNA was performed by real time PCR on DNA extracted from 200ul of blood.
Results: Of 32 patients, 18 patients had a soft tissue infection, 10 a deep tissue infection and 4 endocarditis. The *S. aureus* specific BDL was above the detection limit in 19 patients (59%). All endocarditis patients had a positive PCR while there was no significant difference between the percentages of positives in the other 2 groups. The median BDL in the endocarditis group was 422 cfu/ml (range 92–759), clearly higher than the median BDL of deep tissue infections and superficial tissue infections being 12 cfu/ml (range 3–34) and 4 cfu/ml (range 2–74) respectively.
Conclusion: In patients with *S. aureus* bacteraemia, the BDL is higher in patients with a severe infection (endocarditis) compared to patients with other causes of *S. aureus* bacteraemia. Measurement of the BDL could potentially help clinicians recognise patients at risk of having endocarditis. In patients with superficial and deep tissue infections around 50 percent is detected by our PCR and height of BDL does not differ between these groups. This suggests these infections are characterized by lower amount of DNA in the blood stream or intermittent circulation of bacteria.

O212 The distribution of enterotoxin and immune evasion cluster genes among *Staphylococcus aureus* isolates causing bloodstream infection

S. McNicholas, A. FeTalento, J. O'Gorman, M. Hannon, M. Lynch, A. Shore, D. Coleman, H. Humphreys, D. Fitzgerald-Hughes (Dublin, IE)*

Objective: Bloodstream infections (BSI) caused by *S. aureus* often have unpredictable outcomes. Enterotoxin genes and the newly identified immune evasion cluster genes (IEC) (sea, sak, chp and scn) are thought to contribute to the severity of *S. aureus* infection. We determined the distribution of enterotoxin and IEC genes across 12 clonal complexes (CC) of both methicillin-resistant *S. aureus* (MRSA) (n = 13) and methicillin-susceptible *S. aureus* (MSSA) (n = 45) isolates recovered prospectively from patients with BSI.
Methods: *S. aureus* isolates causing BSI over a two year period, (2008–2010) were collected. A DNA microarray (Alere Technologies, Germany) which detects 185 *S. aureus* genes (and allelic variants) was used for molecular characterisation and strain affiliation of the isolates.
Results: The enterotoxin gene cluster (egc) (seg, sei, sem, sen, seo and seu) was associated with CC5, CC9, CC22, CC25, CC30, CC45 and CC121, the staphylococcal enterotoxin genes sec/sel were associated with CC22 and CC45. Sek/seq was found in CC1 and CC8, while sed/sej/ser was found in CC8. While sec/sel, sed/sej/ser and sek/seq were associated with only some isolates within a given clonal complex, egc was common to all isolates within each CC when it was present. CC15 and CC398 carried no enterotoxin genes. Both egc and sec/sel, were more prevalent in MRSA than MSSA ($p < 0.05$) due to the preponderance of CC22 in MRSA. The IEC genes were found in all CCs examined. The majority of isolates belonged to IEC type B (35, 60%) with IEC types A and E also represented in a significant number of isolates (11, 18.9% and 8, 13.7% respectively). Only 3% of isolates (2/58) harboured IEC types D or E. All isolates within a given CC belonged to the same IEC type, with the exception of CC30 and CC8 which showed variation in IEC type. CC30 isolates harboured IEC types A (5/9), B (3/9) and C (1/9), while CC8 isolates harboured IEC types B (2/3) and E (1/3). All MRSA isolates belonged to IEC type B, while all IEC types were seen among the MSSA isolates with type B being the most prevalent.
Conclusion: The presence of egc in seven clonal complexes suggests that both vertical and horizontal transmission of this genomic island has occurred. The prevalence of egc and sec/sel among MRSA isolates is probably due to the prevalence of the ST22-MRSA-IV clone.

O213 Invasive infections caused by a novel species within the *Streptococcus mitis* group

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Objectives: Twelve clinical cases were observed with infections caused by a novel streptococcal species within the *Streptococcus mitis* group. The corresponding ATCC 15914 strain was previously assigned to *Streptococcus mitis*, but molecular and phenotypic analyses showed a clear distinction from the established species within the *S. mitis* group.
Methods: Microbiological and clinical data were analysed retrospectively. 16S rRNA gene sequencing and subsequent homology analyses in the GenBank database was performed for the clinical streptococcal isolates (n = 16) and the strain ATCC 15914. Additionally, a 313 bp fragment of the recA gene was amplified with a specific set of recA primers for sequence analysis. Biochemical data of the strain ATCC 15914 were obtained by API Strept 20 (bioMérieux, Marcy-l'Etoile, France). For differentiation within the *S. mitis* group, type strains of the most closely related species were analysed, i.e. *Streptococcus infantis*, *S. mitis*, *Streptococcus oralis*, *Streptococcus pneumoniae* and *Streptococcus pseudopneumoniae*.
Results: The isolates (n = 16) were obtained from different body sites, e.g. blood, aortic/mitral valve, cerebrospinal fluid, from 12 patients with invasive infections such as sepsis, endocarditis and meningitis. Partial 16S rRNA gene sequences of the 16 clinical isolates and the strain ATCC 15914 showed sequence homologies of >99.5%. Phylogenetic analyses revealed a clear differentiation from the closest related species within the *S. mitis* group. 16S rRNA gene sequence similarities of the ATCC strain 15914 were 98.3% with *S. infantis* (AY485603) and *S. oralis* (AY485602), 98.0% with *S. mitis* (AY485601), 97.8% with *S. pneumoniae* (AY485600) and 97.9% with *S. pseudopneumoniae* (AY612844), respectively. recA sequence similarities of the ATCC strain 15914 were 94.2% (*S. oralis*), 91.7% (*S. mitis*), 91.4% (*S. pseudopneumoniae*), 90.7% (*S. pneumoniae*) and 90.1% (*S. infantis*), respectively.

Biochemical characteristics confirmed the accurate differentiation of the strain ATCC 15914 from *S. mitis* and its closest related species within the *S. mitis* group.
Conclusion: We propose an emended description of the ATCC strain 15914, which was formerly assigned as *S. mitis*. We found a clear distinction within the *S. mitis* group based on 16S rRNA gene, recA and biochemical characteristics. The pathogenic potential of this strain was demonstrated in 12 clinical cases with severe infectious diseases.

O214 Fitness cost of widely disseminated conjugative plasmids carrying Tn1546-vanA among *Enterococcus faecium* and *Enterococcus faecalis*

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Objectives: Tn1546-vanA is located in conjugative plasmids containing replication proteins of RepA_N family in *Enterococcus faecium* (Efm, Inc18, pRUM, pLG1) and *Enterococcus faecalis* (Efc, pheromone responsive plasmids). Little is known about the fitness cost of either Tn1546 or Tn1546-plasmids in enterococci. The aim of this study was to address these issues by analyzing representative plasmids encoding vancomycin resistance in Efm and Efc.
Methods: We studied Tn1546-plasmids from Efm (n = 6, Inc18, pRUM, pHTB and pLG1 derivatives) and Efc (n = 2, pAD1-like). They are mosaic plasmids of Efm (2 megaplasmids of 150 and 240kb, 1 rep-pRUM/relpEF1 of 90kb; 1 repInc18/reppRE25/reppRUM/relpEF1/w-e-z of 50kb; 1 repInc18/reppRE25/reppRUM/relpEF1/axe-txe of 40kb and 1 repInc18/reppHTB/relpHTB of 85kb) and Efc (75kb repInc18/relpAD1; 110kb reppRE25/reppAD1/par). Plasmids were introduced in plasmid free strains of Efm (ST515-GE-1, ST172-BM4105) and Efc (ST8-JH2–2) by conjugation. Growth curves were performed as described by Foucault et al. using isogenic plasmid-free strains as controls. Relative growth rates (RGR) in presence/absence of vancomycin were used to determine fitness cost.
Results: Efm plasmids did not show significant fitness cost in either Efm bacterial background tested in absence of vancomycin (RGR±1). However, experiments induced with vancomycin revealed cost for the expression of Tn1546 on particular plasmids and host backgrounds. While the carriage of Tn1546 had a relatively low fitness cost for Efm-BM4105 (ST172) (<6%), this value varied in Efm-GE-1 (ST515) from 14–17% for plasmids carrying modules of pRUM (rep, TA), Inc18 (repI, repII) and pEF1 (rel) to >57% for plasmids carrying modules of pRUM (rep), Inc18 (repI, repII, PSK) and pHTB (rep, rel). Efc Tn1546-plasmids frequently suffered reorganizations during conjugation. They conferred a relatively high fitness cost for the host in the absence of vancomycin (13%-22%). However, the expression of Tn1546 had a low cost for the host (<7%).
Conclusions: The low fitness cost of Tn1546-carrying plasmids in Efm, in the absence of selective pressure, might determine the wide dissemination of vancomycin resistance in this species. The instability of Efc Tn1546-carrying plasmids as well as the relatively high cost of these plasmids might determine their loss in the absence of selective pressure and there for, up to some extent, explain the low prevalence of vancomycin resistance among Efc.

Genetics of NDM-1

O215 Complete DNA sequence of blaNDM-1 gene cassette integrated into the chromosome of *Acinetobacter baumannii*

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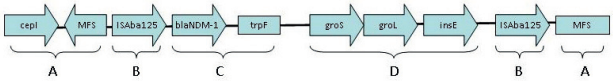
Objectives: New Delhi metallo-β-lactamase 1 (NDM-1), first reported in a *Klebsiella pneumoniae* isolate in Sweden, has now been detected in other Enterobacteriaceae species from Europe, Kenya, Australia and India. More recently it has been detected in *Acinetobacter baumannii*. The genetic location of NDM-1 is assumed to be a plasmid but there are reports of non-transferability which suggests that in some cases it is chromosomal. In the present study we have investigated the genetic location of blaNDM-1 detected in *A. baumannii* isolated from a patient in Frankfurt in 2007.
Methods: Transfer of blaNDM-1 was tested by conjugation into *Escherichia coli* J53 and transformation with plasmid DNA into *A. baumannii*. Whole genomic DNA was isolated and shotgun-cloned into *E. coli* NEB 5-α and selected with ticarcillin. Plasmid-inserts of transformants were amplified by PCR and sequenced by primer walking.
Results: Transformation of *A. baumannii* with native plasmid, and conjugation into *E. coli* failed to transfer blaNDM-1. However, shotgun cloning of chromosomal DNA transferred blaNDM-1. Sequencing of the insert confirmed that the NDM-1 gene cassette was chromosomally located, and was integrated into a gene encoding a putative *A. baumannii* major facilitator superfamily (MFS) metabolite/H+ symporter (Figure, feature A). Adjacent to the MFS gene was a chromosomal homoserine lactone synthase (cepI). Primer walking from both ends of the MFS gene revealed that blaNDM-1 was on a 10.5kb gene cassette bracketed by the insertion sequence ISAba125 (Figure, feature B). BLAST analysis of ISAba125 revealed that this element appears to be common in *A. baumannii*. The IS26 transposase upstream of blaNDM-1 gene that was described in several reports was missing in the *A. baumannii* sequence. The phosphoribosyl anthranilate isomerase gene (trpF) was truncated (Figure, feature C). In addition, there was a 4kb element 94% similar to that described in *E. coli* plasmid pEH4H encoding the chaperonin subunits groS and groL, and the transposase insE (Figure, feature D). No significant DNA homology or open reading frames were detected between trpF and groS.
Conclusion: These data show that in our isolate the blaNDM-1 gene was located on the chromosome of *A. baumannii*. Since it is flanked by insertion elements, it is likely that blaNDM-1 can be shuttled between plasmid and genome.



O216 Diversity of genetic structures at the origin of acquisition and expression of the blaNDM-1 carbapenemase gene

L. Poirel, R. Bonnin, L. Villa, A. Carattoli, P. Nordmann (Le Kremlin-Bicêtre, FR; Rome, IT)*

Objectives: NDM-1-mediated resistance to carbapenems is increasingly reported worldwide. The blaNDM-1 gene is mostly plasmid-located and mostly from *Klebsiella pneumoniae* and *Escherichia coli*. The blaNDM-1 gene was initially identified associated to mosaic structures containing remnants of different genes and insertion sequences. The aim of our work was to characterize the genetic structures surrounding the blaNDM-1 gene among our collection of NDM-1-producing isolates recovered from various countries.
Methods: A series of blaNDM-1-positive isolates were investigated. Cloning, PCR experiments and whole plasmid sequencing were performed to identify the upstream- and downstream-located regions of the blaNDM-1 gene in isolates from different geographical origins and from different species. Isolates studied included *E. coli* and *Citrobacter freundii* from France, *K. pneumoniae* from Sultanate of Oman, *K. pneumoniae* from Kenya, *E. coli* from Australia, and *K. pneumoniae* from India.
Results: Among the six NDM-1-producing isolates studied, different genetic structures surrounding the blaNDM-1 gene were detected. A remnant of insertion sequence ISAba125 (previously identified in different *Acinetobacter baumannii* isolates) was systematically identified upstream of blaNDM-1, but different truncations of this IS were observed. The remaining part of this ISAba125 element contained two base pairs that were always part of a −35 promoter sequence for blaNDM-1 expression. Downstream of blaNDM-1, a gene encoding a putative bleomycin resistance determinant was systematically identified, then followed either by genes of unknown functions, insertion sequences,

or complex sul1-type integrons comprising arrays of resistance genes and the ISCR1 element. Detailed analysis of the plasmid support of the blaNDM-1 gene among the different isolates showed a diversity of plasmid scaffolds, including IncN-type, IncA/C2, and IncL/M features.
Conclusion: This works identified original structures at the origin of acquisition of the blaNDM-1 gene identified in non-clonally-related enterobacterial isolates. It shows that the current emergence of this resistance gene is associated with a common ISAba125 sequence which was truncated at various levels.

O217 Whole genome sequencing of NDM-1-producing *Escherichia coli*: focus on antibiotic resistance

L. Poirel, R. Bonnin, P. Nordmann (Le Kremlin-Bicêtre, FR)*

Objectives: NDM-1-mediated resistance to carbapenems in Enterobacteriaceae is increasingly reported worldwide. We recently identified the blaNDM-1 gene from an *Escherichia coli* isolate recovered in Australia from a patient transferred from Bangladesh. The aim of our work was to characterize the genomic features of that *E. coli* strain, with a special focus on antibiotic rresistance genes and their associated genetic structures.
Methods: Complete sequencing of the genome of *E. coli* 271 was performed by the DNA Vision company (Gosselies, Belgium). Annotations and contig assemblage were performed with softwares availble over internet.
Results: *E. coli* 271 was resistant to all β-lactams, including carbapenems, all aminoglycosides, fluroquinolones, nitrofurantoin, and sulfamides. It belonged to the ST101 sequence type. It harboured five plasmids, on which numerous resistance genes were identified. Plasmid p271A carried the blaNDM-1 gene bracketed by two novel insertion sequences, but no additional resistance genes. Interestingly, that plasmid possessed the perfect scaffold of an IncN-type plasmid known to disseminate the carbapenemase gene blaKPC-3, but the replicase gene was a different and novel one, that makes that plasmid untypeable by PCR techniques. Plasmid p271B carried the extended-spectrum β-lactamase blaCTX-M-15 gene that was associated with blaTEM-1. Plasmid p271C of IncF type carried the rmtB methylase gene together with blaTEM-1. Plasmid p271D of IncI1 type carried the armA methylase gene together with blaTEM-1. Additional β-lactamases blaOXA-1 blaOXA-9 blaOXA-10 were identified, together with aminoglycoside resistance genes aadA6, aphA1, and aac(3′)-II, macrolide resistance genes ermB, mph2, and mel, trimethoprim resistance genes dfrA1 and dfr12, rifampin resistance gene arr2, chloramphenicol resistance genes cmlA5 and catA, sulfonamide resistance gene sul1, and quinolone efflux gene qepA. Resistance to fluoroquinolones was also explained by substitutions in the chromosmally-located gyrA and parC topoisomerase genes.
Conclusion: This works identified an high number of resistance genes that explained the multidrug resistance pattern. Most of these resistance genes were plasmid-borne. This suggests successive accumulations of multiple resistance determinants that might be driven by antibiotic selective pressure.

O218 Spread of blaNDM-1 gene identified in enterobacterial isolates in Switzerland: a molecular analysis

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Objectives: The blaNDM-1 gene that encodes resistance to carbapenems is increasingly reported worldwide. The aim of our work was to characterize the genetic structures surrounding the blaNDM-1 gene, to precise its plasmid support, and identify the genetic background of the clinical isolates in a series of enterobacterial isolates recovered from Switzerland from patients originated from the Indian subcontinent and Serbia.
Methods: Four blaNDM-1-positive isolates recovered from three patients were investigated, being one *Escherichia coli*, two *Klebsiella pneumoniae*, and one *Proteus mirabilis* isolates. PCR experiments were performed to identify the upstream- and downstream-located regions of the blaNDM-1 gene. Multi Locus Sequence Typing (MLST) was used to identify the strains at the sequence type level. Mating-out assays were performed with *E. coli* J53 as recipient using a selection based on cefoxitin (10 μg/ml) and azide (100 μg/ml). PCR based replicon typing was used to determine the plasmid types.
Results: Among the four NDM-1-positive isolates, different genetic structures surrounding the blaNDM-1 gene were detected. The insertion sequence ISAba125 was systematically identified upstream of the blaNDM-1 gene. Downstream of the blaNDM-1 gene, a gene encoding a putative bleomycin resistance determinant was identified in all but the *E. coli* isolates. Transconjugants were obtained with all donor strains, and analysis of the plasmid support of the blaNDM-1 gene among the different isolates showed a diversity of the plasmid scaffolds. In the *E. coli* isolate, the blaNDM-1 plasmid was 100 kb in size and belonged to the IncF type. By contrast, the three other blaNDM-1-plasmids were ca. 160 kb in size and belonged to the IncA/C2 type. Two of these IncA/C2 plasmids co-harboured the blaCMY-16 gene together with blaNDM-1.
The *E. coli* was an ST410 strain, co-producing TEM-1, OXA-1, and CMY-30. The *P. mirabilis* co-produced TEM-1, OXA-1, OXA-10, and CMY-16. The two *K. pneumoniae* were clonally unrelated, belonging to ST25 and ST147 types, respectively, and were ESBL positive. The two *K. pneumoniae* co-expressed CTX-M-15, SHV-11, TEM-1, OXA-1, and OXA-10, and one additionally produced OXA-9 and CMY-16.
Conclusion: This works identified a diversity of genetic structures at the origin of acquisition of the blaNDM-1 gene among non-clonally-related enterobacterial isolates. It further underlines the threatening accumulation of β-lactamase determinants in NDM-1-producers.

O219 A novel ICE mobilising DHA-1 in NDM-1 harbouring clinical isolates of *E. coli* and *Klebsiella*

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Objectives: NDM-1 producing clinical isolates from India are resistant to all antibiotics with the exception of tigycycline and colistin. Since NDM-1 has the ability to give resistance to all β-lactam antibiotics with the exception of aztreonam, the resistance to aztreonam has to be due to another resistance mechanism or mechanisms. Plasmid sequencing strategies indicated that many NDM-1 isolates harboured the class C β-lactamase DHA-1 and the class D β-lactamase OXA-10 in addition to NDM-1. Since these enzymes are both able to confer resistance to aztreonam we sought to understand the mechanism of mobilisation of these resistance genes.
Methods: NDM-1 and DHA-1 transconjugants were prepared by mating clinical isolates of *E. coli* and *Klebsiella pneumoniae* with azide resistant J53 overnight without selection followed by growth on meropenem/azide or ceftazidime/azide selective plates. The genomic location of NDM-1 and DHA-1 in parents and transconjugants was then investigated by S1 digestion of macro DNA followed by Pulsed field gel electrophoresis and probing with 32P labelled NDM-1 and DHA-1 probes. PCR using primers designed to the phage integrase gene and toxin/anti-toxin genes of SXT were used to investigate whether the ICE SXT was present in these isolates.
Results: Selection of transconjugants on merpenem/azide plates followed by S1 genomic mapping indicated that all parents and transconjugants harboured NDM-1 on various different sized plasmids. However, probing of the same parents and transconjugants with DHA-1 indicated that all parents and transconjugants harboured DHA-1 on the chromosome. Similarly all transconjugants selected on ceftazidime/azide plates harboured DHA-1 on the chromosome whereas NDM-1 was only transferred in 20% (2/5) of these transconjugants. All PCR reactions for SXT genes were negative.
Conclusions: The broad resistance profile of NDM-1 harbouring clinical isolates is clearly due to many resistance mechanisms in addition to NDM-1. Here we demonstrate that the class C β-lactamase DHA-1 is mobilised from chromosome to chromosome from *E. coli* and *Klebsiella*

clinical isolates to *E. coli* J53 consistent with that of integrative and conjugative elements. In addition our initial experiments indicate that the ICE responcible for the movement of DHA-1 is not SXT and the mechanism of mobilisation of DHA-1 is more efficient than that of NDM-1.

The future of hospital antibiotic stewardship – what should we achieve by 2020?

S233 Interactive E-learning

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Education of antimicrobial prescriber's is one of the key tools that underpins the implementation of antimicrobial stewardship programmes. The technical and non-technical competencies that are required to prescribe appropriately have been described and form a useful basis for developing teaching. The delivery of this education has primarily been through traditional models of educational delivery. Examples include: face to face learning e.g through didactic lectures or workshops with varying levels of interaction, self directed learning packages, video or satellite broadcasts to groups, one to one individual training[less common as very resource intensive] and others. A more recent method has been the use of a range of online learning sites or resources which provide education through the Web. The quality, transparency and appropriateness of these resources are variable as is their applicability to many different healthcare settings.

E-learning is the use of various technological tools that are Web based, Web distributed or Web capable that support the development, exchange, and application of knowledge, skills, attitudes and aspirations or behaviours for the purpose of improving teaching and increasing learner achievement.

For those wishing to undertake the development of e-learning a number of fundamental principles should be adhered to. For example, it is important to recognise that e-learning is a means of implementing education that can be applied within varying education models and philosophies, it can enable unique form of education that can fit into existing paradigms, the choice of e-learning tools should reflect rather than determine the pedagogy of a course, can be used for presenting educational content and or facilitation of educational processes, should only be use once the benefits and trade-off of online v off line have been considered, should consider how users will engage with the opportunities and that the development of material should fall within the context of the curriculum objectives and not determined by the technology.

This presentation will focus on the application of e-learning methods to support resources available for antimicrobial stewardship. The author will illustrate the development, implementation and evaluation of some resources developed as part of a national stewardship programme in Scotland [(http://www.scottishmedicines.org.uk/SAPG/Scottish_Antimicrobial_Prescribing_Group__SAPG_]

Influenza H1N1 pandemic and complications

O236 Aetiology of community-acquired pneumonia in the adult and the H1N1 influenza pandemic

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Background: Viral causes of CAP in the adult are poorly characterized. Polymerase chain reaction (PCR) based methods have been developed for many respiratory pathogens. Since the start of the past flu pandemic, we added PCR tests to standard cultures in all patients with CAP; also, we compared the clinical and laboratory features of patients with bacterial (BP) vs. those with viral pneumonia (VP).

Methods: All adults with CAP admitted for at least 24h in a 500-bed acute care hospital from November 2009 to October 2010 were included. Demographics, Charlson score, pneumonia severity index (PSI), C reactive protein (CRP) and procalcitonin (PCT) values, clinical features and in-hospital mortality were recorded. Microbiological standard methods included blood and sputum cultures and antigen urinary detection. New methods included sputum analyzed by Multiplex PCR for *M. pneumoniae*, *L. pneumophila*, *C. pneumoniae* and *B. pertussis* and for 15 respiratory viruses. A specific PCR for H1N1 in nasopharyngeal samples was included during the pandemic.

Results: A total of 169 patients were given the diagnosis of CAP. Sputum was obtained in 131. Using only standard methods we identified an etiology in 51.1% (67/131) of cases. In this group, when PCR was included, a microbiological agent was identified in 69.4% (91/131) of cases; 44 had BP (33.5%), 24 had VP (18.3%) and 23 (17.5%) had co infection (BP and VP). *S. pneumoniae* [53/91 (58.2%)] was the most frequent pathogen followed by Rhinovirus 16.5% (15/91, we diagnosed Influenza A 8.8% (8/91). No differences were found in terms of age, gender, Charlson score, PSI, CRP levels, radiological involvement and mortality between VP and BP patients. PCT median values were 4 mg/L and 0.8 mg/L ($p = 0.01$) in BP and VP, respectively. Curb score [1.4 vs. 0.7 ($p = 0.01$)], shock [18.2% vs. 0% ($p = 0.04$)] and shaking chills [56.3% vs. 6% ($p = 0.002$)] were significantly more frequent in BP as compared to VP respectively.

Conclusions: *S. pneumoniae* is still the leading causative agent in CAP, however more than one third of patients presented a viral infection. Surprisingly, H1N1 was infrequently identified as a CAP causative or co infecting agent. Clinical presentation and PCT values could help to discriminate between VP and BP.

O237 Vaccination against pandemic influenza A/H1N1(2009) among healthcare workers: lessons for the next pandemic

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Objective: To investigate the factors associated with receiving or refusing pandemic influenza vaccines among physicians and nurses of a tertiary care university hospital.

Methods: The study was conducted at a 2000-bed tertiary-care hospital with a health care worker (HCW) population consisted of 1234 physicians, 1125 nurses, 385 nurse-asistants and 549 medical technicians. Only physicians and nurses were included in the study.

After the termination of vaccination campaign, a total of 472 HCWs (236 vaccinated, 236 unvaccinated) were randomly selected from the employee list of human resources and data about demographics, professional category and work year, history of seasonal influenza vaccine uptake in previous year, reasons for accepting or refusing the pandemic influenza A/H1N1 vaccine, the source of knowledge on pandemic vaccines of these HCWs were collected by a structured survey.

Categorical data were analyzed by chi-square test, whereas Student's t-test was performed for continuous variables. Bivariate analysis was done to evaluate the effect of each independent variable on pandemic influenza A/H1N1 vaccine uptake. A multivariate logistic regression analysis was performed to determine independent predictors of refusal of the pandemic influenza A/H1N1 vaccine. All statistical analysis was done by a software (STATA-9.0, Tx, USA) and a P-value of <0.05 was considered as statistically significant.

Results: A total of 472 HCWs (236 vaccinated and 236 unvaccinated) were included in the study. Of the 472 HCWs, 333 (70.5%) were female, 253 (53.6%) were physicians and 149 (32.6%) were working in the surgical wards. Characteristics of surveyed HCWs and vaccination rates for pandemic influenza A/H1N1 according to demographics and professional variables are summarized in Table 1.

By using a multivariate logistic regression modelling; being a nurse in a surgical department (OR:0.19; 95% CI:0.08–0.45; $P < 0.001$), receiving seasonal influenza vaccine in the previous year (OR:2.59; 95% CI:1.13–5.95; $P = 0.024$), using internet as the main source of information (OR:0.31; 95% CI:0.13–0.74; $P = 0.009$) and being informed

by attending the meetings held at the hospital (OR:4.54; 95% CI:1.14–17.9; P < 0.031)were found to be independently associated with pandemic vaccine uptake.
Conclusion: Interactive informational meetings at institutional level and high credibility of the policies of national health authorities are essential factors for promoting vaccination against influenza.

Variable	Total (n=472)	Unvaccinated	P-value
Gender N (%)			0.363
Male	139 (29.5)	65(46.8)	
Female	333 (70.5)	171(51.4)	
Mean Age years (min-max)	35.8 (20-70)	36.5 (21-66)	0.118
Mean working experience, years (range)	13.6 (1-42)	14.2(1-42)	0.161
Profession N(%)			0.782
Physician	253 (53.6)	128(50.6)	
Nurses	219 (46.4)	108(49.3)	
Ward N(%)			**0.002**
Surgical	149 (32.6)	90 (60.4)	
Medical	308 (67.4)	138 (44.8)	
Having child at home	260 (55.1)	120 (46.1)	0.064
Living with a CUD person	58 (13.5)	26 (44.8)	0.475
Receiving seasonal influenza vaccine	190 (40.4)	77 (40.5)	**0.001**
Source of knowledge on flu pandemic N(%)			
Scientists' briefings on TV	312 (73.4)	171 (54.8)	**0.006**
Politicians' talks on TV	120 (29.3)	86 (71.7)	**<0.001**
Internet	277 (66.7)	159 (57.4)	**<0.001**
Newspapers and/or magazines	244(59.8)	143 (58.6)	**<0.001**
Friends	289 (70.8)	159 (55)	**0.017**
Scientific articles and/or magazines	372 (87.3)	195 (52.4)	0.109
Informative meetings at the hospital	331 (77.4)	148 (44.7)	**<0.001**

O238 The impact of European vaccination policies on seasonal influenza vaccination coverage rates in the elderly

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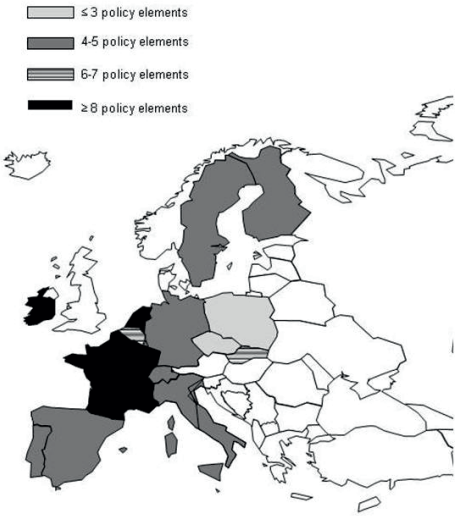
Background: Despite strong recommendations from the WHO and the European Union Council, seasonal influenza vaccination coverage rates (VCRs) remain insufficient in European countries, even in key at-risk groups such as the elderly. There is a need for understanding the impact of vaccination policies and to identify ways of improving seasonal influenza VCRs. The main aim of this study was to identify essential elements of vaccination policies and the influence of policy-related driving factors on vaccine uptake rates among the elderly.
Methods: European National Vaccine Industry Groups were included in a survey (n = 16) to make an inventory of vaccination policies implemented at a national level. The questionnaire was structured around four different topics: management of vaccination programmes; influence of health care workers (HCWs); role of information and communication campaigns; and access to vaccine. The information retrieved was put in relation to current national vaccination rates among the elderly ⩾65 years of age. Correlation coefficients between policy elements and VCRs were calculated.
Results: The number of implemented policy elements quoted in the survey varied considerably across countries assessed (Fig 1). Countries with good monitoring systems regarding VCRs (Spearman's ρ = 0.639, p = 0.010) or sending personal letters offering free vaccination (ρ = 0.728, p = 0.002) showed on average higher vaccine coverage rates among the elderly than countries with less developed vaccine management systems. The association with annual VCRs was stronger in presence of additional policy items such as defined national VCR objectives, incentives to HCWs, vaccination reimbursement systems and awareness campaigns in the mass media. A combination of three elements, namely flyers in medical waiting rooms, personal invitation letters for free flu vaccine, and reimbursement of vaccination, showed one of the strongest associations with VCR among the elderly seen in this study (ρ = 0.820, p < 0.05).
Conclusion: There are several ways to increase influenza vaccination uptake in elderly, but only few countries take advantage of all possibilities. This study indicates that key elements of vaccination policies at national level may include broad information and reminding systems, strong official recommendations as well as free and easy access to flu vaccination. The implementation of these policy elements is expected to help achieving improved influenza VCRs among the elderly.

Figure 1. Implementation of policy elements covered by the survey questionnaire



O239 Influenza vaccination of healthcare workers: a case-control study of its effect on laboratory-confirmed, hospital-acquired influenza among patients

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Objectives: No evidence of a protective effect of healthcare workers vaccination on proven hospital-acquired influenza in patients has been demonstrated. The objective of our investigation was to ascertain the effectiveness of influenza vaccination of HCW on laboratory-confirmed, hospital-acquired influenza among patients.
Methods: A prospective surveillance study enrolled all ILI patients hospitalized in 36 units of Edouard Herriot Hospital (Lyon, France),a tertiary care university hospital, between October 15 and April 15 in the years 2004–05, 2005–06 and 2006–07. A matched case-control study based on this surveillance compared 11 patients with laboratory-confirmed, hospital-acquired influenza to 44 randomly selected controls with influenza-like illness but not related to influenza, matched per influenza season (2004–05, 2005–06 and 2006–07). ILI was defined as rectal or axillary temperature ⩾37.8ºC, in the absence of antipyretics, with cough or sore throat. Cases were patients with virologically-confirmed influenza occurring ⩾72 hours after admission in the unit, controls were patients with ILI during hospitalization with negative results for influenza after nasal swab testing. All patients underwent nasal swabbing. A conditionnal regression analysis was done to assess factors associated with confirmed hospital-acquired influenza among patients.
Results: Overall, 55 patients were analyzed (Table 1). Median age was 77.3 years, and the male/female gender ratio was 0.34. The median proportion of vaccinated healthcare workers in the units was 36% (0% to 67%) with a mean of 34%. A proportion of vaccinated healthcare workers of ⩾35% in the unit protected against hospital-acquired influenza among patients (odds ratio=0.046; 95% confidence interval 0.004–0.58, P = 0.017), independently of patient age and potential patient or healthcare worker influenza source in the unit.
Conclusion: Our study indicates a protective effect of a high proportion of vaccinated HCW on hospital-acquired influenza in patients. Other studies based on experimental design are needed to demonstrate the effectiveness of HCW vaccination in the control of influenza in healthcare settings.

Table 1. Characteristics of the controls and patients with laboratory-confirmed, hospital-acquired influenza and of the factors associated with hospital-acquired influenza in Edouard Herriot Hospital, Lyon (France), in influenza seasons 2004-05, 2005-06 and 2006-07

Characteristics	Patients with laboratory-confirmed, hospital-acquired influenza (N=11)	Controls (N=44)	*P*	Crude OR[a] (95% CI)	*P*	Adjusted OR[a] (95% CI)	*P*
Gender			1				
Male	3 (27)	11 (25)		1.0		-	
Female	8 (73)	33 (75)		0.89 (0.22-3.72)	0.88		
Age			1				
<65 years	5 (45)	19 (43)		1.0		1.0	
≥65 years	6 (55)	25 (57)		0.91 (0.23-3.59)	0.89	10.56 (1.01-109.88)	0.049
Potential source of influenza[b]			0.042				
No	4 (36)	31 (70)		1.0		1.0	
Yes	7 (64)	13 (30)		4.06 (1.08-15.33)	0.039	7.03 (1.27-38.76)	0.025
Individual influenza vaccination[b,c]			0.11				
No	8 (80)	23 (52)		1.0		-	
Yes	2 (20)	21 (48)		0.34 (0.069-1.70)	0.19		
Proportion of vaccinated HCW in the unit			0.078				
<35%	8 (73)	19 (43)		1.0		1.0	
≥35%	3 (27)	25 (57)		0.16 (0.018-1.32)	0.088	0.046 (0.004-0.58)	0.017

NOTE. Data are reported as numbers (%) under descriptive analysis and as OR (95% CI) under logistic regression analysis. Controls were hospitalized patients with ILI and confirmed exclusion of influenza diagnosis after nasal swabbing; they were matched by influenza season (2004-05, 2005-06 and 2006-07). OR, odds ratio; CI, confidence interval; HCW, healthcare workers.
[a] Potential sources of exposure to influenza (other patients or HCW) for 5 days before ILI or influenza occurrence in the same ward
[b] For the current influenza season
[c] Individual influenza vaccination was not given in 1 patient
[d] After conditional logistical regression

O240 Assessment of the frequency of emergence of oseltamivir resistance in influenza A during therapy

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Objective: The H275Y mutation was reported to have emerged in a patient infected with pH1N1 within 48 hours of exposure to oseltamivir (1). As most of the literature on H275Y is from pre-treatment samples, this work was carried out to assess how frequently resistance, as defined by the presence of the H275Y mutation, emerges during therapy with oseltamivir.
Method: Patients found by RT-PCR to be infected with Influenza A pH1N1 between May and June 2009 were offered oseltamivir 75mg bd and isolated until pH1N1 was no longer detectable by RT-PCR. Samples, comprised of combined nose/throat swabs, were collected daily. RNA extracted from these samples was stored at −80ºC. With IRB approval we compiled a list of patients' samples from the laboratory computer system and subjected the last available 'pH1N1 positive' sample collected from each subject to pyrosequencing to detect the H275Y mutation as previously described (1).
Results: Stored RNA was recovered from 93 patients. The number of subjects tested after variable periods of exposure (days) were 3 subjects, 1 day; 25, 2: 19, 3; 18, 4; 11, 5; 9, 6; 1, 7; 4, 8; 2, 9; 0, 10 and 1, 11. H275Y was not detected in 91 samples. A weak signal, 6%, was initially detected in two samples but in both cases it became zero on repeat testing so all 93 were considered 'H275Y not detected'.
Conclusion: The rate of emergence of H275Y was uncommon, about 1% if we include the original case. Minor resistant populations may have been missed either due to RNA degradation or as Pyrosequencing cannot reliably detect sequences that comprise <10% of the population. The exposure to oseltamivir before sample collection appears short, with 50% of samples being collected after fewer than four days of oseltamivir exposure, but this reflects the disappearance of the virus, not a failure to collect samples. The subsequent sample from each case did not have any detectable pH1N1 by RT-PCR so further consumption of oseltamivir in these cases may not equate with further exposure of virus to selective pressure. Extended treatment of patients with prolonged shedding, such as in immunocompromised patients, is likely to yield a higher rate of emergence of H275Y than that reported here.

Reference(s)

[1] Emergence of Oseltamivir-Resistant Pandemic (H1N1) 2009 Virus within 48 Hours of Treatment. Inoue M, Barkham T, Leo Y-S, Chan K-P, Chow A, Wong CW, et al. Emerg Infect Dis 2010; Vol. 16, No. 10; 1633–1636 DOI: 10.3201/eid1610.100688

O241 Hospitalised patients with pandemic influenza A/H1N1 virus infection had similar outcomes independently of hospital characteristics

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Background: The outcome of patients with influenza A (H1N1) virus infection taking into account hospital type has not been elucidated.
Objective: To compare risk factors, clinical features, and outcome of patients admitted to 3 public hospitals with different levels of health care in Cantabria (Spain).
Methods: Prospective cohort study of all non-pregnant adult patients admitted to 3 public hospitals in Cantabria (Spain), with confirmed influenza A (H1N1) virus infection, from June 1 to December 30, 2009.
Results: 111 patients with a mean age of 49 years (15–89) were recruited: 52 in hospital 1 (900-beds tertiary teaching hospital), 33 in hospital 2 (315-beds secondary hospital) and 26 in hospital 3 (150-beds primary care hospital). Patients in hospital 1 and 2 were younger than in hospital 3 (P = 0.02). Overall 80% of patients had at least 1 comorbid condition with no main differences between hospitals. Symptoms or signs on admission were similar except for cough (P = 0.01) present more frequently in patients in hospital 1; and dyspnea (P = 0.05), myalgia, arthralgia (P = 0.04) and hypoxemia (P = 0.009) in patients in hospital 2. More than half of patients in hospital 2 had pneumonia (P = 0.04). In-hospital mortality rates were lower in hospital 2 (6.1%) and 3 (3.8%) than in hospital 1 (11.5%). Level of hospital was not a risk factor for mortality nor to a crude level (hospital 2 vs. hospital 1: RR 0.52, 95% CI 0.11–2.44; P = 0.40); (hospital 3 vs hospital 1: RR 0.33, 95% CI 0.04–2.62; P = 0.26), or in the adjusted analysis for comorbidities. Moreover, in the stepwise analysis, independent predictors of mortality were pneumonia on admission and cardiac complications during hospitalization.
Conclusions: The outcome of patients with Pandemic Influenza A(H1N1) virus infection was independent of hospital-level characteristics. Mortality was influenced for clinical conditions on admission and complications during hospitalization.

O242 How many lives were saved during the A/H1N1 pandemic in 2010 in Europe?

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In countries with temperate climate, mortality increases during the cold season, part of which is attributed to contagious respiratory infections, including influenza. Elderly are the most affected by seasonal over-mortality. In the UK and in France, and more widely in the northern hemisphere, the A/H1N1 pandemic stopped at the end of the year 2009. Interestingly it was not accompanied or followed by the common seasonal fly peak. This may be related to high prevalence of asymptomatic infections and/or to a significant proportion of elderly possessing protective antibodies due to ancient contact with an antigenically-related viral strain. We have examined 2 databases on global mortality in the UK (number of deaths per 1,000 population) and in France (number of deaths) to evaluate whether the absence of seasonal influenza epidemic had consequences on mortality. We compared the cold season over-mortality in the whole population and in people aged 75–84 in England and Wales during the first quarter of 2010 with the death rate observed during the preceding years. In the UK, in the whole population during the cold months (January to march), a reduction of 3% in the death rate was observed in males and 1.9% in females when the death rate of 2010 was compared to the mean rate of 2007, 2008, and 2009. the reduction in the death rate was much higher when the analysis was restricted to the 75–84 age group with a reduction of 8% in males and 7.7% in females of cold-seasons over-mortality.
France, we compared the mean number of deaths for the month of January for the years 2007 to 2009 to the number of deaths in January 2010. A mean number of 53,342 deaths were observed for the year

2007 to 2009 whereas 51,300 deaths were recorded in January 2010, representing a 3.8% decrease in mortality. The overall mortality rate decreased from 0.83 per 1000 population for the 2007–2009 period to 0.80 per 1000 population for January 2010 (p = 0.002). Therefore we suspect that there is a relation of cause and effect between A/HN pandemic, the lack of seasonal epidemic, and a reduction of fatality rate in 75–84 year-old people in 2010 in the UK and in the overall population in France. Our conclusion is that A/H1N1 pandemic paradoxically and unexpectedly resulted in a reduction of mortality in elderly, usually the most affected group of people. This underlines our inability to predict the consequences of epidemic phenomenon in one direction or the other.

O243 Pandemic influenza A/H1N1 vaccine in solid organ transplant recipients: immunogenicity outcomes after whole virus vaccination

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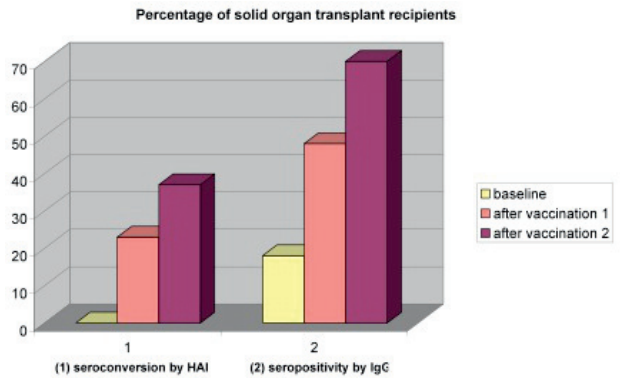
Objective: During the 2009–10 pandemic the vaccination against H1N1 influenza was recommended to immunocompromised patients as the key preventive measure by The American Society of Transplantation and other health organisations. However, no data regarding the efficacy of any of the recommended vaccinations were available at that time, neither in healthy, nor in immunocompromised subjects. The objective of this study was to evaluate the immunogenicity of the pandemic influenza vaccine A/California/07/2009 (H1N1)v in solid organ transplant recipients.
Methods: Patients were recruited from the Medical University Hospital of Vienna, Austria. The vaccination schedule consisted of 2 doses of the vaccine Celvapan (whole virion, Vero cell derived, inactivated, A/California/07/2009 (H1N1)v) given with a 3 weeks interval. Serological analysis was performed by hemagglutination inhibition assay (HAI) on blood samples obtained before the inclusion and after each vaccination (bonostix GmbH & Co. KG, Kornwestheim, Germany). The primary immunological end point was the seroconversion rate, which was defined as the proportion of subjects with an individual 4-fold increase in hemagglutination inhibition titer and a postvaccination hemagglutination inhibition titer of more than 1: 40. H1N1-Influenza specific immunoglobulin was determined by ELISA (Pandemic New Influenza A ELISA IgG/IgA Testkit, Genzyme Virotech GmbH, Rüsselsheim, Germany). Seropositivity was postulated when the measured immunoglobulin levels were above 11 Virotech Units (VE).
Results: Twenty-five organ transplant patients (16 males, 9 females), aged 25 to 79 years were vaccinated and had samples taken for serological analysis. Time since tranplantation was 10 months to 25 years (mean: 9 years; 95% CI: 6–13 years). After two vaccinations 37% demonstrated seroconversion in the HAI as defined above and 70% of the patients had an IgG titer above 11 VE. Among the HAI vaccine responders were 6 of 14 heart transplant recipients and 1 of 4 liver transplant recipients. Number or kind of immunsuppressive agents did not significantly differ in their effect on immune response.



Conclusion: Our results show that the pandemic A/California/07/2009 (H1N1)v vaccine induced limited but useful protective immune responses in adult organ transplantat recipients.

O244 Immunogenicity, efficacy, and safety of the pandemic influenza A/H1N1 vaccine in solid organ transplant recipients

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Objectives: To asses the immunogenecity, efficacy, and safety of the pandemic H1N1–2009 vaccine in solid organ transplant recipients (SOTR).
Methods: We performed a multicenter prospective study in SOTR receiving one-dose of the pandemic vaccine. Immunological response was determined in serum samples by microneutralization assays, and immunoglobulin levels by ELISA. All patients were followed up 90 days after vaccination, or ten months if adverse effects were detected.
Results: Three-hundred and forty-six SOTR (29.2% kidney, 28.9% liver, 24% heart, 16.5% lung, and 1.4% combined transplantation) were included. Median time after transplantation in patients receiving the pandemic vaccine was 4.7 years (IQR: 1.7–8.9) and 24 patients (6.9%) received the pandemic vaccine between two, and six months after transplantation. Pre-existing seroprotection was detected in 13.6% of cases. Overall rates of seroconversion, and seroprotecion after vaccination were 73.1%, and 82.9%, respectively. Multivariate analysis demonstrated that age (OR 1.03, 1.00–1.05, P = 0.01), liver disease (OR 0.27, 0.10–0.70, P = 0.007) and m-TOR inhibitor therapy (OR 0.42, 0.18–1.00, P = 0.048) were independent factors associated with seroprotection. Patients with chronic liver disease seroconverted less frequently (P = 0.02). Factors associated with GMT-postvaccination correlated with factors related to seroconversion and seroprotection. Lower antibody titers were found in younger patients, and in those with liver disease or receiving m-TOR inhibitors. Patients with antibody titers at baseline (GMT 8.1) reached higher antibody levels (GMT 339.4 vs. 121.4, P < 0.001) and a higher rate of seroprotection after vaccination than those without previous antibody titers (80.3% vs. 100%, RR1.3 CI95% 1.2–1.3, P < 0.001). Four of the patients vaccinated (1.1%) were diagnosed with pandemic influenza A(H1N1)-2009 infection in the follow up period. There were no vaccination major adverse effects, or rejection episodes.
Conclusions: Pandemic vaccine is safe in SOTR, and elicits an adequate response although lower than in healthy individuals, especially in young patients, with liver disease, and m-TOR inhibitor therapy.

O245 Immunogenicity and evaluation of antibodies persistence of a single-shot of 2009 pandemic influenza vaccine in HIV-infected and HIV-uninfected subjects living in a residential drug-rehabilitation community

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Objective: To evaluate the immunogenicity and the antibodies persistence of a single-shot of 2009 pandemic influenza vaccine in HIV-infected and HIV-uninfected subjects living in a residential drug-rehabilitation community.
Methods: 109 (49 HIV-infected and 60 HIV-uninfected) subjects living in a residential drug-rehabilitation community (San Patrignano, Rimini, Italy) received intramuscularly a single-shot of a MF59-adjuvanted 2009 pandemic influenza vaccine, containing 7.5ug hemagglutinin of A/California/7/2009(H1N1v).
Subjects (26 women and 83 men) had a mean age of 39.2±11.5 years. HIV-infected patients were mainly (98%) on highly active antiretroviral therapy (HAART) regimens. They had a mean CD4+ cell count of 386.9±225.6 cells/ul and a suppressed viremia in 91.8% of the cases.

Blood samples were taken at the time of vaccination, and 1 month and 6 months post-vaccination and were evaluated for anti-A/H1N1v antibody titers by hemagglutination-inhibition (HI) test.

Results: On baseline evaluation, 4.1–5% had HI-titers to A/H1N1v of at least 1:40 in both groups. Seroconversion, defined as at least a 4-fold increase in antibody titers from baseline, was achieved in 49/49 (100%) HIV-infected subjects and in 59/60 (98.3%) HIV-uninfected ones, with the overall proportion of patients with protective titers ($\geq$1:40) being 93.9% (46/49) and 100% (60/60), respectively. The geometric mean titers (GMTs) significantly ($p < 0.001$) increased in both HIV-infected and HIV-uninfected subjects.

Six months after vaccination the percentage of HIV-infected subjects with protective titers was lower ($p < 0.05$) than that found in HIV-uninfected subjects. Similarly, 6 months post-vaccination GMTs significantly ($p < 0.01$) decreased in both groups, but were significantly ($p < 0.05$) lower in HIV-infected subjects than in HIV-uninfected ones (Table).

For HIV-1-infected individuals, no significant changes either in the CD4+ cell count or in viremia were observed at any time point.

Conclusions: A single-shot of influenza A/H1N1v 2009 MF59-adjuvanted vaccine generated antibody responses possibly associated with protection one month after immunization in successfully HAART-treated HIV-infected adults, with responses comparable to those achieved in healthy adults. This 6-month follow-up suggests that protective antibody titers can be detected after a single vaccine injection in both HIV-infected and HIV-uninfected patients but with a more appreciable decrease of antibodies titers in HIV-infected ones.

Table. Immune responses (seroconversion rates, seroprotection rates and GMTs) before and after the administration of one dose of MF59-adjuvanted 2009 pandemic A/H1N1v influenza vaccine in HIV-infected and HIV-uninfected subjects.

	HIV-infected (N=49)	HIV-uninfected (N=60)	P-value [a]
Subjects with seroconversion [b] % (95% CI)			
1 month	100.0	98.3	>0.05
Subjects with protective HI titer (≥1:40) [d] % (95% CI)			
Baseline	4.1	5.0	>0.05
1 month	93.9	100.0	>0.05
6 months	52.9	78.0	**0.0191**
Geometric mean titer value (95% CI)			
Baseline	6.36	6.16	0.4416
1 month	153.35	316.32	0.1293
6 months	33.29	75.68	**0.0156**

[a] Comparison between the two groups: Chi square test based on binomial distribution (seroconversion and seroprotection rates) and the paired t test (GMTs). A p-value <0.05 was considered significant (2-tailed test).
[b] N.A.: not applicable.
[c] Proportions of subjects who had a seroconversion (a pre-vaccination HI antibody titer ≤1:10 and a post-vaccination titer ≥1:40 or a pre-vaccination titer ≥1:10 and an increase in the titer by a factor of four or more).
[d] Proportions of subjects who had a HI antibody titer of 1:40 or more.

Mycobacterial disease to Epi and beyond

O246 Extensively drug-resistant tuberculosis at a tuberculosis specialised hospital in Shanghai, China: clinical characteristics and treatment outcomes

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Objectives: Extensively drug-resistant tuberculosis (XDR-TB) has recently emerged as a global public health problem. To investigate the clinical characteristics, management, and outcomes of patients with XDR-TB and HIV-negative at a Tuberculosis Specialized Hospital in Shanghai, China.

Methods: XDR-TB was defined as TB with resistance to at least isoniazid, rifampin, a fluoroquinolone, and 1 of 3 injectable second-line drugs (amikacin, kanamycin, or capreomycin). We analyzed TB Patients with culture-proven MDR-TB from January 2007 to June 2009 at a Tuberculosis Specialized Hospital in Shanghai.

Results: Among 1156 TB cases with culture-positive for *M. tuberculosis* complex, 785 cases (67.9%) were drug resistant (DR), 494 cases (42.7%) were classified as having MDR-TB, accounting for 62.9% of DR-TB. 126 cases (10.9%) were XDR-TB, accounting for 16.1% of DR-TB, 25.5% of MDR-TB. The percentage of female from XDR group was 42.1%, significantly higher in comparison with that from other MDR group ($\chi^2 = 7.560$, $P = 0.006$). Median ages in XDR and other MDR groups were 46 (33.8, 58.0) and 41 (31.3, 51.0) years, respectively. Significant difference in the age could be found between the two groups ($Z = 3.184$, $P = 0.001$). 22.2% of drug resistance belonged to newly diagnosed patients. At least three lung fields were involved in 90.5% of XDR patients and in 80.7% of other MDR patients ($P = 0.008$). 40.5% of XDR patients were complicated by diabetes, lung disease, liver disease, which was significantly higher as compared with that of other MDR patients ($\chi^2 = 9.298$, $P = 0.002$). The resistance rates of all drugs except isoniazid and rifampicin were significantly higher in patients with XDR-TB than in other MDR patients ($P < 0.001$). Treatment failure was more common in patients with XDR-TB than in those with other MDR-TB (72.3% vs. 36.0%, $p < 0.001$). Cure or treatment completion was more common in other patients with MDR-TB than in patients with XDR-TB (53.1% vs. 14.9%; $p < 0.001$), whereas the mortality and default rates did not differ significantly.

Conclusion: The prevalence of XDR-TB is serious in some areas in China. Because of its seriousness and shortage of our options, patients with XDR-TB are more likely to show no response to therapy and their clinical treatment outcome is usually very poor.

O247 Pulmonary and extrapulmonary infections caused by non-tuberculous mycobacteria: report of 31 years in a tertiary hospital, Madrid, Spain

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Objective: To describe the epidemiology of non-tuberculous-mycobacteria (NTM) causing active infection in our Hospital during the last 31 years.

Patients and Methods: All patients attended in our Hospital between 1978 and 2009 with disease caused by NTM were included. For pulmonary disease, only those patients with ATS criteria were included. Only the first episode of disease was considered in each patient (first isolate). Samples were processed and seeded on appropriate media [Löwenstein-Jensen, Coletsos, ESP II system (BIOMEDICS, Francisco Soria-Melguizo, Madrid Spain), and Myco F-Lytic medium (BD, Madrid, Spain). Cultures were maintained at 37ºC for at least eight weeks, except skin samples (30ºC) and those samples suspecting of *Mycobacterium xenopi* (42–45ºC). Identification was performed by classical phenotypic methods and also with a specific gene probe for *Mycobacterium avium* complex (MAC) (Accuprobe, GenProbe Inc., San Diego, USA), and in the last 6 years PCR amplification followed of nucleotidic sequentiation was also applied.

Results: In the last 31 years we diagnosed 3,623 patients, 3,372 of them with *Mycobacterium tuberculosis* and 251 with a NMT (7%) with a decrease during the last years. From this 251 patients, 146 (58%) were HIV+ and 167 (66.5%) had an extrapulmonary infection. Considering only those from pulmonary origin (33.5%), 55% of them positive for MAC followed by *Mycobacterium kansasii* (32%). Extrapulmonary patients were two thirds of the total, corresponding more than 62% to HIV+ patients. A high proportion were associated with a disseminated disease ($n = 97$, 58%) and in 14.3%. were recovered from lymphoadenopathy ($n = 24$). MAC was isolated in 70% of extrapulmonary patients, followed by *Mycobacterium chelonae* (11.3%) and *Mycobacterium scrofulaceum* (4.9%). From 105 HIV– patients, 103 were positive for MAC, whereas in the HIV– group, the most prevalent specie was *Mycobacterium chelonae* (31%) followed by *Mycobacterium fortuitum* (22.6%) and in third place MAC (21%).

Conclusion: NMT prevalence decreased in the last decade, especially among the HIV patients. In the respiratory infection, MAC was the most prevalent specie followed by *Mycobacterium kansasii*. In the extrapulmonary infections, prevalence of *Mycobacterium fortuitum*/chelonae group increased in the last years, whereas MAC decline, mostly due to better management of HIV patients.

O248 Novel small RNAs in *Mycobacterium tuberculosis*: experimental genome-wide discovery and computational target prediction

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Objectives: This study aims to identify novel smallRNAs (sRNAs) in *Mycobacterium tuberculosis* (MTB) and to computationally predict their targets in post-transcriptional regulatory networks.
Methods: Genome-wide identification of sRNAs was performed by Illumina sequencing on sRNA-enriched fraction (RNAs up to 300 nt in length) from MTB H37Rv. A custom bioinformatic analysis pipeline of reads was developed to extract putative sRNAs considering expression levels, sequence conservation, length, and secondary structure stability. Candidates were then validated using CombiMatrix microarray technology. A p-value based on a t-type statistic and computed by mean of permutations was calculated for each candidate using microarray results. Candidates with a p-value $\leqslant 0.05$ were further analyzed for target prediction.
Target prediction of candidates was carried out in three main steps:

i. locating sRNA binding sites in the transcriptome using hybridization tools that can predict accessible sites in interacting RNA molecules based on their secondary structures;
ii. ranking sRNA:mRNA interactions using a "binding score" that considers both an energy term and a location term;
iii. selecting the most promising interactions using p-values and corresponding false discovery rates (FDR) for each sRNA:mRNA hybridization.

Results: A total of 9′711′909 reads were obtained by Illumina sequencing. For the selection of sRNA candidates filtering parameters and FDRs were applied to intergenic regions that uniquely matched reads, resulting in 1925 candidates.
We confirmed 331 candidates by microarray analysis. Among these, 122 candidates with a p-value $\leqslant 0.05$ were selected for further characterization by in silico target prediction.
sRNA:mRNA interactions showing a FDR < 0.1 were used to construct a post-transcriptional regulatory network. Computational results showed that sRNAs may target from 1 gene up to 99 genes. The putative function of the genes included in the network was evaluated for functional enrichment based on the Database for Annotation, Visualization and Integrated Discovery (DAVID).
Conclusions: We identified novel sRNA candidates in MTB using two independent approaches. Moreover, computational sRNA target prediction and post-transcriptional regulatory network assembly allowed us to identify functional pathways regulated by 19 sRNAs in MTB.

O249 Clinical characteristics and treatment outcomes of tuberculosis in hospitals of two large cities in Thailand

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Background: Tuberculosis (TB) is a major public health threat of large cities in many countries. The current clinical situation of TB in large cities of Thailand has not been well established.
Methods: A retrospective cohort study was conducted among patients diagnosed active TB in 6 hospitals in Bangkok and Nonthaburi between January 2008 and December 2008. Patients were classified into HIV sero-positive, sero-negative, and undetermined sero-status; and were followed until December 2009.
Results: A total of 813 patients were identified. Mean±SD age was 41±14 years, 62% were male, and mean±SD body weight was 53±11 kilograms. The first three co-morbidities included 38% HIV infection, 6% diabetes, and 2% chronic liver disease. Of all, 66% were diagnosed isolated pulmonary TB. Median (IQR) duration from first presentation to TB diagnosis was 3 (0–10) days. Of all, 43% of patients performed TB culture and 16% did anti-TB drug susceptibility test. Isoniazid, rifampicin, and multi-drug resistance were found in 11%, 6% and 5%, respectively. For 1-year TB treatment outcomes, 52.1%, 19.4%, 11.9%, 8.7%, 6.8%, 1.0% were cured/completed treated, transferred out, defaulted, on-going treated, dead and failed, respectively. Survival rates at 2, 6, 12 and 24 months were 93%, 85%, 81%, and 81% in HIV sero-positve group; 96%, 94%, 92%, and 92% in HIV sero-negative group; 98%, 97%, 97%, and 97% in undetermined sero-status (log rank test, $P < 0.001$). Cox's proportional hazard model showed that death was associated with TB/HIV co-infection ($P \leqslant 0.001$, HR = 2.801), low body weight ($P = 0.005$, HR = 1.637), old age ($P = 0.005$, HR = 1.439), and extrapulmonary/disseminated TB ($P = 0.021$, HR = 2.184).
Conclusions: HIV infection and diabetes are the common co-morbidities in TB patients. Unfavorable outcome of TB treatment is relatively high, particularly in patients with HIV. Thus, further strategic management and collaborative treatment is needed to improve TB services.

O250 Optimisation of follow-up samples for patients with pulmonary tuberculosis

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Objectives: Patients with AFB smear positive respiratory samples who are diagnosed with *Mycobacterium tuberculosis* require three consecutive AFB smear negative follow-up samples before they can be removed from airborne precautions. This study was to review the current follow-up respiratory samples to assess compliance with the protocol and to determine if a more streamlined algorithm could be developed as currently AFB microscopy and culture are performed on all samples submitted.
Methods: The initial diagnostic and follow-up cultures from all patients in the province of Manitoba, Canada who were diagnosed with pulmonary tuberculosis in 2010 were reviewed. The AFB smear and culture results were assessed to determine how long it took for patients to become AFB smear and culture negative.
Results: There were 53 patients diagnosed with pulmonary TB in 2010. There were 27/53 (51%) who were AFB smear positive on at least one of their original three diagnostic respiratory specimens. Of these 22/27 had follow-up samples submitted. Of the 22 there were 17 who did become AFB smear negative, 3 with no follow-up and 2 whose follow-up is ongoing. The median time to smear negativity for the 17 who become smear negative was 22 days (range 6 to 78 days). An algorithm was developed that provides expedited detection of rapid converters by initiating the follow-up specimens on day 12 of therapy. Only 2/17 (11.8%) patients who were originally AFB smear positive were AFB smear negative (3 samples) by day 12. Even by day 21 of therapy only 41.2% were AFB smear negative. Despite the follow-up specimens being AFB smear negative, 100% of the samples (17/17) were still culture positive. Our data indicate that although AFB microscopy is needed that culture is not needed on all three follow-up specimens.
Conclusions: The continued culture positivity of 17/17 patients despite being AFB smear negative on three consecutive follow-up samples raises questions regarding potential infectivity of these patients. We recommend a follow-up algorithm where AFB smear is prepared on all specimens submitted but culture is only performed on the last of the three samples submitted.

O251 Population differences in immune responses following BCG vaccination in different African settings

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Background: Live-attenuated *Mycobacterium bovis* Bacille Calmette-Guérin (BCG) is currently the only vaccine available to protect against tuberculosis (TB). Administration of the vaccine in early life induces a TH1-type immune response and secretion of the cytokine IFN-γ. BCG protects against severe childhood cases of tuberculous meningitis and miliary TB but confers variable protection against pulmonary TB later in life, particularly in TB endemic areas. Earlier studies attempting to study mechanisms behind this discrepancy have shown that *Mycobacterium tuberculosis* (M.tb) PPD-stimulated IFN-γ concentrations in 6 day diluted whole blood cultures were much higher in UK BCG vaccinated infants compared to age-matched Malawian infants given the BCG vaccine. We have now tested another group of infants and their mothers using identical laboratory protocols in another TB endemic country, The Gambia.
Methods: Three-month-old infants vaccinated with BCG in the first week of life and their mothers were recruited into the study. Venous blood samples were taken from infant-mother pairs for the detection of IFN-γ production by enzyme-linked immunosorbent assay (ELISA) following diluted whole blood assay stimulation with an array of tuberculous antigens over 6 days.
Results: 93% of Gambian infants (28 of 30) made a positive IFN-γ response (>62pg/ml) to M.tb PPD stimulation, comparable to previously studied UK infants (93% vs. 100%), and higher than the proportion of responders previously detected in Malawi (93% vs. 53%; Lalor MK et al. JID 2009; 199:795–800). However, the median IFN-γ response from Gambian infants was lower than median IFN-γ responses from UK infants (310pg/ml vs. 1,779pg/ml; $p \leqslant 0.0001$). The median IFN-γ responses in Gambian and Malawian infants were comparable (310pg/ml vs. 289pg/ml; $p = 0.7036$). The response measured in the Gambian infants was not associated with the response measured in their mothers.
Conclusions: The study findings indicate quantitative differences in cytokine secretion in different African settings in response to M.tb PPD antigen stimulation. The data are consistent with published evidence demonstrating lower infant immune responses post-BCG vaccination by latitude, and the underlying mechanisms influencing cytokine secretion post-vaccination warrant further investigation.

O252 Paradoxical worsening in the treatment of tuberculosis after discontinuation of anti-TNF in patients with inflammatory diseases

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Introduction: Paradoxical response in the treatment of tuberculosis is well-known since the use of active antituberculous antibiotics. This phenomena is now well described under highly active antiretroviral therapy in HIV-patients and known as the Immune Reconstitution Syndrome (IRS). Since the use of anti-TNFα, some cases of paradoxical worsening of tuberculosis have been reported in these patients.
Methods: We retrospectively report cases of paradoxical aggravation of tuberculosis in patients treated with anti TNFα from 2 different sources: a French registry called RATIO collecting all cases of tuberculosis in France from 2004 to 2006, and a call for cases on internet.
Results: Six cases have been collected: five women and one man. The median age at diagnosis of tuberculosis was 64 years old (47–87). They suffer from various inflammatory diseases: rheumatoid arthritis for 2, psoriasis for 2, ankylosing spondylarthritis for 1 and horton's disease for 1, and they received different anti-TNFα: adalimumab (3), infliximab (2) and certolizumab (1). Mean duration of anti-TNF treatment was 5 months (7 weeks-36 months). Three patients receive also steroids and three receive méthotrexate.
At diagnosis, there were 4 disseminated, one extrapulmonary and one pulmonary tuberculosis. Five diagnosis were proven on bacteriological exams, one on histology. All patients discontinued anti TNF treatment. They all experienced improvement of their tuberculosis under antibiotics. But in a mean delay of 17 weeks (6 weeks-39 weeks), they presented fever, asthenia and inflammatory syndrome. For 3 of them, lymph nodes increased and fistulas appeared. We noticed also two cavitations in lungs, one cold abscess, and one pleurisy. Four of them suffered from reactivation of their inflammatory diseases.
Treatments were: steroids for three, and surgery for three: bone resection, lymph node fistulas excision. The mean duration of antibiotics has been 17 months (8–23). All patients have been cured.
Conclusion: Paradoxical response in patients treated with anti TNF who develop a tuberculosis is a new and real phenomena (11 published cases). This is a complication in the course of the disease causing morbidities. Diagnosis is difficult without any criteria or specific sign. Incidence, risk factor, treatments of such complications needed to be studied.

O253 IGRAs on paper – specific diagnosis of latent TB sent via mail

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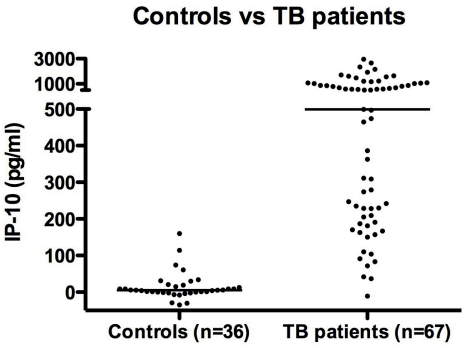
Objectives: IFN-γ release assays (IGRAs) such as the Quantiferon test (QFT) are currently the most accurate diagnostic tests for latent tuberculosis (LTBI). The analysis of IFN-γ depends on a very sensitive ELISA and a laboratory close to the patient, or cooling/freezing capacity during transportation of the samples. Means to simplify these assays and moving the diagnosis of tuberculosis closer to the patient is one of the main targets of the WHO.
IP-10 is a chemokine expressed in concert with IFN-γ and we have demonstrated that the diagnostic accuracy of IP-10 is at par with IFN-γ. IP-10 is released in >100 fold higher levels compared to IFN-γ, which may allow us to use alternative methods of storing and transport of samples.
The aim of this study was to investigate if filter paper could be used as an alternative means of storing stimulated whole blood samples and if IP-10 in plasma from *M. tuberculosis* (MTB) antigen stimulated whole blood could be used to diagnose infection with MTB.
Methods: Whole blood from 67 patients with confirmed TB and 36 healthy unexposed controls was stimulated in the QFT in tube test. Following incubation the plasma was dried on Whatman903 filter paper and stored in sealed gas-impermeable bags with desiccant for at 6 weeks at room temperature. 2 discs were cut from the filter paper using a normal stationary 5.5mm hole punch. IP-10 was measured using an in-house ELISA by direct elution of the plasma from the discs in the ELISA plate. Stability of the method was tested on mitogen-stimulated whole blood from healthy donors.



Results: Median IP-10 responses in TB patients and controls were 474pg/ml (IQR 176–1022pg/ml) and 5pg/ml (IQR 0–18pg/ml) respectively, ($p < 0.0001$) (figure 1). The Area Under the ROC curve was 0.96 The IP-10 filter paper results were relative to IP-10 and IFN-γ measured in plasma ($r2 > 0.75$). Stability testing showed that the recovery

of IP-10 dried on filter paper was >90% for >9 weeks storage at room temperature, and up to 4 weeks at >37ºC.
Conclusion: Plasma from in vitro stimulated whole blood can be dried on filter paper, stored at room temperature for a prolonged period of time and analyzed directly using ELISA.
These results move us closer towards a more simplified test for the diagnosis of MTB infection.
*for the conference we will present data from a cohort of 100 TB patients and 100 controls and data on IFN-γ dried on filter paper (in preparation).

O254 Rapid detection and susceptibility testing of *Mycobacterium abscessus* by microcalorimetry

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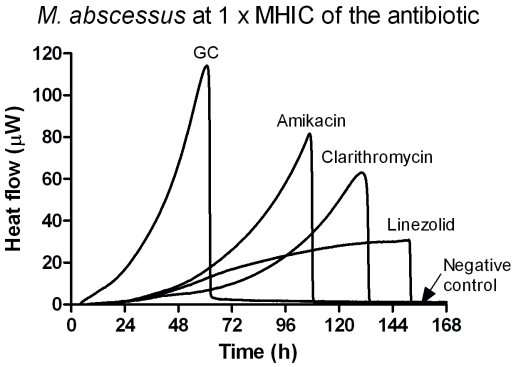
Objectives: Susceptibility testing of mycobacteria is time-consuming and not well standardized. We evaluated a new method for rapid detection and susceptibility testing of mycobacteria by real-time measurement of their growth-related heat production in comparison with the standard microbroth dilution method.
Methods: A clinical isolate of *M. abscessus* was used. MIC was determined by microbroth dilution method after 120 h-incubation. Microcalorimetry was performed by adding 50 µl containing $2\pm0.7 \times 10^7$ CFU/ml (= $1\pm0.5\times10^6$ CFU/ampoule) *M. abscessus* on Middlebrook 7H10 agar supplemented with 10% OADC. For susceptibility testing, serial two-fold antibiotic dilutions were added to agar. Heat production was measured at 37ºC and defined positive at 5 microW. The minimal heat inhibition concentration (MHIC) was defined as the lowest concentration delaying the heat detection (compared to growth control [GC]) for 24 h. Experiments were performed in duplicate.
Results: The mean detection time of *M. abscessus* without antibiotics was 12.8 h (range, 10.4–15.2 h), reaching a peak of 109 microW (range, 104.8–114.1 microW) after 62.9 h (range, 60.8–65.0 h). With antibiotics, the heat production was delayed proportionally to antibiotic concentration (Figure 1). The MHIC/MIC were (mg/L): 2/4 (clarithromycin), 4–8/8 (amikacin) and 12/8 (linezolid).
Conclusions: Microcalorimetry allowed rapid detection (within 1 day) and susceptibility testing (within 2 days) of *M. abscessus*. The MHIC correlated with MIC values within one two-fold dilution. Microcalorimetry might be extended to other mycobacteria, including slow-growing such as *M. tuberculosis*. This method has the potential to accelerate the current diagnosis and antibiotics susceptibility testing of mycobacteria.



O255 In vitro, ex vivo and in vivo studies indicate that the neuroleptic thioridazine cures multidrug-resistant tuberculosis

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Objectives: To assemble data from in vitro, ex vivo and in vivo studies that examined the utility of the neuroleptic thioridazine (TZ) in treatment of multi-drug and extensive drug resistant tuberculosis (MDR and XDR-TB).
Methods: The effects of TZ in separate use and in combination with antibiotics have been studied in vitro via the use of the Bactec 460 and 960 systems; ex vivo in a non-killing human macrophage model; in vivo in therapy of MDR-TB infected mice; and by the treatment of XDR-TB patients infected with strains that are non-responsive to most of the anti-tuberculosis drugs currently in use.
Results: TZ at concentrations that range from 15 to 20 mg/L inhibits the in vitro replication of all tested resistant strains of *Mycobacterium tuberculosis*. These in vitro minimum inhibitory concentrations (MIC) are well above what is clinically achievable. However, concentrations as low as 0.1 mg/L which are well below the plasma concentrations seen in patients treated with TZ for psychiatric disorders have been shown to promote the killing of antibiotic susceptible, multi-drug and extensive drug resistant *M. tuberculosis* bacilli that have been phagocytosed by human non-killing macrophages. Mice infected with antibiotic susceptible and multidrug resistant strains of *M. tuberculosis* are cured of the infection after treatment with TZ (monotherapy or in combination with antibiotics). Lastly, 10/12 patients with XDR-TB were cured within a few months after treatment with TZ in combination with 3 anti-tuberculous drugs.
Conclusion: There is increasing evidence that TZ used in combination with anti-tuberculous drugs to which the strain is considered resistant can lead to cure from antibiotic resistant pulmonary tuberculosis infections. Because TZ is cheap, has been in use for more than four decades, and the doses anticipated for therapy of tuberculosis are far smaller than those used for chronic therapy of psychiatric disorders (5 to 75 mg/day vs 600 to 1000 mg/day), this minimizes the risk of cardiac toxicity and, the risk-benefit profile of TZ is shifted in favour of using this agent for the salvage therapy of patients with extensively drug-resistant tuberculosis, and potentially for the treatment of MDR-TB and for the shortening of drug susceptible TB. Nevertheless, patients treated with TZ should be carefully monitored during treatment for cardiac adverse effects associated with phenothiazines, particularly prolongation of QT interval.

Bad bugs, which drugs? Superior potency for better outcomes in Gram-positive infections

S264 Applied microbiology: the importance of potency

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Recent advances in pharmacodynamics emphasize relationships between MIC and outcome. According to the antibiotic, the critical parameter is variously: (i) the duration for which drug level exceeds MIC; (ii) the maximum concentration/MIC ratio or, (iii) the area under the concentration–time curve/MIC ratio. Nevertheless, other factors related to 'potency' (an elastic term) are important for some settings or drugs, not just the MIC. Bactericidal, rather than inhibitory activity is critical in endocarditis, severe bacteraemia and in infections where the host defences are severely compromised (e.g. neutropenic fevers). Thus, daptomycin, a rapidly cidal antibiotic, is clinically effective as monotherapy in staphylococcal endocarditis [1]; linezolid, a bacteriostatic drug, is not [2]. Nevertheless linezolid is more effective than vancomycin, a weakly bactericidal antibiotic, in methicillin-resistant *Staphylococcus aureus* pneumonia [3], where cidality is less crucial. In addition, rapid cidal potency is not, in itself, a guarantor of efficacy: polymyxin is rapidly cidal for Gram-negative bacteria, but few would argue that it is highly potent; likewise, although aminoglycosides are useful and rapidly cidal, they are not appropriate as monotherapy outside the urinary tract. In some settings, most notably those involving biofilms on synthetic surfaces (e.g. prosthetic devices) the MIC is a poor predictor of outcome. This largely reflects the presence of dormant, difficult-to-kill cells in the stratified depths of the biofilm, but perhaps also the physical

challenge of penetrating the matrix. Some antibiotics with growth-rate-independent killing, again including daptomycin, have in vitro potency against sessile cells in biofilms, but it remains to be established whether this translates into efficacy in device-related infections. Lastly, there are a few situations where antibiotics with poor in vitro activity are clinically potent, either because they interfere with bacterial signalling processes or concentrate in particular compartments, the best example being the utility of azithromycin in pseudomonal infection of cystic fibrosis patients and in gastrointestinal disease. In summary, although pharmacodynamic analysis is a good predictor in many settings, it does have limits, and potency – although an ambiguous term – is also critical in some settings.

Reference(s)

[1] Fowler VG Jr et al. New Engl J Med 2006; 355: 653–665.
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S265 Hit hard, hit early to improve outcomes in Gram-positive bacteraemias

A. Soriano (Barcelona, ES)*

Effective empirical antibiotic therapy is essential to reduce the morbidity and mortality associated with *Staphylococcus aureus* bacteraemia. Effective therapy means the selection of an antibiotic with in vitro activity and administering it at the correct dose to attain, within the first 24 hours, the pharmacodynamic (PD) target that predicts its efficacy. The high mortality rate in *S. aureus* bacteraemia treated empirically with the standard vancomycin dose (1 g/12 hours) is, in part, due to the low probability of achieving the PD target (AUC_24/MIC $\geqslant$400) within the first 24 hours. It is possible to increase the probability of target attainment by obtaining a trough serum concentration of 15–20 mg/l when the MIC is $\leqslant$1 microgram/ml. In order to achieve this goal within the first 24 hours, it is necessary to infuse a loading dose of 25–30 mg/kg and a maintaining dose of 15–20 mg/kg every 8–12 hours. This means, in general, a daily dose of vancomycin >3–4 g, which has been associated with a higher rate of nephrotoxicity. In addition, even with this body-weight-adjusted regimen, when the MIC is 2 micrograms/ml the probability of PD target attainment is low. These concepts are supported by clinical experience showing a deleterious impact of MIC = 2 micrograms/ml on the efficacy of standard vancomycin treatment and the lack of benefit, in these cases (MIC = 2 micrograms/ml), by increasing the dosage to reach a trough serum concentration $\geqslant$15 mg/l [1]. In addition, recent data has shown a higher frequency of tolerance to glycopeptides and vancomycin heteroresistance in methicillin-resistant *S. aureus* (MRSA) strains with a MIC >1 microgram/ml than in strains with a MIC $\leqslant$1 microgram/ml [2]. Taking these data into account, plus the high prevalence of MRSA strains with high vancomycin MICs (2 micrograms/ml) and the poor results with vancomycin against methicillin-susceptible *S. aureus*, even after switching to β-lactams, alerts about the need for new anti-staphylococcal agents not only as a definitive therapy but also empirically in severe infections where *S. aureus* is suspected.

Reference(s)

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S266 Challenges of treating cSSTIs, biofilms and secondary complications

M. Bassetti (Genua, IT)*

Skin and soft tissue infections (SSTIs) are among the most common human bacterial infections that result in significant morbidity and mortality among patients, as well as increased healthcare costs. Complicated SSTIs (cSSTI) can pose considerable diagnostic and therapeutic challenges. *Staphylococcus aureus* is the most common organism isolated from SSTIs, and methicillin-resistant *S. aureus* (MRSA) is a rapidly increasing hospital- and community-acquired pathogen. Where the prevalence of methicillin resistance is high, an antibiotic with activity against MRSA is mandatory. Glycopeptides (vancomycin or teicoplanin) remain the gold standard of therapy for serious MRSA infections. However, there is controversy over the current utility of these agents. There is a growing body of evidence indicating that the glycopeptide MIC has a real impact on patient outcomes [1]. Whether this phenomenon is causing serious problems in the treatment of cSSTI is not clear. Vancomycin and teicoplanin have been also associated with relapsing bacteraemia and slower clinical response rates [1]. A large number of novel antibacterial agents have been or are being developed for the treatment of cSSTIs. Only linezolid, tigecycline and daptomycin are available for clinical use. The clinical decision-making process around antibiotic choice in the empirical setting or where cultures are available is dependent on a variety of factors. Important parameters that appear to determine the clinical effectiveness of an antibiotic for cSSTIs include the severity of the illness, patient co-morbidities, presence of biofilm-associated infections and/or secondary complications, whether the patient receives appropriate antimicrobial therapy at the onset of illness and whether this is given as a single-agent or combination approach to cover a broad range of likely causative organisms. In this context, daptomycin, a cyclic lipopeptide that is rapidly bactericidal and active against almost all Gram-positive cocci including MRSA, offers some interesting advantages. Two studies reported that significantly fewer patients with cSSTIs required prolonged treatment in the daptomycin arm and that clinical cure was faster than with comparators [2].

Reference(s)

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[2] Arbeit RD et al. Clin Infect Dis 2004; 38: 1673–1681.

Pathogenesis of *Chlamydia* infections

S278 Pathogenesis and evolution of *Chlamydia*: lessons from genomics of novel chlamydiae

G. Greub (Lausanne, CH)*

Genomic analysis is a powerful tool to get insight into the biology of *Chlamydia* and *Chlamydia*-related bacteria, since there is no genetic tool to manipulate these obligate intracellular bacteria. To date, three genomes of *Chlamydia*-related bacteria have been released: *Waddlia chondrophila*, an emerging agent of miscarriage, *Parachlamydia acanthamoebae*, a possible agent of pneumonia and *Protochlamydia amoebophila*, a microorganism of yet unknown pathogenicity. These *Chlamydia*-related bacteria possess genomes ranging from 2100 kb to 3000 kb, respectively, i.e. two to three times the genome length of classical *Chlamydia*. Their genomes display numerous repeated sequences indicating a different genome dynamic than that of *Chlamydia*, which almost completely lack repetitive elements. Noteworthy, *P. amoebophila* exhibits a tra operon encoding a type IV secretion system likely involved in DNA conjugative transfer. This tra operon is encoded on a 100kb genomic island, which also contains a very large GC-rich ORF (lgrE), with repeats that evolved by serial duplications.

Genomes analyses revealed that *W. chondrophila*, *P. acanthamoebae* and *P. amoebophila* exhibit many virulence factors present in classical *Chlamydia*, including a functional type III secretion system and a gene homologous to that encoding CPAF, a secreted protein that degrades human transcription factors important for expression of major histocompatibility complexes. Contrarily to classical *Chlamydia*, all three *Chlamydia*-related bacteria possess a large number of factors likely implicated in the resistance to environmental toxic compounds. Moreover, these *Chlamydia*-related bacteria exhibited a higher metabolic ability than that of *Chlamydia*, suggesting that the common ancestor of the modern *Chlamydia* may have been less dependent to their eukaryotic host.

Recently, using Solexa and/or 454 technologies, we sequenced the genomes of three additional *Chlamydia*-related bacteria: *Protochlamydia amoebophila*, *Criblamydia sequanensis* and *Estrella lausannensis*, allowing further comparative genomic analyses and further insight in the evolutionary history of the members of the Chlamydiales order.

In conclusion, the recent availability of the genomes of *Chlamydia*-related bacteria represents an opportunity to better understand the evolution and the biology of chlamydiae.

S279 Immunogenetics: why do not all women become infertile following chlamydial infection?

S. Morré and the FP6 EpiGenChlamydia Consortium

Chlamydia trachomatis (CT) infections are the world leading cause of blindness (trachoma) and the most prevalent sexually transmitted disease which is strongly associated with pelvic inflammatory disease, ectopic pregnancy, and tubal infertility. Twin study-based findings of members of the FP6 EpiGen*Chlamydia* Consortium identified a 40% genetic predisposition to CT infections. The EpiGen*Chlamydia* Consortium structured transnational research to such degree that comparative genomics and genetic epidemiology on large numbers of unrelated individuals can be performed to identify genetic markers to be used for patient profiling.

Subfertility poses an enormous burden on healthcare and society throughout the world. Worldwide, 15% of couples trying to conceive suffer from subfertility. Subfertility generally described as a failure to conceive after one year of unprotected, regular sexual intercourse. Approximately half of the couples suffering from subfertility will conceive spontaneously, or after simple treatment. The other half needs more complex treatment, such as in vitro fertilization (IVF) or other assisted reproduction procedures.

There are several causes of subfertility, which can be classified as ovulation disorders, male factor subfertility, tubal damage, unexplained subfertility, and other causes, such as endometriosis and fibroids. In women, one of the major causes of female subfertility is tubal pathology, with a prevalence of around 30%. In all of cases of tubal pathology, *C. trachomatis* is the single most common cause for infertility.

The reference standard for diagnosing tubal pathology in subfertile women is laparoscopy performed usually after a CT IgG positive antibody test which is only positive in 50–60% of women with tubal pathology. Despite the effectiveness, laparoscopy is costly, invasive and has a low positive predictive value. Therefore there is a unmet medical need for better diagnosing tubal pathology in women with subfertility, a need which could potentially be met by adding genetic profiling to current diagnostic approaches. The current presentation will discuss immunopathological mechanisms and host based genetic diagnostic approaches.

Next generation sequencing and clinical microbiology

S287 Possible use of next generation sequencing in routine clinical microbiology

M.R. Capobianchi, I. Abbate (Rome, IT)*

The error prone nature of HIV-1 and HBV reverse transcriptase, combined with the high replicative activity of these viruses, results, in each infected individual, in the formation of many genetically related viral variants referred to as quasispecies, in which most viral sequences slightly differ from each other. This variability is the substrate for the selective pressure exerted by the immune system or by drugs, leading to the continuous viral evolution in the infected host and to the occupation of replicative space in different body compartments.

Traditional analysis of quasispecies, based on the sequencing of clonal PCR products, has a low resolution power, due to the limited amount of clones that can be handled in each experiment.

In particular, it is not possible, by traditional approaches, to detect and quantify minority genomes present in viral quasispecies, that, indeed, may have biological and clinical relevance. This includes reappearance of founder viruses during natural history, shifts in coreceptor usage at different phases of the infection, evolution of drug resistance and re-emergence of hidden genomes after treatment interruptions.

NGS, with the ultra-deep pyrosequencing (UDPS) approach, provides a unique opportunity to investigate viral quasispecies. In fact, for each patient, it provides a data set of clonally amplified PCR products that is some order of magnitude higher than any previous conventional approach. Hence, NGS represents a breakthrough tool to investigate previously inaccessible aspects of HIV-1 and HBV dynamics, such as to explore the contribution of different viral reservoirs to replicating virus along the natural history of the infection, to identify coreceptor usage in minority viral populations harboured by different cell lineages, and to investigate the dynamics of development of drug resistance along antiviral treatment.

Application of NGS to clinical microbiology is in its infancy, but promising results have been obtained in the detection and characterization of novel viruses (arenaviruses, pandemic influenza and others), in the investigation of the dynamics of resistance development (HIV abd HBV), and in the establishment of viral tropism at quasispecies level (HIV).

Equipment simplification, methods standardization, development of user friendly bioinformatic tools represent urgent needs to be met in order to render more accessible to clinical microbiology the new, potent tool represented by NGS.

Clinical and microbiological update on antibiotic-resistant *P. aeruginosa*

O288 *Pseudomonas aeruginosa* carbapenem resistance mechanisms in Spain: impact on the activity of imipenem, meropenem, and doripenem

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Objective: To investigate the mechanisms of carbapenem resistance in the 175 *P. aeruginosa* isolates (39%) showing nonsusceptibility, according to EUCAST breakpoints, to imipenem (MIC >4 mg/L, 35%), meropenem (MIC >2 mg/L, 33%), or doripenem (MIC >1 mg/L, 33%), recovered in 2008–2009 from 16 Spanish hospitals during the COMPACT study.

Methods: MICs were determined by Etest and clonal relatedness by PFGE. The production of metallo-β-lactamases (MBL) was evaluated with Etest MBL strips and PCR followed by sequencing. Mutation-driven resistance was investigated in 60 of the 175 isolates, according to the distribution of doripenem MICs. Fifteen isolates from each of the following MIC groups were randomly selected: ⩽1 mg/L (susceptible), 2–4 mg/L (intermediate), 8–16 mg/L (resistant but potentially treatable depending on PK/PD parameters), and ⩾32 mg/L (high level resistant). MBL producers were excluded from this analysis. The expression of ampC and the genes encoding the 4 major efflux pumps (mexB, mexY, mexD, and mexF) were determined by real time RT-PCR. The presence of inactivating mutations in oprD was explored through PCR and sequencing.

Results: 12 (6.9%) of 175 *P. aeruginosa* isolates were found to be MBL producers, including 9 VIM-20 (one clone disseminated in a single hospital), 2 VIM-2, and 1 VIM-13. All MBL producing isolates showed high level resistance (MICs >32 mg/L) to the 3 carbapenems. Regarding mutation-driven resistance, all but 1 of the 60 isolates were nonsusceptible to imipenem, linked to oprD inactivating mutations. In addition, 30 (50%) isolates overexpressed ampC, 20 mexY (33%), 19 mexB (32%), and 9 mexF (15%), while none overexpressed mexD. Increasing prevalence of ampC overexpression correlated with increasing doripenem MICs (⩽1, 13%; 2–4, 53%; 8–16, 60%; ⩾32, 73%) while overexpression of mexB (27%, 27%, 53%, and 20%), mexY (7%, 20%, 73%, and 33%), and mexF (7%, 13%, 27%, and 13%) correlated only with moderate doripenem resistance. As presented in the Table, doripenem showed higher activity than meropenem in the complete

collection of isolates, as well as in those overexpressing ampC, and, specially, mexB or mexY.

Conclusion: Although the prevalence of MBLs is increasing, mutation-driven resistance was still far more frequent. Imipenem resistance was driven by OprD inactivation, while additional AmpC, and, particularly, efflux pumps hyperproduction had a lower impact on the activity of doripenem when compared to meropenem.

Table. Activity of meropenem and doripenem in isolates showing different mechanisms of resistance

Isolates	Cumulative No. of isolates inhibited at each concentration (mg/L) meropenem (%)/ doripenem (%)				
	<=1	2	4	8	16
All (n=60)	6 (10)/15 (25)	13 (22)/ 24 (40)	19 (32)/30 (50)	26 (43)/39 (65)	31 (52)/45 (75)
ampC (n=30)	2 (7)/ 3 (10)	2 (7)/ 8 (27)	7 (23) /12 (40)	12 (40) /18 (60)	14 (47) /22 (73)
mexB (n=19)	1 (5)/ 4 (21)	4 (21)/ 7 (37)	5 (26)/ 8 (42)	7 (37)/ 12 (63)	8 (42)/ 16 (84)
mexY (n=20)	1 (5) / 1 (5)	1 (5) / 2 (10)	1 (5) / 4 (20)	4 (20) / 10 (50)	7 (35)/ 15 (75)

O289 IMP-29, a novel metallo-β-lactamase in *Pseudomonas aeruginosa*

K. Jeannot, M. Robert Nicoud, P. Plésiat (Besançon, FR)*

Objectives: *Pseudomonas aeruginosa* may become highly resistant to carbapenems and almost all β-lactam antibiotics by horizontal transfer of metallo-β-lactamase genes (MBLs). To date, 6 types of MBLs (IMP, VIM, SPM, GIM, AIM et DIM) have been reported in this organism. This study describes a novel IMP-type enzyme produced by two *P. aeruginosa* clinical strains collected at the teaching hospital of Besançon (France).

Methods: Strains 10266 and 10298 were isolated from a rectal screening sample and a blood culture of two patients admitted in 2010, respectively. Drug susceptibility testing, strain genotyping by PFGE, MBL production, PCR amplification, cloning and sequencing experiments were performed according to standard protocols.

Results: Double disc synergy tests using imipenem and EDTA indicated MBL production in these two genotypically related strains only susceptible to aztreonam and colistin. A gene encoding an enzyme distantly related to IMP-8 (identity <70%) and referred to as IMP-29 was amplified by PCR and sequenced. In strain 10298, blaIMP-29 was located in a typical class 1 integron downstream of gene cassettes aacA4 et dfrII. The genetic environment of blaIMP-29 appeared to be very different in 10266 as the gene lied alone with the P2 promoter of intL1 on a transferable plasmid of 20 kb. Interestingly, the PU21 tranconjugants were susceptible to piperacillin (MIC = 8 μg/mL), of intermediate susceptibility to imipenem (8 μg/mL) and higly resistant to meropenem (⩾128 μg/mL), doripenem (64 μg/mL), ceftazidime (⩾128 μg/mL) and ticarcillin (⩾128 μg/mL). A second class 1 integron harboring gene blaPSE-1 flanked by gene cassettes aacA4 and aadA2 was detected in the two strains. Finally, the presence of a stop codon in gene oprD accounted for the high level resistance to imipenem in both isolates.

Conclusion: This new transferable MBL identified in France provides *P. aeruginosa* with an atypical MBL resistance phenotype which can be misidentified when additional resistance mechanisms are present (penicillinase PSE-1, loss of porin OprD). The genetic element carrying blaIM-29 is subject to significant rearrangements.

O290 Carbapenem-resistant *Pseudomonas aeruginosa* after short exposure to therapeutic concentrations of ertapenem

S.J. Vainio, M.L. Schepens, A. Wilhelm, C.M. Vandenbroucke-Grauls, J.L. Murk, Y.J. Debets-Ossenkopp (Amsterdam, NL)*

Objectives: Since the introduction of ertapenem, concerns have been raised about the potential of this drug to select for resistance to other carbapenems in nosocomial pathogens, most importantly *P. aeruginosa*. In vitro studies have shown that under certain conditions, such cross-resistance can develop, but conditions resembling clinical settings have not been tested. In this study, we determined the effects of exposing *P. aeruginosa* to a range of ertapenem concentrations that mimics the in vivo serum concentrations during ertapenem therapy.

Methods: 12 clinical isolates of *P. aeruginosa* (all with meropenem MICs <0.25 mg/L and imipenem MICs <1.5 mg/L; 10 with ertapenem MICs 0.064–4 mg/L, categorized as ertapenem sensitive, and 2 with ertapenem MICs >32 mg/L; categorized as ertapenem resistant) were incubated at 37ºC in RPMI-medium with ertapenem concentrations reflecting the in vivo concentrations achieved with the standard daily dose (1 h in each 16, 8, 4 and 2 mg/L, 4 h in 1 mg/L and 16 h in 0.5 mg/L of ertapenem) for three consecutive days. As a control, the isolates were incubated following the same scheme but in the absence of ertapenem. MICs for ertapemen, imipenem and meropenem were determined by E-tests, and sensitivity to other antibiotics was by Vitek-2.

Results: After the incubation with ertapenem, nine of the ertapenem sensitive *P. aeruginosa* isolates exhibited markedly elevated ertapenem MICs (>32 mg/L). Moreover, all these strains displayed diminished sensitivity or frank resistance to imipenem (MICs 4–>32 mg/L) and/or meropenem (MICs 1.5–>32 mg/L) and in some cases, the MICs for ceftazidime and piperacillin were also elevated. The phenotype was stable after one month of continuous culturing in the absence of antibiotics in all but one strain. In the case of one ertapenem sensitive isolate (ertapenem MIC 0.064 mg/L), no viable cells were recovered after the ertapenem treatment. The incubation with ertapenem had no effect on the carbapenem MICs of the two ertapenem resistant isolates, and none of the tested isolates showed changes in the sensitivity to non-βlactam antibiotics.

Conclusions: These data show that exposing *P. aeruginosa* to clinically relevant concentrations of ertapenem can rapidly select for diminished sensitivity to all carbapenems. Thus far, there is no evidence that such selection would occur in clinical practice, but these results disclose the need for continued surveillance.

O291 Exposure of *Pseudomonas aeruginosa* to sub-MIC concentrations of chlorhexidine leads to increased resistance, marked phenotypic changes, overexpression of MexG, and cross-resistance to antipseudomonal antibiotics

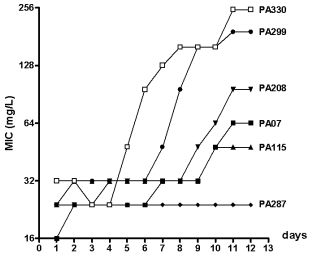
Q. Tan, T. Nguyen, F. Bilocq, D. De Vos, J. Pirnay, P. Cornelis, F. Van Bambeke, P. Tulkens (Brussels, BE)*

Objectives: Biocides carry a risk of resistance and cross-resistance with antibiotics that still needs to be assessed (Meyer & Cookson, J Hosp Infect. 2010, 76:200–5). Our aim was to examine whether exposure of *P. aeruginosa* (PA) to sub-MIC concentrations of CHX could lead to resistance to CHX, modify the bacterial phenotype, cause increased expression of efflux transporters, and trigger cross-resistance to anti-PA antibiotics.

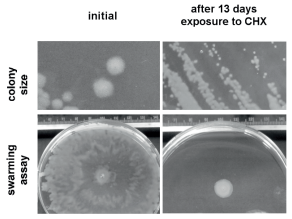
Methods: (i) 6 fully susceptible and 18 multi-resistant isolates of PA (VAP and burned patients) with initial CHX MIC ⩽32 mg/l were exposed to CHX at 0.5 MIC with daily measurement of MIC (microdilution) and readjustment of the CHX concentration to 0.5× the new MIC value, for up to 14 days, followed by 10 subcultures on CHX-free agar (revertants). Clonality was checked by Repetitive Extragenic Palindromic-PCR [DiversiLab] >95% similarity [Riou et al. IJAA 2010, 36:513–22]). Bacteria were examined for change in CHX MIC, swarming (agar plates containing CHX at 0.5 MIC), pyoverdine production (405/660 nm absorbance ratio), and susceptibility to antipseudomonal antibiotics (MIC; CLSI methods). Expression of 3 genes (mexA, mexX, mexG) part of clusters encoding 3 efflux pumps was measured by RT-PCR.

Results: All isolates showed an increased CHX MIC (2 to >8-fold; up to >256 mg/L) after 13 days exposure (see Figure for 6 selected strains). Most CHX-exposed isolates showed reduction in colony size, almost complete suppression of pyoverdin production, and marked reduction of swarming. 3 isolates showed increased MIC to amikacin (2 to 4 x), 1 to piperacillin (2 to >8-x), 3 to cefepime (2 to 4-x), and 5 to ciprofloxacin (2 to 16-x), nut 1 (PA330) showed a decrease in ciprofloxacin MIC (64 to 0.5 mg/L). There was a variable overexpression of mexA, mexX, but mexG was overexpressed in all cases (MexGHI-OpmD facilitates

cell-to-cell communication, confers resistance to vanadium, promotes virulence and growth in *P. aeruginosa* but increases susceptibility to many antibiotics [Aendekerk et al. Microbiology 2002, 148:2371–81]) Reversion for all the above parameters was partial only.
Conclusion: Exposure to non-lethal concentrations of chlorhexidine fosters the development of strains with reduced susceptibility and with cross-resistance to antibiotics. The multiple genotypic and phenotypic alterations need to be critically assessed, but the data call for caution against using CHX at non-lethal concentrations.



Change in MIC of CHX during exposure to 0.5 MIC with daily concentration readjustment



Typical change in colony size and swarming abilities after 13 days of exposure to 0.5 MIC

O292 Levofloxacin-doripenem combination prevents emergence of resistance among *Pseudomonas aeruginosa* expressing mechanisms of resistance impacting susceptibility to one or both drugs in the combination

P. Lister (Omaha, US)*

Objectives: Previous studies have demonstrated the ability of levofloxacin-imipenem to prevent the emergence of resistance among isolates of *P. aeruginosa* in a two-compartment in vitro pharmacodynamic model (IVPM). Data from those studies suggested that other carbapenems could be effective in the combination. Therefore, this study was designed to evaluate the pharmacodynamics of a levofloxacin-doripenem combination against *P aeruginosa* in an IVPM. The primary focus of the study was to evaluate the ability of this combination to eradicate and prevent emergence of higher levels of resistance from characterized isolates of *P. aeruginosa* expressing one or more mechanisms of resistance impacting susceptibility to the drugs in the combination.
Methods: The strains used in this study included a fully-susceptible clinical isolate, 4 characterized strains overexpressing mexAB-oprM, mexCD-oprJ, mexEF-oprN, or mexXY, and one strain lacking a functional OprD porin. Log-phase cultures (~10^7 to 10^8 cfu/ml) were inoculated into the peripheral compartment of the IVPM and treated with simulated human doses of 750mg levofloxacin, 500mg doripenem (1h infusion), or the levofloxacin-doripenem combination. Pharmacokinetics were adjusted to account for protein binding. Levofloxacin was dosed at 0h, and doripenem was dosed at 0, 8, and 16h. Pharmacodynamics were evaluated over 24h, and drug-selection plating was used to evaluate emergence of resistant subpopulations.
Results: In studies with the susceptible clinical isolate, the OprD-deficient strain, and strains overexpressing mexAB-oprM, mexCD-oprJ, and mexXY, the levofloxacin-doripenem combination decreased viable counts over 6 logs and achieved eradication from the IVPM by 8 to 24h. Furthermore, the combination prevented inoculum regrowth and emergence of resistance that was observed with each drug alone. Although levofloxacin-doripenem did not eradicate the strain overexpressing mexEF-oprN (co-regulated decrease in oprD), viable counts decreased 4.5 logs by 8h and were maintained at this level through 24h. This was in contrast with the emergence of more resistant subpopulations with each drug alone.
Conclusions: These data demonstrate the ability of levofloxacin-doripenem to effectively prevent emergence of resistance among *P. aeruginosa*, even when the target strains express one or more resistance mechanisms impacting drugs in the combination. Further studies and clinical evaluation are warranted.

O293 Linear increasing of resistance rates to nine antibiotics of *Pseudomonas aeruginosa* strains within a nosocomial outbreak

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Objectives: *Pseudomonas aeruginosa* (PA) has been recognized as a common cause of nosocomial infections, mainly in critically ill or immunosuppressed patients. Its dramatic ability to exhibit multidrug-resistance is related to inherent mechanisms and the ready acquisition of new ones, making PA uniquely dangerous. The aim of this study was to describe and quantify the increasing of resistance rates to several antibiotics of serially recovered PA strains from inpatients with prolonged infection and/or colonization within a nosocomial outbreak at a University Hospital in Madrid.
Methods: We conducted an observational retrospective study of 822 PA isolates from 371 adult inpatients who were admitted to 'La Princesa' University Hospital during one year. PA strains were isolated from respiratory samples, urine, stool, intravascular devices, blood and other sterile fluids. Nine drugs were tested: amikacin (AKA), gentamycin (GEN), tobramycin (TOB), imipenem (IMI), meropenem (MER), ciprofloxacin (CIP), piperacilin-tazobactam (TZP), ceftazidime (CAZ) and cefepime (CPE). Several PA isolates were sequentially recovered from each patient. First PA isolation was marked as "Day 0". Susceptibilities were determined by a broth microdilution method and interpreted according to the CLSI breakpoints. Resistance rates are expressed as proportions. Correlation was studied with Pearson coefficient and a linear regression model was built. Confidence intervals of 95% have been used and P-values lower than 0.05 were considered statistically significant.
Results: All R-coefficients resulted over 0.8, but not for AKA (R = 0.46) between day 0 and day 18. Since resistance rates stabilize around day 20, changes found from day 21 are irrelevant. CAZ, IMI and MER showed the best fitness to the linear model (R^2=0.95, 0.76, 0.74). Thus, every 3 days, the likelihood of resistance increases [4.4–8.5%] for CAZ, [1.5–11.8%] for IMI and [1.1–11.1%] for MER. TZP, GEN and TOB are the less affected ([0.2–9.8%], [0.3–9.3%] and [0.1–9.4%]).
Conclusions: (1) Resistance rates linearly increased in time for all antibiotics until day 18 except for AKA. (2) Susceptibility to AKA remained stable. (3) The likelihood of resistance to CAZ, IMI and MER increases markedly every 3 days. (4) This is less evident for TZP and aminoglycosides. (5) Short-time treatment with CAZ and carbapenems must be considered. (6) A good initial combination treatment should include an amynoglycoside (mainly AKA) and TZP.

Linear correlation between time of isolation and resistance rates

Antibiotic	R	R^2	p	A [CI 95%]	B [CI 95%]
AKA	0.46	0.21	0.36	0.015 [-0.025 – 0.054]	0.117 [-0.035 – 0.270]
TOB	0.82	0.67	0.047	0.048 [0.001 – 0.094]	0.597 [0.416 – 0.779]
GEN	0.83	0.68	0.043	0.048 [0.003 – 0.093]	0.677 [0.5 – 0.854]
IMI	0.87	0.76	0.024	0.066 [0.015 – 0.118]	0.359 [0.157 – 0.561]
MER	0.86	0.74	0.028	0.061 [0.011 – 0.111]	0.345 [0.150 – 0.540]
CIP	0.84	0.71	0.036	0.043 [0.004 – 0.081]	0.655 [0.505 – 0.805]
TZP	0.82	0.68	0.045	0.050 [0.002 – 0.098]	0.098 [-0.089 – 0.286]
CAZ	0.97	0.95	0.001	0.065 [0.044 – 0.085]	0.189 [0.108 – 0.270]
CPE	0.84	0.70	0.038	0.054 [0.005 – 0.103]	0.412 [0.222 – 0.603]

Linear regression model: Y = A*X + B. "Y"= Resistance rate, "X"= Time of isolation (each unit of "X" indicates a period of 3 days), "A" and "B" are constants.

O294 Antibiotic resistance trends of *Pseudomonas aeruginosa* and *Acinetobacter* spp. isolates at a teaching hospital in Istanbul, 2004–2010

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Objectives: Antibiotic resistance by intensive care unit pathogens especially *Pseudomonas aeruginosa* and *Acinetobacter* spp. is increasing, but variations do exist among different countries and hospitals. The local

resistance data is essential for appropriate therapy of ICU infections. In this study we reported the antibiotic resistance rates of *Pseudomonas aeruginosa* and *Acinetobacter* spp. strains and alterations in years in a teaching hospital, Istanbul.
Methods: From January 1, 2004 to December 20, 2010, we obtained a total of 1583 *P. aeruginosa* strains and 1855 *Acinetobacter* spp. strains from various clinical specimens of patients followed in ICU, which were regarded as infectious agents. Identification of species was performed with standart methods. Antibiotic susceptibilities of isolated strains were investigated by Kirby-Bauer disk diffusion method according to CLSI criteria. Chi-square test was used for statistical analysis.
Results: In 2004 and 2010, resistance to certain antibiotics of *Pseudomonas aeruginosa* was revealed as follows: 48%, 58% ($p = 0.2023$) for piperacillin/tazobactam, 66%, 92% ($p < 0.0001$) for ceftazidime, 25%, 62% ($p < 0.0001$) for imipenem, 25%, 56% ($p < 0.0001$) for meropenem, 2%, 21% ($p < 0.0001$) for amikacin, and 72%, 59% ($p = 0.0743$) for ciprofloxacin, respectively. The resistance rates of *Acinetobacter* spp. were as follows: 48%, 97% ($p < 0.0001$) for ampicillin/sulbactam, 2%, 80% ($p < 0.0001$) for cefoperazone/sulbactam, 23%, 89% ($p < 0.0001$) for imipenem, 24%, 92% ($p < 0.0001$) for meropenem, 71%, 38% ($p < 0.0001$) for gentamicin, and 92%, 90% ($p = 0.8048$) for ciprofloxacin, respectively.
Conclusion: While the resistance to cefoperazone/sulbactam, ampicillin/sulbactam, imipenem, meropenem have significantly increased from 2004 to 2010; gentamicin resistance has decreased for *Acinetobacter* spp. The resistance rates of *Pseudomonas aeruginosa* to imipenem, meropenem and amikacin have increased; although ciprofloxacin resistance has decreased. In 2010 the resistance rate of *P. aeruginosa* to tazobactam/piperacillin has not changed compared with 2004. As known, insufficient infection prevention strategies and the unnecessary use of broad spectrum antibiotics are the cause of high-level antibiotics resistance. Therefore, infection control and antibiotic management strategies should be reconsidered in our ICU.

O295 Characterisation of resistance mechanisms in clinical isolates of *Pseudomonas aeruginosa* with reduced susceptibility to carbapenems

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Objective: To characterise the mechanisms of resistance to carbapenems in clinical isolates of *Pseudomonas aeruginosa* (Pa) non-susceptible to these agents collected during an 8-year period.
Material and Methods: Clinical isolates of Pa with MIC of impenem (IMP) and/or meropenem (MPM) $\geqslant$8 mg/l were collected from January 2002 to December 2009. Identification and preliminary susceptibility testing were performed with the Vitek 2 System (bioMérieux). The MICs of IMP and MPM were confirmed by broth microdilution (CLSI guidelines). MICs of doripenem (DOR) were determined by Etest method. Production of metallo-β-lactamase was studied by broth microdilution, Etest and multiplex PCR. The expression of the OprD porin was analyzed by SDS-PAGE, and inactivating mutations were determined by sequencing of the entire oprD gene amplified by PCR. Both ampC hyperproduction (hipAmpC) and mexB overexpression (overmexB) expression levels were assesed by real-time PCR. The clonal pattern of the strains was studied by REP-PCR.
Results: A total of 109 carbapenem-resistant Pa from different patients were collected in the indicated period, and 26 isolates representative of the different clonal patterns were further studied. The $MIC_{50/90}$ (mg/l) for these 26 strains were 32/64 for IMP, 16/32 for MPM and 32/>32 for DOR, respectively. VIM-2 was detected in 5 (19.2%) isolates (in 2 cases combined with OprD loss and in 1 case with overmexB). Thirteen isolates had only one of the studied resistance mechanism, including 9 (34,6%) isolates with OprD loss, 2 (7,7%) with hipAmpC and 2 (7,7%) and the 2 (7.7%) with VIM-2 alone. Loss of OprD plus hipAmpC, and combination of OprD loss, hipAmpC and overmexB were observed in 30.8% and 7.7% of the isolates, respectively.
Conclusion: The combination of different resistance mechanism was the main cause for developing resistance to carbapenems, highlighting the loss of OprD porin as the most prevalent mechanism.

O296 Wide dispersion of ST175 clone despite a high-genetic diversity of carbapenem-non-susceptible *Pseudomonas aeruginosa* clinical strains from 16 Spanish hospitals

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Objective: The prevalence of carbapenem non-susceptible clinical *Pseudomonas aeruginosa* isolates detected during the COMPACT-Spain surveillance study was 35% for imipenem (MIC >4 mg/L), 33% for meropenem (MIC >2 mg/L) and 33% for doripenem (MIC >1 mg/L). The aim of the present work was to analyze the genetic diversity and the population structure of these carbapenem-resistant isolates.
Methods: A collection of 175 carbapenem non-susceptible clinical *Pseudomonas aeruginosa* isolates recovered from 16 Spanish Hospitals between October-2008 and March-2009 was included. Antibiotic susceptibility was performed by the standard CLSI-microdilution method and Etest. Genetic diversity was studied by PFGE-XbaI and a dendrograme based on the Dice's coefficient was constructed with the Phoretix 5.0 software. One strain per pulsotype and per hospital were further selected for MLST typing (n = 84).
Results: All 175 *P. aeruginosa* isolates showed non-susceptibility to, at least, one carbapenem (imipenem, meropenem and/or doripenem) according to EUCAST criteria (2010). Resistance to other antibiotics were: 51% piperacillin/tazobactam, 47% ceftazidime, 57% cefepime, 73% ciprofloxacin, 77% levofloxacin, 68% gentamicin, 48% tobramycin, 58% amikacin, 82% fosfomycin, and 0% colistin. Sixty-five different PFGE patterns were observed, and from the 84 isolates that were selected for MLST, a high genetic diversity was observed: 23 strains corresponded to a unique ST, 5 of them newly described in our study (ST1014–1018). The ST175 was the most represented MLST-type corresponding to 18 isolates recovered from 7 hospitals. Within these isolates, doripenem exhibited significantly lower MIC values (mean geometric MIC, 16 mg/L) when compared with meropenem (33.3 mg/L) or imipenem (50.1 mg/L). Other STs that were found in different hospitals were: ST244 and ST111 (4 in each hospital), ST446 (3 hospitals) and ST235, ST253, ST646, ST308, ST313 (2 in each hospital).
Conclusion: All carbapenem non-susceptible clinical *Pseudomonas aeruginosa* isolates in Spain recovered through the COMPACT study remained susceptible to colistin, whereas high rates of resistance were detected for other antibiotics. Doripenem MICs were less affected than those of meropenem and imipenem. Moreover, within this population and despite a high genetic diversity, the ST175 clone was highly disseminated.

O297 Comparison of chromogenic and selective media for the detection of *Staphylococcus aureus* and *Pseudomonas aeruginosa* in respiratory samples from cystic fibrosis patients

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Objectives: A hallmark of cystic fibrosis (CF) patients is chronic respiratory infection caused mainly by *Staphylococcus aureus* (SA) and *Pseudomonas aeruginosa* (PA). The microbiology of CF is complicated by the emergence of abnormal phenotypes such as small colony variants (SCV) of SA and PA. The aim of this study was to compare the performance of chromogenic media to conventional media for the detection and the presumptive identification of SA and PA, including normal (N) and SCV phenotypes.
Methods: 159 respiratory samples from 64 CF patients were plated onto Columbia agar (COL), *Haemophilus* agar (HAEM), MacConkey agar (MAC), Mannitol Salt agar (MAN), *Burkholderia cepacia* selective agar (BCSA), SA chromID (SAID, bioMérieux), PA chromID (PAID, bioMérieux). Plates were incubated at 35ºC for 5 days and examined

daily. Suspected SA and PA colonies, according to the manufacturer's interpretative criteria, were subcultured on COL for phenotypic identification. Identification of SA isolates was confirmed by multiplex PCR for 16S rRNA, mecA and nuc genes. PA isolates showing atypical biochemical profile were characterized by 16S rRNA sequencing.
Results: 79 SA isolates, including 58 N and 21 SCV phenotypes, were found from 48 samples. The sensitivities of SAID (89.9%) and MAN (81%) were not significantly different ($p = 0.107$) for SA detection. The specificities of SAID and MAN were 67.2% and 77.1% respectively. SA SCV isolates were more frequently recovered on SAID ($n = 21$) than on MAN ($n = 18$). 133 PA isolates were found from 72 samples. PAID showed significantly higher sensitivity than MAC (88.7% vs 75.9%; $p = 0.013$) for PA detection. The specificities of PAID and MAC were 90.8% and 64.5% respectively. PA SCV isolates were also more frequently recovered on PAID than on MAC ($n = 44$ vs $n = 31$; $p = 0.02$) but the mucoid phenotype of PA was less expressed on the chromogenic medium. On PAID, mucoid colony variants produced frequently translucent colonies, which could go undetected following to the manufacturer's recommendations.
Conclusion: In our CF patients centre, both SAID and PAID chromogenic media demonstrated better performances than conventional media for the detection of SA and PA isolates, especially in the recovery of atypical variants. However, mucoid translucent colonies on PAID should be identified to exclude PA.

Tracking antibiotic-resistance in Gram positive cocci

O298 Colonisation and transmission of resistant bacteria in 13 European intensive care units

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Objective: To quantify colonization on admission and ICU-acquired acquisition with multidrug resistant organisms (MDRO) in 13 Intensive Care Units (ICUs) throughout Europe participating in the MOSAR ICU-trial. This is a cluster-randomized trial evaluating different interventions to limit the spread of MDRO in ICU. MDRO included MRSA, VRE and ESBL-producing Enterobacteriaceae (ESBL). This abstract refers to the 6-month baseline period in which no interventions were implemented.
Methods: Participating ICUs were in France, Greece, Italy, Latvia, Luxemburg, Portugal, Slovenia and Spain. The first ICU started this trial in May 2008 and the trial ended in Oct 2009. Screening swabs were taken from the nose (MRSA), rectum (VRE, ESBL) and wounds (MRSA, VRE and ESBL) of all patients admitted for $\geqslant$3 days (long-stay patients, LS) on admission and twice a week, as well as from a sample of patients admitted for <3 days (short-stay patients, SS). Samples were processed using a standardized lab protocol, without feedback of culture results. All screening swabs were dispensed in cryopreservative fluid (microbanks) and frozen at −80ºC. After a delay of at least 2 months, to prevent feedback to the clinic, the samples were thawed and plated onto BBL CHROMagar MRSA II plates (MRSA), ECCV chromagar plates with 8 microg/ml vancomycin (VRE) or Brilliance 2 ESBL plates (ESBL). All plates were read by a local expert, and suspected colonies were picked for confirmatory testing. Individual patient data were obtained using a web based data-capturing protocol (ResearchOnline(R)).
Results: In total, 2887 patients were enrolled, with a median age of 64 yrs (IQR 48–76 yrs), a mean LOS of 11 days (range 1–263 days) and a median APACHE II (5 sites) of 15 (IQR 10–21) and median SAPS II (8 sites) of 35 (IQR 22–50). Main admission diagnosis was surgical in 73.9% of admissions (range 37.0–98.6%) and non-surgical in 26.1% (range 1.4–63.0%). Admission and ICU acquired colonization rates are depicted in Table 1.
Conclusions: Throughout Europe, colonization with MRSA, VRE and ESBL on admission is generally low. Acquisition during admission occurs relatively frequently, especially with ESBL. Large differences exist between different ICUs in different countries in Europe. Quantifying transmission makes it possible to design and evaluate effective interventions. Future data from the MOSAR trial will give more insight in the effect of different interventions on the transmission of these MDRO in these ICUs.

	Colonisation on admission (%, range per hospital)	Colonisation during stay (%, range per hospital)
MRSA	4.3 (0.8-14.1)	5.3 (0.6-15.8)
VRE	2.9 (0.0-11.4)	5.6 (0.0-22.3)
ESBL	5.1 (0.0-13.6)	12.2 (1.2-41.8)
Any	10.9 (3.5-22.8)	18.0 (2.3-51.1)

O299 Emergence and evolution of heteroresistant vancomycin intermediate *Staphylococcus aureus* in a single hospital over a 12-year period

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Objectives: To determine the evolution of heteroresistant vancomycin intermediate *Staphylococcus aureus* (hVISA) in a single institution.
Methods: All methicillin-resistant *Staphylococcus aureus* (MRSA) blood stream infection (BSI) isolates between1997 and 2008 were included in the study. All isolates underwent modified population analysis profiling (PAP-AUC) with hVISA confirmed if the AUC ratio of the isolate to the reference (Mu3; ATCC 700698) was >0.9. All isolates were typed by pulsed field gel electrophoresis (PFGE) using HARMONY criteria against known multi-locus sequence typed (MLST) controls. Isolates were considered ST239 MRSA if they 1) resembled the ST239 control; 2) were multi-resistant (resistant to >2 B-lactam antibiotics); and 3) had a coagulase PCR restriction length fragment polymorphism pattern of 24. Relationships between PFGE banding patterns was examined using BioNumerics® (version 6.1).
Results: Of the 465 isolates identified over the 12 years, 54 (12%) were classified as hVISA by PAP-AUC. hVISA emerged in 2000 with a "biphasic" epidemic curve. PFGE typing categorised 360 (77%) isolates as ST239, the remaining isolates resembled ST22 [43]; ST1 [20]; ST93 [11]; ST30-like [11]; unclassified [13]. hVISA was only identified in ST239 ($p < 0.01$). A UPGMA dendogram based on PFGE patterns of ST239 isolates revealed large diversity with 61 sub-pulsotypes clustering into 6 distinct clusters. The diversity in individual clusters was large ($\geqslant$6 sub-pulsotypes) with two clusters (1 & 4; 296/360; 82%) and 8 sub-pulsotypes (CL1–1; CL1–2; CL1–3; CL1–4; CL4–1; CL4–7; CL4–9 & CL4–14; 231/360; 64%) predominating. Temporal relationships existed for all clusters with cluster 4 peaking in 2002 and replaced by 2008. In contrast, cluster 1 first appeared in 2000 and was the predominant cluster from 2006 onwards. Although hVISA occurred in 5 of the 6 clusters, hVISA mainly occurred in cluster 4 (44/54; 81%) and principally restricting to a single sub-pulsotype (CL4–1; 23/44; 52%). Of note, hVISA did not occur in the most common sub-pulsotype (CL4–7; 0/53; 0%) but was exclusively found in CL4–5 (4/4; 100%).
Conclusion: The proclivity of different MRSA types and within specific MRSA types to select hVISA varies greatly. Furthermore, factors associated with hVISA emergence remain unclear but are probably related to evolutionary pressures on the predominant vancomycin susceptible clones.

O300 Tracking antibiotic resistance along the Silk Road

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Objectives: Information on the occurrence of antibiotic resistance in relatively pristine ecosystems is still scarce. Accordingly, the goal of this study was to evaluate if wild or feral animals, most of which facing extinction, inhabiting remote areas of the globe (from the Tibetan plateau to the Gobi desert), carry bacteria that harbour antibiotic resistance genes.

Methods: During the SilkRoad2010 expedition (CIBIO and Chinese Academy of Sciences), faecal samples were collected from five iconic mammal species (Mongolian wild ass, Przewalski horse, Grey marmot, Bactrian camel and Dhole), most of which endangered or in the verge of extinction. Samples were pre-enriched in peptone water and 0.1ml were plated in Slanetz-Bartley agar without and with antibiotics (vancomycin-4mg/L, ampicillin-16mg/L, ciprofloxacin-4mg/L, gentamicin-125mg/L, streptomycin-1000mg/L, tetracycline-8mg/L, chloramphenicol-8mg/L). Susceptibility to 12 antibiotics was tested by disk diffusion method (CLSI). Species identification (*E. faecium*-Efm, *E. faecalis*-Efl), characterization of antibiotic resistance genes (tetM, tetL, tetO, tetK, tetS, ermB) and putative virulence genes (esp, hylEfm, acmEfm) were done by PCR. purK gene was amplified and sequenced for specific Efm-isolates.
Results: Enterococci were present in all samples, identified as Efm (14), Efl (3) and *Enterococcus* sp (3). Overall a high incidence of antibiotic resistance was detected: tetracycline-85% (17/20), minocyclin-45% (9/20), erythromycin-40% (8/20), ciprofloxacin-35% (7/20), High Level of Resistance (HLR) to gentamicin-15% (3/20), HLR-streptomycin-5% (1/20) and ampicillin-10% (2/20). Resistance to tetracyclines was associated to tetM+tetL (13 Efm) or tetM (3 Efl), while erythromycin resistance was linked to ermB (6 Efm). esp and acmEfm genes were detected in 3 Efl from a Przewalski horse and in all Efm, respectively. purK1 was identified in 2 Efm highly resistant to ampicillin and ciprofloxacin, from a Bactrian camel.
Conclusion: Even though inhabiting remote areas with extremely low human pressure, the critically endangered Silk Road fauna surprisingly harbours bacteria carrying antibiotic resistance and virulence determinants. Moreover, the unexpected detection of genetic traits commonly associated with worldwide nosocomial epidemic strains (purK1, ampicillin and ciprofloxacin resistance, acmEfm and esp) shows that drug resistance, far from limited to hospitals, may be spreading into the most remote areas of the globe.

O301 Spread of vancomycin-resistance among *Enterococcus faecalis*: the influence of pAD1 and Inc18-plasmids (1987–2005)

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Objectives: Vancomycin resistance (VR) is more often associated with *Enterococcus faecium* than *E. faecalis* (Efc). However, the spread of VREfc is of concern since vancomycin-resistant *Staphylococcus aureus* (VRSA) have mostly been isolated in patients co-colonized with Efc containing Inc18-like VanA plasmids. Plasmid diversity among VREfc was analyzed in order to better understand the scarce dissemination of VR within Efc and the potential transferability to other hosts.
Methods: 33 VREfc (28 vanA, 4 vanB, 1 vanG) representative of clonal outbreaks in 13 countries (Argentina, Australia, Brazil, Chile, Cyprus, Hungary, Italy, Pola nd, Portugal, Serbia, Spain, UK, and USA; 1987–2005) were analyzed. They correspond to 19 PFGE types/17 STs, mostly of CC2, CC9 and CC87. Van-Tns were typed by PCR and sequencing. Plasmid analysis included transferability assays, determination of size and plasmid content (PFGE-S1), comparison of EcoRI/ClaI-RFLP profiles of van-plasmids, identification of relaxases (rel), replication initiator proteins (rep), and 5 toxin-antitoxin systems (TA) by PCR. Results were confirmed by S1/I-Ceu-I-hybridization and sequencing.
Results: VREfc contained a variable number of plasmids (1–4/cell; 20–200kb) with variable sequences: rep [rep-pAD1 (91%), rep-Inc18 (58%), rep-pRE25 (42%), rep-pAMx1 (21%), rep-pAM373 (12%), rep-pTEF1 and rep-pHTB (9% each)], rel [rel-pAD1 (91%), rel-Inc18 (20%), rel-pAMx1 (36%), rel-pCF10 (24%), rel-pHTB (6%), rel-pRUM (3%)], and TA systems [par (70%), Axe-Txe and ω-ε-ζ (9% each)]. While vanB-Tn5382 and vanG were chromosomally located, vanA-Tn1546 was identified on plasmids (30–200kb), some types identified in different countries over years. Most vanA-plasmids hybridized with rel, rep and/or par genes from pAD1 in different combinations, and often with rep-Inc18. Plasmids from VREfc isolated besides vanA-*S. aureus* in Michigan-USA contained rel-Inc18 which was identical to those of pRE25. Differences in VanA plasmid size and rep/rel/TA content between wild types and transconjugants were frequent (50%).
Conclusions: Most vanA-plasmids from VREfc are mosaic derivatives of pheromone-responsive pAD1 and Inc18 plasmids. Both the pAD1 narrow host range and the frequent rearrangements observed during conjugation are consistent with the maintenance of pAD1-Tn1546 within the Efc species. The transferability of VREFc plasmids to *S. aureus* seems to be associated with the broad host conjugative module of Inc18 plasmids.

O302 Emergence of MRSA ST130 in humans

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Objective: *Staphylococcus aureus* ST130 was reported so far from mastitis in cattle in UK (1), also MRSA ST130 containing a new mec-determinant was described (2). Here we report the characterization of mecA negative MRSA ST130 isolates from infections in humans.
Methods: MRSA isolates ST130 were obtained from 6 patients treated in different German hospitals (5 wound infections needing surgical intervention, 1 colonization). Typing by means spa- typing und MLST, and microbroth assay for AST were performed as described previously (3). For detection of sets of resistance- and virulence associated genes the microarray platform array-mate "Staphtype" from Alere was used. Primers for the detection of an alternative mec gene were deduced from the whole genome nucleotide sequence of *S. aureus* LGA 251 ([2], Sanger centre databank).
Results: Of the isolates investigated 5 exhibited MLST ST130, one a new ST; spa- types were t843, t1736, and t1773. The isolates were phenotypically resistant to oxacillin and to oxacillin/sulbactam only. PCR for the alternative mec-gene known for MRSA ST130 from UK (2) was positive. The isolates were negative for sak, chp, and scn, and as expected positive for hla, untruncated hlb, and hld, they furthermore contained edinB, aur, slpA, slpB, slpE. From genes coding for surface and cell wall associated products the ica-operon, cap8, clfA, clfB, ebpS, fnbA, fnbB, sdrC were detected but not cna. The isolates were positive for a number of set determinants and negative for enterotoxin genes and tst, as well as for eta, and etb. agr-type was III.
Discussion: The alternative mec gene and the pattern of virulence associated genes widely correspond to *S. aureus*/MRSA ST130 from cattle in UK (1,2). Therefore transmission from animal sources and a zoonotic potential with respect to infections in humans seem likely. The lack of the innate immune evasion operon is typical for most of *S. aureus* isolates of primarily animal origin, probably other enzymes (aur) take over this function. For the interdisciplinary surveillance of further spread proper AST and confirmation by PCR for the alternative mec gene are important.

O303 High prevalence of EMRSA-15 in Portuguese public buses: a worrisome finding

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Objectives: The nosocomial prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) in Portugal remains one of the highest in Europe and is currently over 50%. Transmission of *S. aureus*, including MRSA, occurs principally by direct human-to-human skin contact. However, *S. aureus* can survive for long periods on inanimate objects, which may represent an important reservoir for dissemination as well. The aim of the present study was to explore the role of hand-touch surfaces in Portuguese public buses as a reservoir of MRSA and provide insights into the genetic background of the isolates.
Methods: Between May 2009 and February 2010, handrails of 85 public urban buses circulating in Oporto, Portugal, were screened for the occurrence of MRSA. All isolates were tested for antimicrobial susceptibility and for the presence of mecA and Panton-Valentine leukocidin (PVL) genes. Moreover, the isolates were characterized by pulsed-field gel electrophoresis (PFGE), staphylococcal cassette

chromosome (SCC) mec typing, spa typing, and multilocus sequence typing (MLST).
Results: Twenty-two (26%) out of the 85 buses showed MRSA contamination. The molecular characterization of a total of 55 MRSA clustered the isolates into three clonal types. Nevertheless, the overwhelming majority (n = 50; 91%) of the isolates belonged to a single non-multidrug resistant clone (PFGE A, spa types t747, t032, t025 or t020, ST22, SCCmec type IVh). This clone exhibits the characteristics of the pandemic EMRSA-15, currently the major lineage circulating in Portuguese hospitals, namely in the Oporto region. Two additional clones were found but in much lower numbers: (i) PFGE B, ST5, spa type t002, SCCmec IVa (n = 3), and (ii) PFGE C, spa type t008, ST8, SCCmec IVa (n = 2). None of the 55 isolates was PVL positive.
Conclusions: Public buses in Oporto are an important reservoir of MRSA of nosocomial origin, providing evidence that the major hospital-associated MRSA clone in Portugal is escaping from the primary ecological niche of hospitals to the community environment. Infection control measures are urgently warranted to limit the spread of EMRSA-15 to the general population and avoid a massive increase of MRSA in the Portuguese community, which so far remained low.

O304 Epidemiology of invasive group A streptococci in Canada from 2000–2009

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Objective: The objective of this report is to present invasive Group A streptococcal (iGAS) M typing, antimicrobial susceptibility, iGAS specimen source and ages affected by iGAS disease from isolates collected in Canada from 2000–2009.
Methods: From 2000–2009, The National Centre for *Streptococcus* received iGAS isolates for analysis. This analysis included serological M typing (2000-August 2006) and emm typing (September 2006 to 2009) and patient. Antimicrobial susceptibility was performed using reference disk diffusion methods as per CLSI.
Results: Over the 10 year period, 9725 iGAS isolates were assayed. The age distribution of iGAS cases was 14.3% (<1–15 years), 38.5% (25–45 years), 17.1% (46–59 years), and 30.1% (60–110 years). The ten most prevalent M types were M1 (2046 isolates, 21%), M3 (700 isolates, 7.2%), M12 (624 isolates, 6.4%), M59 (547 isolates, 5.6%), M4 (416 isolates, 4.3%), M89 (379 isolates, 3.9%), M28 (347 isolates, 3.6%), M11 (257 isolates, 2.6%), M5 (245 isolates, 2.5%), and M77 (244 isolates, 2.5%). Nontypeable isolates prior to September 2006 comprised 11.6% (1126) of isolates. The three provinces submitting the highest number of isolates were Ontario (38.1%, 3704 isolates), Alberta (19.9%, 1938 isolates), and British Columbia (15.5%, 1508 isolates). Together, these three provinces made up 63% of the Canadian population in 2009. The four most common M types from blood, brain, and cerebrospinal fluid were M1 (13.7%), M3 (5.4%), M12 (4.3%), and M59 (3.4%). Antibiotic susceptibility testing showed 11.5% resistance to erythromycin and 7.9% had inducible clindamycin resistance (ICR). The highest resistance rates were seen in M12 isolates (10.4% for erythromycin and 5.3% ICR). M1 isolates showed a clear seasonality trend as did M12 isolates. M3 showed regular seasonal variation except between May 2003-December 2004. Overall, there was regular seasonal variation noted of iGAS disease except for the same period as seen for M3.
Conclusions: From 2000–2009, ten different M types constituted 60% of the iGAS isolates M/emm typed in Canada. M1 continues to be the most common circulating M type. Since our last report, there has been an increase in M59 iGAS isolates, accounted for by an epidemic that began in January 2006. An increase in M28 iGAS infections from January 2006 to 2009 compared to 2000–2004 was noted as well. The highest level of erythromycin and ICR was seen in the M12 strains.

O305 Prevalence and molecular characteristics of MRSA colonisation among adult patients visiting emergency department in a medical centre in northern Taiwan

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Objective: To understand the carriage rate of methicillin-resistant *Staphylococcus aureus* (MRSA) among the adult patients visiting emergency department (ED) in a tertiary care hospital in northern Taiwan.
Methods: From May 21 to August 12, 2009, a total of 502 adult patients visiting ED were surveyed for nasal carriage of MRSA. A questionnaire regarding the risk factors for MRSA acquisition was obtained. All MRSA isolates were further characterized.
Results: The overall prevalence of *S. aureus* and MRSA nasal carriage among the patients was 17.3% and 3.8%, respectively. MRSA carriage rate of the patients with risk factors (13/219, 5.9%) was significantly higher than that of those without risk factors (6/283, 2.1%). Patients with urinary complaints, diabetes mellitus (DM), chronic kidney disease and usage of catheters or tubes were significantly associated with MRSA colonization. After multiple logistic regression analysis, only current usage of catheters or tubes was the independent predictor for MRSA nasal colonization. Of the 19 MRSA isolates for molecular analysis, a total of 6 pulsed-field gel electrophoresis (PFGE) patterns were identified and most isolates were clustered in three patterns (A, 21%; C, 32% and D, 26%). Most MRSA isolates belonged to one of two lineages characterized as sequence type 239/SCCmec III/PVL-negative (32%) and ST59/SCCmec IV or VT/PVL-positive (58%). The latter linage, accounting for 83% of 6 isolates from patients without risk factors, is a community-associated (CA) clone in Taiwan, while the former linage is among healthcare-associated clones.
Conclusion: In 2009, 3.8% of adult patients visiting ED of a Taiwanese medical center were colonized with MRSA in nares; the rate was even higher for those with risk factors. Most isolates were CA-MRSA, particularly those from patients.

O306 A case-control study comparing infections due *Staphylococcus aureus* with Panton-Valentine leucocidin (PVL) to those without PVL

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Objective: *Staphylococcus aureus* toxin Panton Valentine Leucocidin (PVL) has been associated with invasive soft tissue infections and necrotizing pneumonia, but its causative role has been questioned. Thus, we aimed at comparing clinical and outcome data of PVL positive (PVL+) and PVL negative (PVL-) *Staphylococcus aureus* invasive infections irrespective of methicillin resistance.
Methods: A retrospective case control study of all PVL+ and a random selection with a 1:1 ratio of contemporaneous PVL− invasive infections was performed in a tertiary referral centre. PVL detection was performed systematically on all methicillin resistant strains and on only invasive infections for methicillin susceptible strains.
Results: From September 2007 to January 2009, 141 PVL+ infections were identified and 148 PVL− were randomly selected. In the PVL+ group 62 (44%) isolates were methicillin resistant (MRSA) compared to 56 (38%, $p = 0.29$) in the PVL− group. PVL+ were significantly younger (median 35 [range 15–86] vs 59 [16–99], $p < 0.01$), more frequently indigenous (34% vs 12%, $p < 0.01$) and intravenous drug users (16% vs 7%, $p = 0.01$) compared to PVL-. Furthermore their infection was more commonly community acquired (62% vs 24%, $p < 0.01$). However, PVL+ less frequently had solid tumours (1% vs 10%, $p < 0.01$) or haematological malignancy (0% vs 4%, $p = 0.02$) or been hospitalised in the past year (36% vs 62%, $p < 0.01$). Skin/soft tissue infection accounted for 82% of PVL+ infection compared to 64% for the PVL− group ($p < 0.01$) whilst bacteraemia was less frequent (9% vs 25%, $p < 0.01$). Need for surgery was more common in PVL+ (73% vs 45%, $p < 0.01$).

PVL+ isolates demonstrated lower resistance rates for clindamycin (12% versus 27%, $p < 0.01$) and erythromycin (14% versus 28%, $p < 0.01$). Clindamycin or linezolid was more frequently added to PVL+ infection (31% vs 13%, $p < 0.01$), however the benefit of this strategy was not evaluable as these antibiotics were probably given to the sicker patients. Although 7 and 30-day mortality were similar between groups, the length of stay was shorter in PVL+ compared to PVL– (median 5 [0–81] vs 14 [0–638], $p < 0.01$).

Conclusion: In our region, more than half of PVL+ S aureus are methicillin susceptible. PVL is strongly associated with skin/soft tissue infections in young, indigenous and/or intravenous drug users and is a burden to our health system as 73% required surgical intervention.

O307 Confounding in determining attributable mortality of methicillin-resistant *Staphylococcus aureus* bacteraemia: a meta-analytical approach

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Objectives: It has recently been estimated that, each year, 5400 persons succumb due to nosocomial MRSA infections in Europe. However, quantifying attributable mortality is difficult as the causal linkage between infection and outcome can be confounded by multiple determinants. In this meta-analysis we aimed to quantify the mortality risk associated with methicillin-resistant [MRSA] vs. methicillin-susceptible *S. aureus* [MSSA] bacteraemia, and to determine the effect of confounding factors hereon.

Methods: PubMed was searched from 1975 through January 2010 using the terms 'aureus' and('bacteraemia' or 'blood stream infection') and('outcome' or 'mortality' or 'death').

Unadjusted odds ratios [OR] and 95% confidence intervals [CI] were calculated on the basis of the primary data reported in the study. We pooled adjusted ORs for those studies that reported adjusted ORs and did subgroup analyses of studies that corrected for key confounding variables (i.e., adequacy of treatment, comorbidity and severity of underlying illness (or length of stay before onset as a proxy)). The attributable mortality risk [AMR], providing information about the excess risk of dying in patients with MRSA compared with patients with MSSA bacteraemia, was calculated with the formula: AMR = p0(OR*(p0/1 – p0)/1 + OR*(p0/1 – p0)), in which p0 = mortality risk for MSSA bacteraemia. AMR for unadjusted and adjusted ORs are provided.

Results: 73 studies were selected: 42 retrospective en 25 prospective cohort studies and 6 case-control studies, comparing MRSA and MSSA related mortality in 6310 and 11812 patients, respectively. The pooled unadjusted odds ratio [OR] was 2.05 (95% CI 1.82–2.30) (I^2 = 39%, number of studies [n] = 73 (18122 patients), with an AMR of 13%. Adjustment for confounders was available for 35 studies. The pooled adjusted OR was 1.66 (95% CI 1.45–1.91) (I^2 = 34%, n = 35 (11383 patients)), yielding an AMR of 9%. Adjustment for the key confounding variables yielded a pooled adjusted OR of 1.25 (95% CI 0.91–1.73) (I^2 = 67%, n = 7 (2774 patients)), corresponding with an AMR of 4%.

Conclusion: The results of our meta-analysis demonstrate the discrepancy between unadjusted and adjusted study results, yielding a decreasing association between methicillin resistance and mortality after adjustment for treatment, comorbidity and severity of illness. Adjustment for these variables is mandatory when attempting to quantify attributable mortality of antibiotic-resistant pathogens.

Table. Subgroup analysis of adjusted results comparing outcome of MRSA versus MSSA bacteraemia

Subgroup	*Nr studies*	*Nr patients*	*Pooled unadjusted OR (95% CI)*	*AMR, % (95% CI)*	*I^2 (%)#*	*Pooled adjusted OR (95% CI)*	*AMR, % (95% CI)*
Adjusted for any variable and adjusted OR provided	35	11383	2.24 (1.94 to 2.59)	14.8 (11.7 to 18.0)	34	1.66 (1.45 to 1.91)	8.6 (6.1 to 11.4)
Adjusted for any of key confounding variables[1]	29	9523	2.22 (1.88 to 2.62)	15.1 (11.5 to 19.0)	47	1.63 (1.38 to 1.91)	8.6 (5.4 to 11.9)
Adjusted for 1 key confounding variable[1]	5	2629	2.19 (1.78 to 2.70)	14.4 (10.1 to 19.2)	0	1.61 (1.17 to 2.20)	8.1 (2.4 to 14.5)
Adjusted for treatment	2	1964	2.05 (1.62 to 2.59)	12.9 (8.2 to 18.1)	0	1.28 (0.86 to 1.90)	3.9 (-2.1 to 11.3)
Adjusted for severity of illness[2]	1	521	3.12 (1.82 to 5.35)	23.0 (10.8 to 36.5)	NA	2.60 (1.40 to 4.90)	18.7 (5.7 to 34.3)
Adjusted for comorbidity	2	144	2.21 (0.97 to 5.07)	13.7 (-0.4 to 33.2)	0	1.97 (0.76 to 5.13)	11.3 (-3.4 to 33.5)
Adjusted for 2 key confounding variables[1]	17	4120	2.29 (1.81 to 2.90)	15.3 (10.3 to 20.8)	49	1.73 (1.39 to 2.16)	9.4 (5.3 to 14.0)
Adjusted for treatment and severity of illness[2]	10	2303	2.94 (2.39 to 3.62)	19.2 (14.8 to 24.1)	0	2.04 (1.65 to 2.53)	11.6 (7.6 to 16.0)
Adjusted for treatment and comorbidity	2	688	2.03 (1.40 to 2.93)	12.1 (5.2 to 20.2)	0	1.38 (0.92 to 2.07)	4.9 (-1.1 to 12.5)
Adjusted for comorbidity and severity of illness[2]	5	2062	1.76 (1.04 to 2.97)	11.2 (0.7 to 23.7)	73	1.34 (0.78 to 2.31)	5.5 (-4.0 to 17.5)
Adjusted for 3 key confounding variables[1]	7	2774	1.96 (1.29 to 2.98)	13.5 (4.6 to 23.6)	67	1.25 (0.91 to 1.73)	4.3 (-1.4 to 9.8)

Note: Nr – number; MRSA – methicillin-resistant *S. aureus*; MSSA – methicillin-susceptible *S. aureus*; OR – odds ratio; 95% CI – 95% confidence interval; AMR – attributable mortality risk; I^2 - I-square. # I^2 describes the percentage of variability in point estimates that is attributable to statistical heterogeneity rather than to sampling error, defined to be low (≤ 25%), moderate (25%–75%), or high (≥ 75%)[3]. [1] key confounding variables: treatment, comorbidity, severity of illness[2] severity of illness at onset of bacteraemia and/or length of stay before the onset of bacteraemia as a surrogate marker for severity of illness.

Paediatric infections: preclinical and clinical data

O308 Viral respiratory tract infection in preschool children: the burden and impact of co-infection

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Objectives: Respiratory tract infection is a frequent problem in childhood. Contemporary studies are required given the identification of emerging viral pathogens and improved diagnostics. The impact of viral co-infection remains uncertain. We undertook to describe the virology of symptomatic respiratory tract infection in preschool children and to determine the burden and impact of viral co-infection.

Methods: An observational study assessing influenza vaccine effectiveness was conducted in Perth, Australia (winter 2008/2009). Preschool children (6 months-5 years) presenting with fever (>37.5ºC) and acute respiratory symptoms were eligible. Demographics, risk factors, clinical features, therapies and outcomes were recorded by parental questionnaire. Antigen detection, PCR and viral culture were performed on per-nasal samples.

Results: Per-nasal samples were available from 919/944 subjects. Frequent symptoms included rhinorrhoea (802; 87.3%), cough (795; 86.5%), poor feeding (687; 74.7%) and irritability (608; 66.2%).

Respiratory viruses were identified in 711 (77.4%) with outcome data available for 566 children. Hospital admission was required in 144/566 (25.6%). Rhinovirus (n = 239), RSV (n = 210) and influenza virus (n = 179) were most frequently detected followed by human bocavirus (n = 79), parainfluenza viruses (n = 73), coronavirus (n = 64), adenovirus (n = 49) and human metapneumovirus (n = 20).

Influenza infection was more frequently associated with hospital admission compared with other viruses (influenza 49/151; 32.45% vs. RSV 27/161; 16.8% vs. other 46/266; 17.3%, both analyses $p < 0.002$). The rate of hospitalisation with influenza in 2008 was not significantly different than in 2009: 29/73 (39.7%) in 2008 compared with 20/78 (25.6%) in 2009.

Viral co-infection was observed in 197/711 (27.70%) with rhinovirus/RSV the most frequent co-infection: 41/197; 20.8%. Clinical features (mean peak temperature; frequency of symptoms) and outcomes (hospitalisation; antibiotic prescription) were not significantly different between co-infected and non co-infected children. Subgroup analysis of children with influenza or RSV didn't demonstrate any significant differences between co-infected and non-coinfected children.

Conclusions: This study demonstrates the frequent identification of emerging pathogens including coronaviruses, human bocavirusand human metapneumovirus. Influenza remains a significant pathogen in young children. Co-infection has little impact on clinical features or outcome.

O309 Influence of the respiratory virus species and multiple viral infections on bronchiolitis severity in hospitalised infants

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Objectives: Bronchiolitis is an important manifestation of viral respiratory tract infections (RTIs). However, the influence of the virus respiratory species and multiple viral infections on bronchiolitis severity is poorly understood because of the limited scope and throughput of conventional viral detection methods.

Methods: We prospectively performed a large viral screening by a RT-PCR microarray system to detect single and multiple RTIs in 138 infants aged less than 12 months and hospitalized for bronchiolitis in Northern East of France from October 2007 to September 2008.

Results: The RT-PCR microarray system detected viruses in a higher proportion of samples (91%) than did classical immunofluorescence and viral culture isolation assays (70%) ($P < 10–3$). The RT-PCR microarray system identified 85 multiple infections cases whereas none was detected

by the classical techniques. The most common associations were: human respiratory syncytial virus (hRSV) and human bocavirus (32%), hRSV and adenovirus (29%), hRSV and parainfluenza 3 (24%) and adenovirus and human bocavirus (13%). Infants infected by hRSV had longer duration of hospitalization in comparison with infants infected by other viruses ($P = 0.006$), and our multivariate analysis adjusted on age indicated an increase in hospitalization length of 1.7 ± 0.6 days ($P = 0.04$). No other virus or association of viruses appeared to have a significant impact on the bronchiolitis severity parameters ($P > 0.12$).
Conclusion: Our findings indicated that only hRSV species could cause more severe bronchiolitis. Given the large panel of multiple viral infections detected here, larger-scale studies will be necessary to assess whether specific viral associations could confer an elevated risk of severe bronchiolitis.

O310 Group C rotavirus infection and VP6 diversity in children, Brazil 2007–2010: monitoring a potential impact of rotavirus vaccination programme

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Objectives: Group C rotavirus (GpCRV) has a worldwide distribution, increasingly recognised as a significant cause of diarrhoea in children, however their epidemiology and ecology are still unclear. It seems important to improve the GpCRV laboratory surveillance system, especially after 2006 when the RIX4414 rotavirus vaccine was included in Brazilian Immunization Program, preventing severe rotavirus gastroenteritis, and inducing significant reduction in the frequency of Group A rotavirus (GpARV) detection in children with gastroenteritis. Moreover, an evidence for a possible zoonotic role of GpCRV has been recently postulated for Brazilian children strains. The aim of this work was to monitor GpCRV in children $\leqslant$15 years with acute gastroenteritis during 2007–2010 national Brazilian rotavirus surveillance, and perform the molecular characterization of the major VP6 capsid protein.
Methods: A total of 3019 faecal samples were first screened for GpARV. A total of 2205 GpARV ELISA negative samples were further tested for the presence of GpCRV by SDS-PAGE, electronic microscopy, and RT-PCR for the VP6 gene. The genetic diversity of GpCRV was carried out by sequencing of VP6 gene.
Results: GpARV and GpCRV infection were detected in 27.0% (814/3019), and 0.3% (8/3019), respectively. GpCRV detection rate increased from 0.2% (1/422) in 2007 to 1.0% (7/708) in 2008, and no GpCRV cases were detected in 2009 and 2010. The phylogenetic analysis indicated that the strains belonged to the human lineage, were related to each other, since they shared 97–100% amino acid identities in the VP6 gene. None of the study sequences was closely related to animal GpCRV strains. GpCRV Brazilian strains showed a genetic relationship with GpCRV strains from Japan, especially from Yokohama city isolated in 2008 and 2009.
Conclusions: This study adds further proof that GpCRV is a minor cause of acute childhood gastroenteritis in Brazil, and does not suggest that GpCRV may be assume epidemiological importance in the future, even after the GpARV vaccine introduction. In particular, the molecular analyses of GpCRV samples in this study do not support the zoonotic hypothesis. The relationship between Brazilian and Japanese GpCRV strains may be a reflect of the significant migration flow. Currently, Brazil is the country with the largest number of Japanese outside Japan.

O311 Congenital CMV infection: clinical outcome according to the serological status of the mother

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Objectives: To determine the type and incidence of sequelae found in children with a congenital cytomegalovirus infection (cCMV) in relation to the serological status of the mother during pregnancy.
Methods: In a prospective 13-year study, 18219 mother/infants pairs were included. In the mother a serological screening was performed consisting in the detection of CMV IgG and IgM antibodies at the first prenatal visit and at birth. In the neonate CMV urine culture was performed to diagnose congenital infection. Late trimester abortion or death in utero were investigated in order to diagnose congenital CMV infection. Live born infants were evaluated for the appearance of auditive and neurological sequelae.
Results: 118 consecutive cases of cCMV were detected (0.64%). 54 (46%) were caused by a primary maternal infection, 33 (28%) by a non primary, and in 31 (26%) the type of maternal infection could not be determined. Of the 118 cases, 111 were live born, 1 resulted in a death in utero and in 6 other pregnancies a termination of pregnancy (TOP) was performed. From the 54 cases of primary infection, 4 TOP were performed from whom 1 with signs of congenital cytomegalovirus on ultrasound (US). From the 50 live born children after primary infection, 1 was born with symptomatic disease, 3 other children developed psychomotoric retardation all or not with hearing deficit, and in 4 children isolated hearing loss was found. In the non primary infection group 1 TOP was performed because of hydrocephalus, 1 infection resulted in a death in utero, 1child suffered from severe mental retardation and 1 child had bilateral hearing loss. In the unclassified infection group, 1 TOP was performed because of US abnormalities, 1 child suffered from severe psychomotoric retardation and 7 demonstrated isolated hearing loss.
Conclusions: In our population the incidence of cCMV is 0.64%. Sequelae are found in at least 16% of the cCMV in 7% the sequelae were considered severe. Severe sequelae are seen in primary as well as in non primary cCMV.

O312 DEVANI UK clinical screening study for maternal carriage of *Streptococcus agalactiae*

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Objectives: *Streptococcus agalactiae* (Group B *Streptococcus*, GBS) is a leading cause of neonatal sepsis, meningitis & pneumonia affecting 1–2 newborns/1000 live births in Europe. The DEVANI (DEsign of a Vaccine Against Neonatal Infections) programme funded through the European Commission Seventh Framework and coordinated by Novartis Vaccines was launched on 1 January 2008 (www.devaniproject.org). The main objective of this 3.5 year project is to assess European GBS epidemiology in order to facilitate the design of a new vaccine capable of conferring broad coverage to immunize neonates against GBS infections through a durable maternal immune response. A key component of the programme was to undertake a multicentre surveillance study of maternal GBS colonisation, maternal GBS antibody levels and neonatal GBS infection in the UK.
Methods: Four UK centres from different geographical locations participated. Maternal vaginal/rectal swabs and serum samples were taken between 34–37 weeks gestation and processed using a standardised microbiological screening protocol. Samples from neonatal cases (early onset disease; EOD & late onset disease; LOD) were processed using local procedures. GBS isolates were characterised at the HPAMSD using standardised serological and molecular typing methods based on the Strep-B-Latex agglutination kit & a novel multiplex PCR assay which detects all ten GBS capsular polysaccharide types (Ia to IX). Maternal sera from colonised & non-colonised women together with maternal and neonatal GBS isolates were submitted to Rome for analysis.
Results: A total of 650 pregnant women were recruited between 34–37 weeks gestation, from June 2009 to April 2010, of which 170 were colonised with GBS (incidence rate ~26%). GBS isolates from 16 neonatal cases (13 EOD & 3 LOD) together with corresponding maternal sera were also obtained. The most common GBS capsular types were III (33%), Ia (26%) & V (17%), respectively.
Conclusion: In contrast to most EU countries, the UK does not offer routine GBS antenatal screening however, results from the clinical studies that we have undertaken should raise more awareness and support routine GBS screening for the prevention of early-onset neonatal GBS

disease in the UK. The maternal incidence rate of 26% is consistent with the rates quoted in other European countries. The final outcomes of the DEVANI programme should significantly contribute to the reduction in neonatal GBS disease incidence in Europe and beyond.

O313 Retrospective study of *Staphylococcus aureus* bacteraemia in an Irish neonatal intensive care unit: epidemiology, risk factors and management

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Objectives: The Rotunda Hospital, Dublin is a maternity hospital with a 39-bedded tertiary level neonatal intensive care unit (NICU). We examined the prevalence and epidemiology of *S. aureus* bacteraemia (SAB) in our NICU, the risk factors for, and management of this infection over a 78-month period (1st January 2004–30th June 2010).
Methods: The laboratory information system was used to identify all neonates with SAB in this time period. Case notes were reviewed for information on demographics, admission days prior to bacteraemia, whether community-acquired (CA)/hospital-acquired (HA) or healthcare-associated (HCA), focus of infection, risk factors, management and outcome.
Results: We identified 54 episodes of SAB in 54 neonates in the 78-month time period. 16/54 (30%) episodes occurred in infants of multiple pregnancies. All *S. aureus* isolates were meticillin-sensitive. HA-SAB accounted for the majority: 48/54 (89%) followed by CA-SAB in 5/54 (9%) and HCA-SAB in 1/54 (2%). The age range at onset of bacteraemia was 2–65 days (average 13 days).
The focus of infection was unknown in 23/54 (43%), peripheral venous catheter-related in 15/54 (28%), central venous catheter-related in 10/54 (19%), skin infection in 4/54 (7%), bone in 1/69 (1.5%) and urinary tract in 1/69 (1.5%).
Many neonates had more than one risk factor for SAB. The most common risk factors included: prematurity in 43/54 (80%), previous antibiotics in 38/54 (70%), peripheral venous catheter (PVC) in 38/54 (70%), central venous catheter (CVC) in 33/54 (61%), mechanical ventilation in 30/54 (56%), and blood transfusion in 18/54 (33%).
Treatment was primarily with intravenous flucloxacillin in 51/54 (94%) neonates. Sterile blood cultures following commencement of antibiotics were documented in 36/54 (67%). No patient experienced a relapse of infection but 10/54 (19%) had complications: osteomyelitis in three, concurrent osteomyelitis and meningitis in one, abscess formation in five. One baby born at 25 weeks gestation with a CVC-related SAB died from extreme prematurity and sepsis. 7 out of 10 (70%) infants with complications had intravascular catheter-related infections.
Conclusions: There were no cases of SAB caused by meticillin-resistant *S. aureus* in the study period. The majority were due to HA-SAB and all were classified as late-onset sepsis. The focus of infection was line-related in 47% of cases.

O314 The prevalence of *Helicobacter pylori* cagA, vacA, iceA, babA genotypes in Polish children with gastroduodenal diseases: impact on histology

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Objectives: Determination of the occurence of selected virulence-associated genes, such as: cagA, vacA, iceA, and babA gene in *H. pylori* clinical isolates and to characterize their association with histopatological changes in the gastric mucosa.
Methods: The study was performed on 137 *H. pylori* strains collected in years 2006–2009. The strains were isolated from antral biopsy specimens of children, aged 2–18 years, diagnosed because of dyspeptic complaints. The diagnosis in children was established on the basis of endoscopic, histopathological and clinical findings. Two groups of patients were evaluated: children with chronic gastritis (n = 114) and with peptic or duodenal ulcers (n = 23). The *H. pylori* infection was was determined by bacteriological and histological examination. The genes encoding specific virulence factors, such as: vacA (s1/s2, m1/m2), allelic variants of iceA gene (iceA1, iceA2), babA were determined by polymerase chain reaction.
Results: The cagA gene was detected in 86/137 (62,7%) of *H. pylori* isolates. The presence of cagA gene was significantly associated with duodenal ulcer and chronic gastritis ($p < 0,05$). Distribution of vacA genotype was as follows: vacAs1/m1 was identified in 46/137 (33,6%) of strains, vacAs1/m2 in 42/137 (30,6%) and vacAs2/m2 in 49/137 (35,8%), respectively. IceA1 gene was present in 59/137 (43,1%) and iceA2 in 31/137 (22,6%) of the samples, respectively. In 41/137 clinical isolates neither iceA1 nor iceA2, alleles were detected. Six *H. pylori* strains isolated from children with chronic gastritis possessed both iceA alleles 6/137 (4,38%). The babA gene was detected in 31/137 (22,6%) strains. The presence of triple *H. pylori* genotype vacAs1/cagA+/iceA1+/babA– was significantly associated with ulcers (17,4%, $p = 0,002$). The predominant genotype in children with chronic gastritis was vacAs2/cagA-/iceA1+/babA- ($21/114, p = 0,00762$). The degree of chronic inflammation significantly correlated with cagA-positive status and the presence of vacA s1/m1 alleles ($p < 0,05$) but not with the *H. pylori* density, activity nor atrophy. A significant correlation was found between the presence of babA and iceA2 genes and degree of chronic inflammation ($p = 0,046$).
Conclusion: CagA, vacAs1 and iceA1 genes are predominant in children with ulcers and cagA/vacAs1 genotype is significantly associated with chronic inflammation but not with other histopathological parameters. The role of babA and iceA *H. pylori* genes in children needs further studies.

O315 Outcome of prenatal toxoplasmosis: retrospective analysis for effectiveness of pyrimethamine/sulfadiazine therapy during pregnancy

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Objectives: Whereas an infection with the protozoan parasite *Toxoplasma gondii* mostly is asymptomatic in immunocompetent patients, primary infection during pregnancy might be health- or even life-threatening to the foetus. Depending on the country, different treatment strategies exist for the prevention of the parasite's transmission from the mother to the foetus or/and for the prevention of clinical manifestations in the child. The German scheme of toxoplasmosis therapy during pregnancy foresees spiramycin until 16th week of pregnancy (WOP) followed by at least four weeks combination therapy with pyrimethamine, sulfadiazine and folinic acid. Infections occurring at later WOPs are directly entering the combination scheme. In other countries such as France, only spiramycin is given unless infection of the foetus is proven. The objective of this study was to evaluate the effectiveness of the German therapeutical scheme of primary *T. gondii* infection during pregnancy.
Methods: Data from 685 women, who showed a serological constellation for primary infection during pregnancy were retrospectively analysed. Thereby, (i) timing of initiation and type of toxoplasmosis therapy, (ii) transmission rates to the foetus, and (iii) the ratio of infected and clinically symptomatic children in utero respectively neonates was examined.
Results: As expected, the transmission rate to the foetus increased with the duration of pregnancy. In our cohort, there seems to be a trend towards lower transmission rates (4.8%) and clinical manifestations in the neonates (1.5%) in comparison to data published by other groups, indicating that the German scheme of therapy seems to be effective. Most important, an interval of more than 4–8 weeks between time of infection and initiation of combination therapy seems to have a negative effect on the clinical outcome of the newborn.
Conclusion: Rapid initiation of combination therapy based on pyrimethamine and sulfadiazine and folinic acid after *T. gondii* infection during pregnancy seems to be beneficial for the child. Optimal screening should therefore be performed at 4 weeks intervals. Prospective studies are needed to confirm our findings.

O316 Clinical presentation and management of deep neck infections in an Italian cohort of children and adolescents

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Objectives: Deep neck infections (DNIs), although relatively rare in children, can cause significant morbidity and potentially life-threatening complications. We analysed clinical and microbiological characteristics of DNIs, including their relationships with age, in a paediatric case series.
Methods: We retrospectively reviewed the medical records of children and adolescents admitted to Regina Margherita Children's Hospital, Turin, Italy, for DNI between 2006 and 2010. DNIs were classified as retropharyngeal, parapharyngeal, peritonsillar and mixed (with parapharyngeal and retropharyngeal involvement), according to the site of infection. Diagnosis of DNI was based on clinical and/or imaging findings on CT scan.
Results: Thirty-one patients (21 males and 10 females, mean age 7.4 years, range 0.3–15.4) were studied: clinical and management details are listed in Table 1. Males were preponderant in retropharyngeal and parapharyngeal abscesses (80% and 100% respectively).
All children initially received intravenous antibiotics and then switched to oral treatment, for a mean total duration of 25.4 days; the longest duration was observed in retropharyngeal abscesses (31.6 days). 48.4% of patients (mostly with a retropharyngeal abscess) underwent surgery with incision and drainage or needle aspiration. No significant difference in length of hospitalization between patients who underwent surgery and those who did not was observed (22 vs 23 days).
Severe complications occurred in 4 patients: two mediastinitis, one sepsis and one abscess rupture. No patient had signs of recurrence of DNI at the latest follow-up visit.
Microbiological cultures from infected sites yielded *Streptococcus* spp. in 5 cases, mixed flora (including anaerobes and aerobes) in 4 and *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Gemella morbillorum* in one case each. Three cultures showed no growth.
Conclusions: Our study confirms that clinical presentation of DNIs in children may be unspecific and may overlap with other common paediatric diseases. A significant relationship between age and site of infection was not observed, although 60% of retropharyngeal infections involved children aged less than 4 years. Considering DNIs aetiology in children, a prolonged antimicrobial therapy with a spectrum of activity including aerobic and anaerobic bacteria is mandatory. Surgery is often required to prevent life-threatening complications.

Table 1. Clinical characteristics and management of patients with DNIs

	Retropharyngeal (*n* = 10)	Parapharyngeal (*n*= 5)	Peritonsillar (*n*= 14)	Mixed (*n*= 2)	Total (*n*= 31)
Mean age, years (range)	6.3 (0.3-15.4)	6.4 (3.9-11.1)	8.7 (2.8-14.5)	6.6 (3.2-10.1)	7.4 (0.3-15.4)
Mean hospital stay (days)	21.1	20.0	10.9	27.5	15.7
Fever, *n* (%)	10 (100)	5 (100)	14 (100)	2 (100)	31 (100)
Sore throat, *n* (%)	8 (80)	4 (80)	13 (92,8)	1 (50)	26 (83.8)
Neck pain, *n* (%)	10 (100)	5 (100)	12 (85.7)	2 (100)	29 (93.5)
Neck swelling, *n* (%)	10 (100)	5 (100)	11 (78.6)	2 (100)	28 (90.3)
Surgical treatment, *n* (%)	8 (80)	1 (20)	4 (28.6)	2 (100)	15 (48.4)

O317 Improving clinical quality and patient safety in cystic fibrosis paediatric patients. First multidisciplinary clinical audit at a large district hospital of northwest England

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Background: Cystic fibrosis (CF) is a multisystem disorder with pulmonary disease the leading cause of morbidity and mortality. Royal Blackburn hospital in northwest England has a large paediatric CFunit. Preliminary to proposing an integrated carepathway for CF management – a multidisciplinary clinical audit was undertaken. Standards were recommendations of cystic fibrosis trust consensus (CFTC) [www.cftrust.org.uk].
Methods: Between Jan–Dec 2008, clinical data and laboratory database on 24 CF patients was audited. Information on demographics, screening, testing protocols, reporting and management of these patients was obtained and compared against guidance from CFTC. The data specific to *Pseudomonas aeruginosa* [PSA], MRSA and *Burkholderia cepacia* complex [Bcc] is presented here.
Results: 24 paediatric patients aged 1–17yrs [male – 37.5% and female 62.5%] were included in this audit. Data on PSA, MRSA and BCC is presented here. 33 organisms were isolated [PSA – 36% (12/33); MRSA – 9% (3/33) and BCC – 6% (2/33)]. Respiratory samples must be screened every 2-months – a compliance of 71% (17/24) was observed. All new isolated of BCC and PSA must be sent to a reference laboratory for genotyping – 100% compliance with BCC and 100% non-compliance with PSA was noted.
Absolute compliance was noted for use of a combination of nebulised colistin and oral ciprofloxacin for 3-weeks for new isolates of PSA. The standard has no firm consensus on screening from non-respiratory sites or eradication of MRSA. Compliance to screening of non-respiratory sites [nose and groin] as per local policy remains variable; however cough swabs and/or sputum are collected on every visit [100% compliance]. IV teicoplanin or vancomycin is used locally for eradication.
62.5% compliance to a turn-around-time of 72-hours was achieved while the average time to reporting of samples was 77.6-hours. Significant non compliance was observed in local standard operating procedures [SOP] for processing of samples from cystic patients [to be presented].
Conclusions: CF is one of UK's most common life-threatening inherited diseases. A multi-disciplinary team initiated integrated care pathway for enhancing clinical quality and patient safety was envisaged. Results from this audit have been used to draw up an action plan. Significant non-compliance were observed with laboratory SOPs; screening of non-respiratory sites for MRSA; referring new PSA isolates for genotyping. Details of result to be presented.

Visceral leishmaniasis: an endemic disease with global impact

S322-S324 Visceral leishmaniasis: an endemic disease with global impact

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Visceral Leishmaniasis (VL) is the second most common protozoal infection after malaria worldwide and affects approximately 2 million people per year predominantly in sub-Saharan Africa and the Indian subcontinent. Co-infection with HIV has become a major public health threat in endemic areas. Therapy has been traditionally prolonged, requiring affected individuals to remain in hospital or under medical supervision for several weeks. Providing access to medicines is problematic given the worst-affected regions tend to be poverty-stricken, geographically remote or affected by conflict which makes it difficult for aid agencies to provide the treatment programmes required to address the burden of care.
Non-governmental agencies such as the WHO, DNDi and MSF in collaboration with VL specialists have been working to provide treatment programmes as well as conduct clinical research with newer or better-tolerated agents in order to establish shorter treatment times, optimise therapeutic strategies and assess the cost-effectiveness of different approaches.
This symposium will address the burden of disease and the steps the NGO's have been taking to manage VL as well as review the literature on VL to discuss optimum therapy. This disease is also endemic in parts of Europe and infects mostly HIV-infected adults and more rarely patients with other T cell immune deficiencies such as solid organ transplant patients or those receiving anti-TNF-α antibodies, although rare. The symposium will also address the issue of VL as a differential diagnosis

in European patients and whether data from studies in India and Africa are relevant in Europe or on other continents.

Hospitalised CAP – penicillin/doxy vs FQ or Ceph3/macrolide?

S339 Penicillin + [doxy or macrolide]

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The treatment of hospitalised CAP is increasingly driven by 'deterministic guidelines'. Guidelines take into account trial outcome data and antibiotic resistance trends, but rarely factor in the 'law of unintended consequences' – ie. that an intervention in a complex system often creates unanticipated and undesirable outcomes. Guidelines provide an evidence-based framework for therapy, but can also create a uniformity that can be deleterious and short-sighted. Hence guideline choice requires vision. Guidelines set standards of care, but these are nuanced by different attitudes to the "philosophy" behind antimicrobial therapy and risk-aversity. As example, using the ceph/quinolone option one can take an outcome-based individual patient care approach ("broader is better at all cost") where fear of failure is paramount. This compares to a more societal advocacy that favours narrow-spectrum therapy and attempts to conserve antibiotics, aiming to avoid the collateral damage of increasing antibiotic resistance (cf: *C. difficile*, MRSA, ESBLs). It is illustrative to speculate on the differences in international CAP guidelines wrt β-lactams and quinolone use, and on the motivations that guide these. Is the perception (risk) of the immediacy of individual patient failure overriding concerns for the long-term loss of efficacy and societal costs? Yes, the use of 3rdG cephalosporins and/or quinolones may guarantee success in CAP. But are these successes any more than when iv penicillin is used with less fashionable drugs like tetracyclines or macrolides? And at what cost? As will be presented, the data does not reveal a difference. But broad-spectrum antibiotic use does not teach judicious antibiotic use to impressionable health professionals – eg. resident doctors and surgeons. It supports a culture of empiricism in ED departments, where many ceph/quinolone treated patients do not have CAP on review, and culminates in the domino effect of cephalosporin use. And it damages the credibility of ID units – paying lip service to the concept of antibiotic stewardship whilst using antibiotics indiscriminately for CAP.

The 'Chaos Theory' explains the behaviour in dynamic (chaotic) systems that are highly sensitive to initial conditions, (of which healthcare is a paradigm) ie. where small differences in initial choices yield widely diverging long term outcomes. Chaos is promoted by broad-spectrum antibiotics in CAP, penicillin presents a way back toward prescribing Order.

MALDI-TOF: is it as good as we hoped

O343 MALDI-TOF identification from BacTAlert blood cultures in Cambridge, UK

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Objectives: MALDI-TOF mass spectrometry on samples direct from positive blood cultures can yield a species identification result within the same time period as it takes to obtain a Gram stain result. We assessed the accuracy of MALDI-TOF direct from blood cultures and the clinical effect of the rapid MALDI-TOF result as compared to that of a Gram stain result.

Methods: Positive BacTalert blood cultures from 92 patients were processed and analysed by MALDI-TOF according to the manufacturers recommendations (Bruker Daltonics Ltd). Four medical microbiologists agreed the clinical actions, or recommendations made, on the basis of the Gram stain result and compared with those retrospectively agreed based on the MALDI-TOF result. The effects of the MALDI-TOF result were categorised relative to the Gram stain result as: No difference, altered patient management for public health purposes, and altered antimicrobial therapy.

Results: Identification: From 92 BacTAlert blood cultures, 83 direct MALDI-TOF species identifications were made, three at the genus level. Five specimens gave no reliable identification. Gram stain and biochemical identification agreed with MALDI-TOF results in 82/83 cases, one *Pseudomonas hibiscola* isolate was identified as a *Stenotrophomonas maltophilia* by biochemical ID. In three cases where >1 organism was present in the blood culture MALDI-TOF identified the primary pathogen.

Clinical impact: Compared with the Gram stain result, a rapid MALDI-TOF result would have triggered changes to patient management in 17/92 cases. In 16/17 cases MALDI-TOF would have resulted in earlier changes to empiric antimicrobial therapy; 9 instances of antimicrobial withdrawal would have included 3 coagulase negative staphylococci, one *Lactobacillus rhamnosus* and *S. maltophilia*, in 9 cases better targeted antimicrobial(s) would have been added for bacteraemias due to e.g. *L. monocytogenes*, *K. pneumoniae* and *E. faecalis*. In 6/17 cases a rapid MALDI-TOF result would have effected changes to patient management for infection control and public health reasons, including two salmonellaceae, two *S. pyogenes* and one *N. meningitidis*.

Conclusion: MALDI-TOF results direct from BacTalert blood cultures provided a rapid and accurate tool to aid laboratory diagnosis and would have positively impacted care in a significant proportion of cases. When used in combination, Gram stain and improved MALDI software algorithms will reliably identify mixed cultures.

O344 Clinical impact of direct matrix-assisted laser desorption ionisation time-of-flight mass spectrometry analysis on positive blood cultures

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Objectives: Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) allows the identification of microorganisms directly from positive blood culture broths. Use of the MALDI-TOF MS for rapid identification of microorganisms from blood culture broths can reduce the turnaround time to identification and may lead to earlier appropriate therapy of bacteremia.

The aim of this clinical trial was to assess the impact of performing direct MALDI-TOF MS on positive blood cultures on turnaround time and antimicrobial management.

Methods: During February and April 2010, direct MALDI-TOF MS was routinely performed on all positive blood cultures. During December 2009 and March 2010 no direct MALDI-TOF MS was used. All blood cultures that were positive during the study period were included. Information on antibiotic therapy was collected from the hospital and intensive care units' information systems.

Results: In total, 253 episodes of bacteremia were included of which 89 during the MALDI-TOF period and 164 during the non-MALDI-TOF period. 284 microorganisms were identified, mostly aerobic Gram positive cocci (68.0%) and aerobic Gram negative bacilli (23.9%). 27 bacteremia episodes were polymicrobial.

The direct MALDI-TOF showed a correct identification (spectral score >1.7) in 56.2% of bacteremia episodes during the MALDI-TOF period (n = 50). No identification was obtained in 32.6% (n = 29, of which 5 polymicrobial), no MALDI-TOF was performed in 4.5% (n = 4) and the MALDI-TOF correctly identified 1 of the microorganisms in 6 (6.7%) polymicrobial blood cultures.

Median time from blood culture positivity until identification was 16.4 hours (IQR 10.3–42.9) with MALDI-TOF versus 45.2 hours (IQR 35.5–55.9) without MALDI-TOF ($p < 0.001$). In the MALDI-TOF period median time until the first switch in antibiotic therapy was 17.5 hours (IQR 9.8–38.8) versus 24.0 hours (IQR 9.5–47.0) in the non-MALDI-TOF period (p0.27).

In 57.3% of bacteremia episodes no switch in antibiotic therapy was made (MALDI-TOF 55.0%, non-MALDI-TOF 58.5%). In 37.2% a switch was made once (MALDI-TOF 41.6%, non-MALDI-TOF 34.8%)

and in 5.5% a switch was made twice (MALDI-TOF 3.4%, non-MALDI-TOF 6.7%).
Conclusions: Routinely performed MALDI-TOF directly on positive blood cultures significantly reduced identification time with 28.8 hours. However, routine implementation of this technique did not significantly accelerate antibiotic switching.

O345 MALDI-TOF mass spectrometry ID of bacteria directly from cerebrospinal fluid – what you see is what you get

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Objectives: Fast and reliable identification (ID) of bacteria in cerebrospinal fluid is of pivotal significance for the correct treatment of patients with infections in the central nervous system. Maldi-Tof MS ID of bacteria from culture is fast and reliable and has in recent years been introduced in many laboratories. The performance of MS on other materials than culture is being investigated. We present results from Maldi-Tof MS ID performed on 183 consecutive clinical spinal fluid specimens, of whom 14 were positive at culture. Further, spinal fluid samples were spiked with common pathogens at different concentrations to evaluate the Maldi-Tof MS performance.
Methods: All Maldi-Tof MS (Bruker Daltonics) were done in parallels. The cerebrospinal fluid samples were analysed by Maldi-Tof MS without an extraction step, both centrifugated (1500 g i 15 min., supernatant discharged) and un-centrifugated for the detection of bacteria. The impact of electrolyte, protein and cell concentration on the MS results were analysed by scatter plots. The 14 culture positive samples were further extracted (ethanol and formic acid) and results were compared to extracted sterile samples (on culture). Randomly chosen clinical samples, sterile on culture, and controls in MQ-water, were spiked with bacteria in 10-fold dilution series (*S. pneumoniae*, *H. influenzae*, *E. coli*, *E. faecium* and *P. acnes*) and undervent Maldi-Tof MS in 5 paralells, with and without extraction to investigate sensitivity and specificity.
Results: All the clinical cerebrospinal fluid samples yielded negative (low score 0,000–1,699) mass spectres, whether centrifugated or not. Extraction of the culture positive samples did not alter the results. The spiked cerebrospinal fluids suggests sensitivity for detection of *S. pneumoniae* and *H. influenzae* to be 105 cfu/Ml, *E. coli* 106 cfu/Ml and *E. faecium* and *P. acnes* 104 cfu/Ml. Protein concentration, electrolyte concentration and number of cells had little impact on the mass spectres, importantly no false positive results were present.
Conclusion: Maldi-Tof MS for direct identification of bacteria in cerebrospinal fluid specimens has a sensitivity comparable to acridine orange microscopy. No false positive identifications were experienced, protein/electrolyte concentrations and number of cells had little impact on mass spectres.

O346 Preliminary results from a comparison of two matrix-assisted laser desorption ionisation–time-of-flight mass spectrometry (MALDI-TOF) systems with 16S rRNA gene sequencing for identification of anaerobic bacteria to the species level

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Objectives: MALDI-TOF MS is a powerful technology for identification of bacteria. However, anaerobic bacteria have not been investigated to a larger extent in detail, apart from the *Bacteroides fragilis* group. A head-to-head comparison was performed on a collection of anaerobic bacteria to evaluate the performance of the Bruker Biotyper and the AnagnosTec SARAMIS MALDI-TOF MS system.
Methods: Clinical anaerobic isolates from blood cultures, other sterile body sites and suspected *Clostridium* spp. and *Fusobacterium* spp. from any site, which had been identified to the species level with 16S rRNA gene sequencing, were included in the study. The isolates were identified simultaneously by the Biotyper and the SARAMIS MALDI-TOF MS systems according to a specified protocol.
Results: One hundred and twenty isolates, representing 39 species, belonging to 13 genera (primarily *Bacteroides* spp., *Clostridium* spp., *Fusobacterium* spp., *Lactobacillus* spp., *Veillonella* spp. and *Propionibacterium acnes*), were examined (the final number of isolates will be approximately 240). Of the 120 isolates, 103 and 102 were included in the databases of the Biotyper and the SARAMIS system, respectively. The number of isolates that were identified correctly to the species level was 70 (58.3%) and 60 (50%) by the Biotyper (score $\geqslant$2.0) and the SARAMIS system (score $\geqslant$80%), compared to the 16S rRNA gene sequencing identification. The Biotyper system did not identify 33 isolates, which were in the database and misidentified nine isolates, which were not in the database, as a closely related species (e.g. *Bacteroides dorei* as *B. vulgatus*). The SARAMIS system did not identify 29 isolates, which were in the database. Only two isolates were misidentified (one was not in the database). Eight isolates were correctly identified to one of two species (e.g. *B. dorei* as *B. dorei*/vulgatus) and four isolates to the correct genus/family level.
Conclusion: MALDI-TOF is a promising technology for species identification of anaerobic bacteria and much cheaper and faster compared to 16S rRNA gene sequencing. However, development and expansion of existing databases needs to be continued. To further optimize the technology, testing of different pre-analytic procedures before MALDI-TOF-MS might be performed.

O347 Rapid detection of *Salmonella* by selective enrichment and mass spectrometry based on MALDI Biotyper

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Objective: *Salmonella* infection causes severe diarrhoea. Employing standard routine procedures the identification of positive specimen takes at least 2 days. Aim of this work was the development of a rapid approach for the detection of *Salmonella* from stool samples.
Methods: *Salmonella* sp. spiked stool samples were incubated in selenite bouillon at 37ºC over night. The supernatant of this culture was applied to 1 ml water and centrifuged. Pellets were washed twice with water. Bacteria were lysed in formic acid and acetonitrile. 1 µl of the cleared lysate was applied on a MALDI sample carrier plate. Dried spots were overlaid with MALDI matrix.
Patient samples were stroked on Hectoen agar. After incubation at 37ºC over night, colonies were directly transferred to a MALDI sample carrier plate and overlaid with MALDI matrix. Selenite cultures were processed as described above and further plated on selective agar (XLD).
MALDI-TOF MS spectra were acquired on a microflex LT and analyzed with the MALDI Biotyper software for identification of microorganisms.
Results: The analysis of the spiked stool samples revealed that inoculation with less than 800 cfu resulted in an overgrowing of *Salmonella* by natural enterobacteria. Spiking with at least 800 cfu led to an unambiguous identification of *Salmonella* sp. The novel approach facilitates the direct analysis of the selective liquid culture without further plating.
This approach was applied to clinical samples. Over a time frame of 9 months 4847 clinical samples were analyzed according to the above described protocol. All samples were plated on selective agar to detect false positive or negative samples. After incubation over night at 37ºC, suspicious colonies were identified by means of MALDI Biotyper analysis. Applying this workflow, 66 samples were directly identified as *Salmonella* positive from the Hectoen agar. The same samples and further 34 samples were detected as *Salmonella* positive from the selective enrichment culture. In total, 100 samples could be identified as *Salmonella* positive already one day after starting culturing. The residual, so far negative samples were plated on XLD-agar. Only 8 additional samples turned out to be *Salmonella* positive after MALDI Biotyper analysis. No false positive samples were identified.
Conclusion: The novel approach facilitates the detection of most of the *Salmonella* positive samples at least one day before the standard routine procedures.

CA-MRSA – what is going on with CA-MRSA?

O348 High genetic diversity of community-associated *Staphylococcus aureus* in Europe

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Objectives: The worldwide emergence of infections caused by MRSA in healthy individuals (CA-MRSA) is worrisome. In the USA, the USA300 clone is responsible for the great majority of MRSA infections in emergency departments. Nonetheless, a global picture of the epidemiology of CA-MRSA in Europe remains unclear. In this study, we aimed to define the population structure of CA-*S. aureus* in Europe.
Methods: Six hundred and eighteen *S. aureus* isolates were obtained from sixteen of the most populous European countries, from outpatients or from patients attending hospitals within the first 48 hours of hospitalization. All isolates were characterized by spa typing. Multilocus sequence typing (MLST) and/or pulsed-field gel electrophoresis (PFGE) were performed for isolates with new or non-specific spa types. mecA and PVL were detected by PCR. Staphylococcal cassette chromosome mec (SCCmec) typing was performed by multiplex PCR. The relatedness of isolates was assessed by eBURP analysis.
Results: Three hundred and thirty two *S. aureus* isolates (52%) were found to be associated to community-associated (CA) lineages. The great majority of isolates (66%) were related to USA300 clone (ST8, 81 isolates), the European clone (ST80, 52), ST15 (29), Taiwan clone (ST59, 28) and Southwest Pacific clones (ST30, 28). Moreover, other ten CA minor lineages were detected. Of all CA isolates, 52% carried PVL and 52% carried the mecA; SCCmec IV was the most common type (80%), but SCCmec types V and VI were also found. A high number of different PFGE types, spa types and SCCmec types were found among clonal lineages CC8, CC15, CC25, CC30 and CC121. Only 50% of CA-MRSA isolates belonged to epidemic clones with the typical genetic features PVL+ and SCCmec IV. eBURP analysis revealed that some CA-MSSA and CA-MRSA isolates were highly related.
Conclusions: A wide genetic diversity was found among CA-*S. aureus* isolates in Europe, in striking contrast with the reality observed in the USA. The conditions which favored this wide diversification still remain to be clarified. Altogether data resulting from this study suggest that while some CA-MRSA might have emerged and evolved in Europe, other seem to have been imported.

O349 Changing trends in the epidemiology of PVL-MRSA in England

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Objectives: The potential for various clones of PVL-MRSA to cause severe disease has been a cause for serious concern. This is especially evident for one clone (so-called USA300) which has reached epidemic proportions in North America, effectively marginalising other MRSAs. We sought to map the burden and molecular epidemiology of PVL-positive MRSA England to examine for trends over a 4 year period.
Methods: Isolates of MRSA recovered from suspected PVL-related disease (SSTIs, necrotising pneumonia, musculoskeletal infections etc) from patients in England between 2007 and 2010, were referred to the national *Staphylococcus* Reference Unit (SRU). All isolates were examined for PVL toxin genes and PVL-positive MRSA were characterised by various techniques including: MLST, spa typing, toxin gene profiling, SCCmec typing and/or pulsed-field gel electrophoresis. MICs of a wide range of antibiotics were determined and susceptibility interpreted according to BSAC/EUCAST criteria. Associated patient demographic and clinical data were analysed.
Results: The data show an approximate 1.5-fold annual increase in the number of PVL-MRSA identified, ranging from 477 in 2007 to >900 in 2010. Patients were aged 0 to 100y (median 31y); the male:female ratio was 1.2:1 and ca. 70% presented with SSTIs. At least 10 different lineages of PVL-MRSA were identified, 3 of which were dominant: European, South West Pacific and USA300 clones (ST80-IV, ST30-IV and ST8-IV respectively). In 2009, USA300 became the major PVL-MRSA clone in England, displacing the European clone which dominated in 2007 and 2008. USA300 strains were typically resistant to erythromycin with variable resistance to ciprofloxacin; representatives of the European clone were usually resistant to tetracycline and fusidic acid. The number of cases due to the multiply-resistant Bengal Bay clone (ST772-V) increased 6-fold from 10 in 2007 to 61 in 2009. Overall, most PVL-MRSA infections were community-acquired and sporadic in nature, although occasional clusters involving transmission of PVL-MRSA in community and healthcare settings were identified.
Conclusion: PVL-MRSA in England are polyclonal, with 3 lineages predominating. The emergence of multiply-resistant strains as a cause of community- and healthcare-associated infection has important implications for recognising, diagnosing and managing such infections. Continued vigilance is essential to map the burden and molecular epidemiology of PVL-MRSA internationally.

O350 High prevalence of hospital-associated *Staphylococcus aureus* epidemic clones in the community in Portugal: evidence for blurring of community/hospital boundaries

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Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA) were traditionally confined to hospitals (HA-MRSA) but in the 1990s emerged in the community (CA-MRSA). CA-MRSA present specific genetic backgrounds, an enhanced virulence potential, carry the smallest staphylococcal cassette chromosome mec (SCCmec IV or V) types and produces Panton-Valentine leukocidin (PVL).
Recent data indicates that the boundaries between the hospital and the community are blurring and that CA-MRSA have already conquered the hospital becoming more resistant to antibiotics. In spite of the CA-MRSA relevance, no data exist concerning the frequency of CA-MRSA in Portugal. In the present study we aimed to evaluate the *S. aureus* population structure in the Portuguese community.
Methods: A total of 139 isolates were collected from infection in individuals with no risk factors for HA-MRSA acquisition. For all isolates pulsed-field gel electrophoresis (PFGE) and spa typing were performed and mecA and PVL genes (lukS-PV and lukF-PV) were detected by PCR. Multilocus-sequence typing (MLST) was carried out to ascertain clonal type in specific cases. SCCmec typing and antimicrobial susceptibility testing for a panel of 22 antimicrobial agents was performed for MRSA isolates. Isolates relatedness was analyzed by eBURP.
Results: We found a relatively low frequency of CA-MRSA causing infection in Portugal (5%, 7/139), related either to predominant CA-MRSA clones (USA300, ST80 and ST398) or to less disseminated clones (ST121, ST22 and ST1810). Two out of seven CA-MRSA found were multidrug resistant. Moreover, *S. aureus* population structure was composed by diverse CA-MSSA clones (41.7%) and an unexpected high proportion of isolates (30.2%; 19 MSSA, 23 MRSA) belonging to HA-MRSA epidemic clones (EMRSA15, New York/Japan, EMRSA16, and Berlin). Only 2.9% of isolates carried PVL. Most of isolates were from SSTI (47%) and pneumonia (7%). CA-MRSA and CA-MSSA isolates with highly related spa types were found.

Conclusion: The majority of CA-MRSA isolates in Portugal belonged to both epidemic and minor clones; the latter might have emerged from an extant MSSA population. The finding of CA-MRSA isolates that were multidrug resistant and of isolates belonging to HA-MRSA epidemic clones recovered from individuals with no health-care related risk factors, suggests that CA-MRSA already entered into the hospital and that HA-MRSA epidemic clones may have already gained a foothold into the community in Portugal.

O351 Rise of USA300 hospitalisations in the US during 2004–2008

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Objectives: To estimate the prevalence and rates of US300 in *S. aureus* hospitalizations in the US for the period 2004–2008 by combining data from a national bank of isolates and a national hospitalization survey.
Methods: Data sources consisted of a randomly selected subset of isolates from the US TSN *S. aureus* surveillance collection (Eurofins Medinet) and the Nationwide Inpatient Sample (Healthcare Cost and Utilization Project). 421 *S. aureus* inpatient isolates were genotyped using multilocus sequence typing to characterize clonal complexes (CC). The Panton-Valentine leukocidin (PVL) status and antibiogram were also obtained. Three major groups, corresponding to 89% of all isolates, were identified: (a) USA300 (CC 8 PVL+ MRSA), (b) CC 5 PVL– MRSA and (c) PVL– MSSA. Hospitalization rates for *S. aureus* and those attributable to each one of the identified groups were computed. Rates, standard errors, proportions and rate ratios were obtained using appropriately weighted data that included the variance of the molecular epidemiology study.
Results: Overall, *S. aureus* hospitalization rates per 1,000 discharges have significantly increased from 14.1 +0.25 in 2004 to 15.1 +0.24 in 2008 ($p < 0.05$). USA300 represented 7.3% of all *S. aureus* hospitalizations in 2004, with a hospitalization rate of 1.03 + 0.08. By 2008 USA300 had increased 3.5 times ($p < 0.001$) and now represented 23.9% of all *S. aureus* hospitalizations with a rate of 3.62 +0.24 per 1,000 discharges. CC5 PVL– MRSA was the most common hospital genotype in 2004 (41.3% of all *S. aureus* hospitalizations, with a rate of 5.83 +0.14), but by 2008 it had declined significantly ($p < 0.01$), becoming the second most common group (28.3%, hospitalization rate 4.28 +0.11 per 1,000 discharges). The polyclonal MSSA PVL– group did not change significantly over time accounting for 39.7% of all hospitalizations (rate of 5.59 +0.19) in 2004 and 38.5% of all *S. aureus* hospitalizations in 2008 (rate of 5.83 +0.19 per 1,000 discharges).
Conclusion: Although the overall *S. aureus* hospitalization rate increased moderately during the period of observation, the share of USA300 isolates dramatically increased from 1 out of 13 *S. aureus* hospitalizations in 2004 to 1 out of 4 in 2008. USA300 partly replaced the CC5 PVL– MRSA, as the latter group declined significantly, while the share of MSSA PVL– remained stable over time. The significant increase of *S. aureus* hospitalizations in the US can be explained by the rise of USA300.

O352 Prevalence of Panton-Valentine leukocidin in methicillin-susceptible and -resistant *Staphylococcus aureus* from Australia and New Zealand, 2009

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Objectives: Panton-Valentine Leukocidin (PVL) is accepted as a marker of disease severity in staphylococcal infections. The aim of this study was to assess the prevalence of PVL among *Staphylococcus aureus* isolated from Australia and New Zealand, and determine any association with specific clonal complexes.
Methods: Non-duplicate *S. aureus* isolated from hospitalised patients with bacteraemia or skin and skin structure infections, collected during the 2009 SENTRY Surveillance were examined. Oxacillin-resistance was determined using broth microdilution (CLSI). The lukF-PV and lukS-PV genes coding for the PVL toxin, were detected using a real-time PCR (Roche LC480). Isolates were assigned to a clonal complex (CC) using high resolution melt analysis of PCR fragments obtained from amplification of six house-keeping gene targets (Minim assay).
Results: A total of 468 *S. aureus*, 112 oxacillin-resistant (MRSA) and 356 oxacillin-susceptible (MSSA) were evaluated. MRSA were more common from AUS than NZ (29% vs 11%). PVL genes were detected in 21.6% of MRSA and 15.0% of MSSA from Australia, and 60.0% and 31.7% from NZ (see Table). In both countries, all MRSA isolates that contained PVL belonged to either ST93 or CC30. More diversity was found among MSSA isolates; in Australia, CC121 and ST93 were the dominant clones, while in NZ, CC121, CC1 and CC30 predominated.
Conclusion: PVL producing MSSA are more common than previously recognised in Australasia, and belong to specific clonal complexes.

		Australia (n=330)		New Zealand (n=138)	
Oxacillin	Source[a]	N	PVL+ (%)	N	PVL+ (%)
Susceptible	All	233	35 (15%)	123	39 (32%)
	BC	54	7 (13%)	29	8 (28%)
	SSSI	179	28 (16%)	94	31 (33%)
Resistant	All	97	21 (22%)	15	9 (60%)
	BC	19	1 (5%)	3	1 (33%
	SSSI	78	20 (26%)	12	8 (67%)

[a] BC: blood cultures
SSSI: skin and skin structure infections

Epidemiology of antimicrobial resistance

O353 High prevalence of ESBL-producing *Escherichia coli* in chicken meat imported into Sweden

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Objectives: Investigate if chicken meat imported into Sweden from South America and Europe may be a potential source for extended spectrum β-lactamase (ESBL) producing *Escherichia coli*.
Methods: In total, 115 samples, 36 South American and 79 European, from imported fresh, tenderized and frozen chicken meat were collected in retail stores and outlets during 2009 and 2010. The meat was subjected to stomaching, put in pre-enrichment broth containing cefotaxime and incubated overnight. Potential ESBL-producing *E. coli* was isolated using CHROMagar™ ESBL and CHROMagar™ supplemented with cefotaxime. ESBL-producing *E. coli* was verified by Etest® ESBL. Antibiotic susceptibility to 11 antibiotics, belonging to 7 classes, was assessed using VetMICTM GN-mo microtitre plates. The presence of antibiotic resistance genes, including the ESBL gene groups, was identified using Identibac AMR-ve ArrayTube™. The specific gene variants responsible for ESBL production were determined by sequencing using BigDye® v1.1.
Results: In total 42% of the chicken meat was identified to contain ESBL producing *E. coli*. The meat from South America was positive in 92% of the cases and in the European meat 19% was positive. The following ESBL gene variants were identified: cmy-2, ctx-m1, ctx-m1 + tem-1, ctx-m2, ctx-m2 + tem1, ctx-m8, ctx-m8 + tem-1, ctx-m14 and tem-135. The ctx-m8 and ctx-m14 were only identified in isolates from South American meat and ctx-m1 and tem-135 were only identified in isolates from European meat. Resistance to chloramphenicol (4 isolates) and florfenicol (1 isolate) were exclusively found among the South American isolates from South American meat. One isolate from Chilean meat was susceptible to colistin only. Overall, isolates from South American meat showed resistance to a wider range of antibiotics and carried more genes encoding antibiotic resistance compared to the isolates from European meat. Isolates from Danish meat were the most susceptible generally showing resistance to β-lactams only.
Conclusion: ESBL-producing *E. coli* were frequently identified in chicken meat imported into Sweden, indicating it as a possible source for gut colonization. Chicken meat from South American had a much higher prevalence of ESBL-producing *E. coli* than chicken meat imported into Sweden from Europe.

O354 High prevalence of ESBL-producing Enterobacteriaceae carriage in Dutch patients visiting their general practitioner

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Objectives: The aim of this study was to determine the prevalence of carriage of ESBL-E in the region of Amsterdam, to characterize the ESBL genes and plasmids involved, and to type the ESBL-positive strains to gain understanding of the epidemiology of this emerging resistance in the Dutch outpatient population.
Methods: Fecal samples were obtained between April 12 and May 19, 2010, from patients presenting to their GP with mild gastrointestinal discomfort. Screening for ESBL-E was performed with selective enrichment broth and inoculation on a selective screening agar, containing cefotaxime and ceftazidime. ESBL production was confirmed with the double disc synergy test with clavulanic acid. Species identification and antibiotic susceptibility testing were performed with the Vitek-2 system (bioMérieux). ESBL genes were characterized by microarray (Check-KPC ESBL Check-Points), PCR, and sequencing. The strains were analyzed for genetic relatedness by AFLP. Identification of plasmids was done by PCR-based replicon typing (Carattoli, J. Microbiol. Meth. 2005).
Results: We obtained 471 samples from 471 patients. The median age of patients was 46 years (range: 0–92), 54% were females. Fifty (10.6%) samples yielded ESBL-E: 49 *Escherichia coli* strains and 1 *Shigella sonnei* strain. Of these isolates, 14/50 (28%) were resistant to gentamicin, 22/50 (44%) to ciprofloxacin, and 33/50 (66%) to co-trimoxazole; 12% (6/50) were multiresistant (to β-lactams, gentamicin, ciprofloxacin and co-trimoxazole). The presence of genes encoding ESBLs was confirmed in 49 isolates; these comprised 4 blaCTX-M-1, 24 blaCTX-M-15, 1 blaCTX-M-14, 7 blaCTX-M-14b, 4 blaCTX-M-27, 7 blaSHV-12, 1 blaSHV-2a, 1 blaTEM-52 genes. AFLP showed a few small clusters of isolates with identical AFLP patterns, although there was no epidemiological relation between the patients. The following plasmids were identified: ColE (20/50), Frep (8/50), R (6/50), FIA (4/50), IncI1 (4/50), and P (1/50).
Conclusion: This high prevalence of ESBL-E in Dutch outpatients was unexpected. We noted a high percentage of co-resistance to ciprofloxacin, co-trimoxazole and gentamicin. Strain typing showed no evidence for clonal spread of particular strains in the community. ColE was the most prevalent plasmid; this plasmid has been found previously in strains of *Salmonella* and *Klebsiella* isolated from humans. The significance of the frequent occurrence of ColE plasmids in *E. coli* in Dutch outpatients needs to be explored further.

O355 Complex epidemiology of KPC-producing Enterobacteriaceae in a university hospital in Madrid (Spain) after an outburst of KPC3-producing *Klebsiella pneumoniae*

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Objective: We previously described the first outburst of KPC-3-producing *Klebsiella pneumoniae* (Kpn) in Spain (Sept-09 to Feb-10) (Curiao et al. JAC 2010; 65:1608–14). Seven patients were infected/colonized by the ST384 KPC-3-Kpn clone and another one with the ST388 Kpn-KPC-3 clone. In this study we present the on-going epidemiology of several KPC-producing Enterobacteriaceae in our hospital after the emergence of KPCs.
Methods: From March-09 to Nov-10, all Enterobacteriaceae isolates recovered from clinical or surveillance cultures with decreased carbapenem susceptibility and a positive modified Hodge test were screened for the presence of carbapenemase genes (PCR and sequencing). Antibiotic susceptibility was performed by the semiautomatic Wider system (Fco. Soria-Meguizo, Spain). PFGE and MLST were achieved to assess clonal relatedness. Clinical records of infected and/or colonized patients with KPC-producing isolates were reviewed.
Results: 16 KPC-producing Enterobacteriaceae isolates [12 Kpn, 2 *Escherichia coli* (Eco) and 2 *Enterobacter cloacae* (Eclo)] were recovered from 15 patients (mean age 64-y; 6 female) during the follow-up period. Patients were admitted in 8 different wards (40% admitted at ICU, 33% in medical and 26% in surgical wards). In the 80% of the cases, KPC-producers were recovered from surveillance cultures. By PFGE, 5 Kpn (identified by MLST as ST20, ST454, and unassigned new STs), 2 Eco and 1 Eclo clones were recognized. The blaKPC-3 gene was detected in 4 Kpn and 1 Eco clones, whereas the blaKPC-2 gene was detected for the first time in our institution in one each Kpn, Eco and Eclo clones. The KPC-3-Eco clone was isolated from one carrier of KPC-3-Kpn. All clones (n = 8) were resistant to all β-lactams, including imipenem and meropenem (MIC range 4–>8 mg/L), and showed co-resistance to fluoroquinolones (n = 6; 75%) and aminoglycosides (n = 1; 12%).
Conclusions: A complex penetration of blaKPC genes involving different genetic lineages of several Enterobacteriaceae were detected after the emergence of a KPC-3-Kpn-ST384 clone in our institution. This allodemic situation was present with a high colonization pressure. Moreover, the co-colonization by two different bacterial species carrying the blaKPC-3 gene suggests the in vivo transfer of this gene.

O356 Epidemiology of invasive disease due to serotype 6A and 6C *Streptococcus pneumoniae* from 2002 to 2009 in France

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Objectives: Since the introduction of the 7-valent conjugate vaccine (PCV7) in 2003 in France, the incidence of serotype 6B invasive pneumococcal disease (IPD) has dramatically decreased from 5.2 and 2.3 cases/100,000 in children <2 years and adults >64 years, respectively, to 0.4 cases/100,000 in 2007–2008 in both age groups. By contrast, during the same period, IPD due to serotype 6A/C slightly decreased in children <2 years (1.1 to 0.6 cases/100,000), but increased in adults >64 years (0.7 to 1.4/100,000). The aim of our study was to compare the prevalence and the susceptibility profile of the newly recognized serotype 6C, a serotype 6A-variant, in IPD between pre- and post-PCV7 period.
Methods: Out of 5501 IPD isolates, 186 (bacteraemia 56%, meningitis 44%) were identified as serotype 6A/C by conventional serotyping method using antisera from the Statens Serum Institut. Among the latter, serotype 6C and 6A were further distinguished using PCR to determine the respective length of their wcin gene (wcin6C = 1.8 kb; wcin6A = 2 kb). Antimicrobial susceptibility testing was performed by agar dilution (penicillin, amoxicillin, and cefotaxime) or disk diffusion method (erythromycin, clindamycin, tetracycline, chloramphenicol, and cotrimoxazole) according to Ca-SFM. Selected representative strains were studied by MLST.

N° of isolates		Pre-PCV7	Post-PCV7			p
	Serotype	2002	2007	2008	2009	2009 *vs.* 2002
All ages	All	1179	1488	1176	1658	-
	6A	30 (2.5%)	36 (2.4%)	13 (1.1%)	24 (1.4%)	0.005
	6C	4 (0.3%)	19 (1.3%)	17 (1.4%)	43 (2.6%)	<0.001
<2 years	All	173	232	205	278	-
	6A	6 (3.5%)	8 (3.4%)	2 (1.0%)	3 (1.1%)	0.02
	6C	0	1 (0.4%)	1 (0.5%)	1 (0.4%)	ns
>64 years	All	438	522	379	502	-
	6A	14 (3.2%)	15 (2.9%)	5 (1.3%)	11 (2.2%)	ns
	6C	2 (0.5%)	9 (1.7%)	7 (1.8%)	22 (4.4%)	<0.001
Penicillin I+R	6A	11	10	6	5	ns
	6C	1	9	12	19	ns
Erythromycin I+R	6A	13	10	2	8	ns
	6C	1	14	12	30	ns
MLST-ST (CC)	6A	460, 473, 1876, 1190 (460,473)				-
	6C	1150, 1692, 2667, 386 (156, 386, 395)				-

Results: The prevalence of serotype 6C IPD, which was low at the pre-PCV7 period, significantly increased in the post-PCV7 period in adults >64 years, but not in the children <2 years. Half of the serotype 6C isolates were penicillin-non susceptible (MIC_{90} = 0.125 mg/L) while yet susceptible to amoxicillin and cefotaxime, and resistant to erythromycin (MLSB inducible) and tetracycline. MLST results indicated that 6C

sequence-types (STs) were distinct from those of 6A strains, and were grouped mainly in the clonal complexes (CCs) 156, 386, and 395.
Conclusions: These results suggested a direct effect of PCV7 on original serotype 6A IPD in children <2 years. The increase in 6A/C IPD observed in adults was due to an increase in the new non-PCV7 serotype 6C related to the spread of different clones, while original 6A IPD remained stable (no PCV7 indirect effect). Thus, serotype 6C has become in 2009 the most prevalent type of the group 6 in adults IPD in France.

O357 Group B streptococcal neonatal infection in the Barcelona area: 18-year surveillance

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Objectives: We undertook the analysis of 212 group B streptococci (GBS) isolates from invasive infections in newborns in the Barcelona area, between 1992 and 2009, with the aim of documenting changes in prevalence of serotypes, antimicrobial resistance patterns and genetic lineages and evaluating their association with either early-onset (EOD) or late-onset disease (LOD).
Methods: All isolates were serotyped and assigned to clones according to their pulsed-field gel electrophoretic (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 isolates were further classified by phenotype and were tested by PCR for the presence of the erm(B), erm(A) and mef genes. Presence of the surface protein genes bca, alp2, alp3, alp4, eps and rib genes was also tested by PCR.
Results: The isolates were grouped into 18 PFGE clusters and serotypes III (ST17) and Ia (ST23/ST24) were the most frequent. PFGE-based clones showed a very good predictive power over serotype (as evaluated by the Wallace coefficient, W = 0.955). Serotype III was found to be significantly associated to LOD. The overall rate of erythromycin resistance was 14.2%, all isolates displaying the cMLSB phenotype (76.7% associated to erm(B) gene and 23.3% to erm(TR) gene). All isolates carried an α or α-like protein, with specific associations between surface proteins and serotypes such as: Ia and eps; Ib and II and bca; III and rib; and V and alp3.
Conclusions: The characterization of the population of GBS causing invasive infections in neonates in the Barcelona region revealed the existence of a large number of genetically distinct lineages that were present over a significant time-span. The stability and dominance of a few lineages that are responsible for the majority of infections, in spite of continuous antibiotic and immune selective pressures, suggest that these are extremely well adapted to their particular niche. Although most of these lineages are widely disseminated worldwide, we have also identified seemingly regionally successful clones, raising the possibility of an ongoing selection and expansion of specific virulent GBS clones.

Lyme borreliosis, toxoplasmosia

O358 Direct detection of early Lyme borreliosis from whole blood

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Objectives: Early treatment for Lyme Borreliosis is crucial for long term prognosis. The ability to provide a physician with diagnostic results from whole blood collected during the initial patient visit will allow for treatment to begin earlier in the infection and potentially reduce the risks of adverse conditions. To address this need we have developed an assay to detect *Borrelia* directly from whole blood collected during the initial patient visit.
Methods: Samples of whole blood from patients with erythema migrans were tested for Lyme borreliosis by the CDC two-tiered test. Nucleic acid extracts from these patient specimens were made using a combination of mechanical and chemical lysis and magnetic bead purification. The extracts were then tested with and without a nested isothermal amplification prior to use in multi-locus broad-range PCR and electrospray ionization mass spectrometry PCR.
Results: The nested isothermal PCR increases the amount of *Borrelia* DNA in the extracts enabling its detecton by broad-range PCR and electrospray ionization mass spectrometry ionization mass spectrometry. Using our methods we detected *Borrelia* in 65% (13/20) of samples that were positive by CDC criteria on either the initial visit with the blood draw or a subsequent follow-up visit 3 weeks later. In patients that were positive by CDC criteria at the time of the blood draw we detected *Borrelia* in 64% (9/14) of the patients. We detected *Borrelia* in 66.7% (4/6) of patients that were negative by the two-tiered test at the time of the blood draw but were positive on the follow-up demonstrating the ability to detect *Borrelia* infection prior to being seroconversion.
Conclusion: We demonstrate that our *Borrelia* detection assay can detect instances of early Lyme borreliosis directly from whole blood specimens. Additionally, we show that we can detect Lyme borreliosis prior to seroconversion when patients are still negative by CDC two-tiered testing. This assay could provide physicians with actionable results while other tests would remain negative or inconclusive. In addition the test can provide genotypic information on the *Borrelia* strain.

O359 Survey of pathogens in nymphal ticks from the Czech Republic

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Objectives: Many tick-borne diseases have similar symptoms and are found in the same vector. Active surveillance of ticks can provide health agencies with data for population risk assessments and inform physicians as to the possible pathogens patients may encounter. We developed and used an Ibis PLEX-ID assay to screen ticks from the Czech Republic to identify the pathogen frequency and landscape.
Methods: We used a multi-locus assay utilizing 11 different loci with to detect a wide range of tick-borne pathogens including bacteria, protozoa, and viruses. Total nucleic acids were extracted from ticks collected at sites in the Czech Republic and screened on the assay. Electrospray ionization mass spectrometry of the PCR amplicons was used to determine their base composition. These base composition signatures were subsequently used to identify the organisms found in the samples.
Results: Extracts from 148 field collected nympal *Ixodes ricinus* ticks were screened for a variety of pathogens. Pathogens were found in 66.8% (99/148) of the ticks. We detected *Borrelia*, *Anaplasma*, *Rickettsia helvetica*, *Babesia*, and a high prevalence of TBEV. Additionally a 25% (25/99) of the ticks containing pathogens contained 2 or more pathogens with TBEV being the most common co-pathogen.
Conclusions: We demonstrate broad-range detection of tick-borne pathogens in a single assay using ticks from the Czech Republic. The Ibis assay detected a diverse range of bacteria, protozoa, and viruses associated with ticks including a high prevalence of TBEV and ticks confected with two or more pathogens. The Ibis PLEXID system, which can be completed within five hours from specimen processing to result reporting, provides rapid and accurate detection and identification of a broad range of pathogens causing tick-borne human infections.

O360 *Toxoplasma* seroprevalence studies: what are we looking at?

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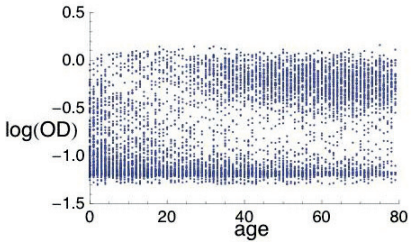
Objective: To discuss the outcome of *Toxoplasma* seroprevalence in different age groups in population based studies in the Netherlands.
Methods: Sera from a nationwide serum bank of the general population were tested with an in house *Toxoplasma* IgG ELISA, using the same

methods in two studies: Pienter study 1995/1996 (1) and Pienter2 study in 2006/2007 (2). The OD value of the samples were compared with a cutoff sample and a ratio was calculated (the OD value of the sample divided by the OD value of the cutoff) In both studies a sample was positive when the OD ratio was ⩾1. The log transformed OD ratios of the serum samples were plotted against the age.
Results: Sera and data of participants of 7521participants in 1996/1997 and 5541 participants in 2006/2007 were available for analysis. The overall seroprevalence was 40.5% in 1995/1996 and 26.0% in 2006/2007. There is a difference between the young age group (<10–20 years) and the older age group (>20 years) in distribution of OD ratios with highle variable OD ratios in the young age groups that are not clearly distinct from low OD ratios, whereas at older ages there seems to be a clear distinction between negative and positive sera.
Discussion: The detection method used is the same in both studies and part of the samples of 1996/1997 were retested in 2010, with the same outcome (data not shown). Both studies show the same pattern of clear distiction of positive and negative results in the older age category, and lack of it in the young. We are looking for an explanation of this phenomenon. As these highly variable OD ratios are found up to ages of about 20 years, maternal immunity or aspecific vaccine effects seem unlikely. Could children be exposed to a different source, with antigen of nonviable oocysts causing seroconversion followed by antibody decay? And does this implicate that seropositive children revert to a seronegative state and are then not protected against toxoplasmosis?



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O361 How to detect significant changes in *Borrelia burgdorferi* antibody reactivity in a follow-up sample

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Introduction: For the laboratory diagnosis of Lyme borreliosis it is not generally recommended to take repeated samples. However this is nevertheless done and may be relevant in selected cases. However no criteria exist to evaluate whether the difference between to measurements of antibody reactivity are significant. The purpose of this study was to analyse repeated routine samples from consecutive patients in order to develop criteria for detection of significant changes, especially a rise, in serum antibody reactivity beyond random measurement error.
Materials and Methods: 22342 samples from 18193 patients were routinely analysed from 2001–2010. Two or more samples were available from 2779 patients retested from 0–3000 days apart. 2038 patients had just two samples taken and above 5 samples were rare. The same ELISA (IDEIA™ *Borrelia burgdorferi* IgM and IgG; Oxoid, Cambridgeshire, UK) based on purified native flagella antigen was used during the whole period. Patients retested less than 7 days apart were assumed to represent random measurement only.
Results: Using 510 samples from patients retested within one week the upper and lower 2.5% quantiles were set as cutoff for the relative change in arbitrary units between the first and any subsequent sample. The upper and lower cutoffs for a relative change in units for IgG was 3.3 and 0.6 and for IgM 2.2 and 0.5. Sample if 0.4 Units of IgG was measured on the first sample then 0.4*3.3=1.32 units or above on the second sample was considered significant. Using these criteria a rise in IgG antibodies was found in 4% of samples, in 90% no change and a decrease in 6%. A rise in IgM antibodies was found in 7% of samples, in 82% no change and a decrease in 11%. Low readings on the first sample below 0.4 units were defined as not decreasing.
Conclusion: A method to establish the minimal detectable change in antibody reactivity is proposed based on statistical criteria, using readily accessible routine samples. In a Danish clinical routine laboratory 15% of patients tested for Lyme borreliosis were tested twice or more. A rise in antibody reactivity was found for IgG in 4% and IgM in 6% of patients. This has of course to be interpreted in the clinical context. The clinical status and symptoms of the patients in this study are not known. However the sample size is large and representative and it is considered safe to conclude that it is not possible to detect a change for IgG in 90% and IgM in 82% of the repeated samples.

O362 Childhood neuroborreliosis does not lead to long-term cognitive disturbances

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Objectives: Adult patients after NB acquired after the age of 18 years have significantly lower z-values for frontal executive functions, and their performance is in all other domains poorer than in the control group, whilst still in the normal range. The aim of this study was to evaluate the long-term cognitive outcome of adults who acquired NB earlier in their childhood.
Methods: 30 patients who had been treated 14.8 + 3.3 years ago at the University hospital of Goettingen, Germany, Dept. of Paediatrics. These patients were examined neuropsychologically with tasks addressing verbal learning and memory, non-verbal learning and memory, attention, executive functions, and visuo-spatial functions. 23 healthy control persons were examined likewise.
Results: The two groups were matched for age (mean + SD 23.2 + 3.7 [NB] vs. 24.0 + 3.5 [C] years, t-test $p = 0.4$) and for the years of scholar education (mean 11.4 + 1.5 [NB] vs. 11.8 + 1.4 [C] years, t-test $p = 0.3$). The gender distribution was matched as well (m/w 19/11 [NB] vs. m/w 15/8 [C]; Fisher's exact test $p \gg 0.05$).
All results of the applied neuropsychological exams of the NB group were comparable with the control group. Significant differences were not detectable between the groups.
Conclusion: In contrast to adults after Neuroborreliosis acquired after the age of 18 years, adults after childhood NB have an excellent outcome with cognitive functions as good as their matched control group.

HIV/AIDS

O363 Genotypic (V3 sequencing) determination of co-receptor usage in HIV-infected patients carrying non-B subtypes

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Objective: To evaluate the success in amplification and the prevalence of non-CCR5 using strains in a panel of non-B subtypes representative of non-B distribution in Southern Spain.
Methods: We have tested a panel of reference pure non-B clones (A1–3, B1–3, C1–4, D1–3, E1–3, F1–2, G1–3 Y H2) and 63 samples of patients infected with non-B subtypes (using rapid subtyping of pol sequences). Amplification was done with primers E80/E105 for RT PCR and ES7/E125 for nested PCR. Sequencing was done with Cy5.0 and Cy5.5 labeled ES7/E125 and the Trugene platform. 46.8% of HIV-1 infected patients were naïve [69,8% male, median age 42,5 (35,5–47,2), median viral load 4.6 [3.9–5.2] log10 copies/ml. copies/ml.

V3 sequences were analyzed using different bioinformatic approaches and matrixes (geno2pheno, web-PSSM) and a combinatorial method previously described by our group (Chueca et al., JMV 2009; 81(5): 763–7).
Results: All reference pure non-B clones were successfully amplified using E80/E105 and ES7/E125. Pol subtype distribution for the HIV-1 infected patients studied was: 29 Circulating Recombinant Forms [CRFs, 46% (22 CRF02_AG, 9 Unique Recombinant Forms – URFs, 14%, 25 pure non-Bs)]. 10 samples could not be successfully amplified (16%); 2 had low viral loads (<1000 copies/ml), 4 were CRFs (3 CRF02_AG), and 6 were from patients carrying pure pol non-Bs (3 C; 2 G, 1 F1). Non CCR5 viruses were detected in 23% of the patients. No significative differences between the detection of non-R5 viruses in naïve (28.6%) or treated (30%) patients, or CD4 count (371.12 cells/μL for R5 vs 335.80 cells/μL for non-R5), and no association between non-R5 usage and recombinant forms was observed.
Conclusions: Successful amplification rate for non-B subtypes using E80/E105 and ES7/E125 set of primers is slightly lower than previous reports for B subtypes. Use of alternate primers, specially for RT-PCR for certain non-B subtypes may be recommended. CRF02_AG infected patients, the most prevalent non-B subtype in our area, are not infected by a higher proportion of non-CCR5 viruses.

O364 Fine epitope specificity of circulating autoantibodies to endogenous erythropoietin, in HIV-1 infected patients, reveals molecular mimicry with HIV-1 p17 Gag protein: a potential pathogenetic mechanism for HIV-1-related anaemia

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Objectives: HIV-1 infection is characterized by autoimmune phenomena, including the production of several autoantibodies. Circulating autoantibodies to endogenous erythropoietin (anti-EPO) were detected in a substantial proportion of patients with HIV-1 infection and represent a risk factor for anemia. The aim of this study was to identify the linear B-cell epitopes on erythropoietin (EPO), recognized by anti-EPO, and to investigate whether these epitopes are similar to those on HIV-1 antigens.
Methods: The EPO epitope mapping was performed using twelve sequential 20-mer synthetic peptides, seven amino acids overlapped each, covering the entire sequence of EPO. Anti-EPO were detected by ELISA; EPO was quantified by radioimmunoassay. Sixteen sera from each of the following groups were tested against the overlapping synthetic peptides: Sera from HIV-1 infected patients with anti-EPO; without anti-EPO; and from age/sex-matched healthy blood donors.
Results: Only sera from HIV-1-positive patients with anti-EPO presented showed binding to the following EPO-epitopes: 1–20aa (Ep1), 54–72aa (Ep5), 147–166aa (Ep12). Inhibition tests confirmed the specific antibody binding to Ep1, Ep5 and Ep12. Structural analysis of EPO revealed that Ep1 and Ep12, which cover the NH2- and C-terminal ends of EPO respectively, are connected with a disulfide bond, comprising the interaction interface with EPO receptor (EPOR). Binding of anti-EPO to this specific region is predicted to block the EPO-EPOR interaction resulting to blunted erythropoiesis. This phenomenon was indicated by the higher EPO [45.23 (28.7) vs. 24.1 (9.2) IU/ml, $p < 0.001$] and lower hemoglobin levels [12 (1.8) vs. 14.3 (1.9) g/dl, $p < 0.001$] of anti-Ep1 positive patients compared to anti-Ep1 negative. Ep1 exhibited a 63% similarity with the 34LVCASRELERFAVNPGLLE52 fragment of the HIV-1 p17gag-matrix protein. All sera binding to Ep1 peptide recognized the HIV-p17gag analogue but not the scrambled peptide constructed from the same region. Furthermore inhibition tests verified the specific binding with values for Ep1 and HIV p17gag as 54% and 52% respectively.
Conclusion: EPO contains linear B-cell epitopes, recognized by anti-EPO antibodies, located in the binding site of EPO to its receptor. Ep1 shows molecular similarity with p-17 matrix protein of HIV-1 suggesting a molecular mimicry mechanism. The presence of Ep1 is associated with anemia and blunted EPO response in HIV-1 infected patients.

O365 The role of hepatitis B virus co-infection in immunologic progression among patients with primary human immunodeficiency virus type 1 infection in an area of hyperendemicity for HBV infection

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Objectives: Hepatitis B virus (HBV) co-infection is common in human immunodeficiency virus (HIV)-infected patients and has been reported to be associated with accelerated progression to acquired immunodeficiency syndrome. However, it remains unclear whether HBV affects the natural history of primary HIV infection. We studied the impact of HBV on immunologic progression among patients with primary HIV infection.
Methods: Patients aged 18 years or greater who did not start antiretroviral treatment during the first 3 months after the diagnosis of primary HIV infection were identified from a prospective observational cohort at two major medical centers for HIV care in northern Taiwan, from January 1997 to December 2010. Primary HIV infection was defined as an interval of 6 months or less between negative and positive HIV serologic tests by enzyme-linked immunosorbent assay; an incomplete Western blot finding; or a negative HIV serologic test in the presence of HIV viremia by real time polymerase chain reaction. HBV infection was defined as presence of hepatitis B surface antigen in the serum. Immunologic progression was defined as the occurrence of a CD4 cell count <350 cells/mm^3 3 months or greater after diagnosis of primary HIV infection. We identified clinical and virologic predictors of immunologic progression by Cox Proportional Hazards model.
Results: In the 14-year study period, 112 patients with untreated primary HIV infection were included for analysis; the majority (95%) of them were men who have sex with men and 15 patients (13.4%) had chronic HBV infection. During a median follow-up duration of 248 days (range, 99–1980), 60 (53.6%) experienced immunologic progression. patients with HBV co-infection had a higher incidence of immunologic progression than those without HBV co-infection patients: 8.37 vs. 0.33 per person-year of follow-up ($P < 0.01$). In multivariate analysis, HBV co-infection (adjusted hazard ratio [aHR], 4.15; 95% confidence interval [CI], 1.23–13.96) and a lower initial CD4 cell count (aHR = 1.74 per 100 cells/mm^3 CD4 decrease; 95% CI=1.18–2.58) were independent predictors of immunologic progression.
Conclusions: The risk of immunologic progression after primary HIV infection is higher in patients who have chronic HBV co-infection and a lower baseline CD4 count. Early initiation of antiretroviral therapy should be strongly considered in this specific group.

O366 Prevalence of high-risk genotypes human papillomavirus in anal samples with cytological changes from homosexual patients attending in a tertiary hospital, Madrid

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Objective: The aim of this work was to determine the prevalence of high risk genotypes of human papillomavirus (HR-HPV) in anal samples from homosexual men with cytological changes, as well as the frequency of cytological lesions and its association with the human immunodeficiency virus (HIV).
Methods: We included 100 homosexual men attended at the Sexual Transmitted Disease Unit of the Ramón y Cajal Hospital from June 2009 to October 2010. Anal samples were collected with cervical brushes (Cervix-Brush®) and stored in PreservCyt® medium. Cytological analyses were performed using the Bethesda classification and HPV genotyping (Linear Array®, Roche Diagnostics, Mannheim, Germany).
Results: Patients' average age was 35.3 years (range 17–65 years), and 47% of them were HIV positive. Cytology lesions were only detected in 46 anal samples from 43 patients, and were as follows: 8.7% Atypic Squamous Cell Unknown Significance (ASCUS), 69.5% Low Squamous Intraepithelial Lesion (LSIL), 8.7% Indeterminate Squamous Intraepithelial Lesion (ISIL) and 13.1% High-Grade Squamous Intraepithelial

Lesion (HSIL). HIV positive patients presented 74% of LSIL and 11% of HSIL versus 63% and 15.7% respectively in the non infected ones ($p > 0.5$). Negative results for HPV were observed in 4 samples with cytological lesions (3 LSIL and 1 ASCUS). From the HPV positive samples, 93% showed a mixed co-infection (from 2 to 11 genotypes), being the coexistence of three different genotypes in the same sample (19%) the most frequent trait. In the overall analysis, HPV-51 was the most frequent genotype detected (30%), followed by HPV-31 and HPV-66 (both 26%), and finally genotypes HPV-16 and 18 were found in 24% and 13%; respectively. An 80.4% of anal samples carried a HR-HPV (68.4% in HIV negative versus 88.8% in HIV positive, $p = 0.08$). The most frequent HR-HPV in HIV negative and HIV positive patients was HPV-31 and HPV-51; respectively. Both HR-HPV-16 and 31 were the most prevalent genotypes in patients with LSIL whereas in the group of HSIL these were represented by HPV-51 and HPV-58.

Conclusions: The most prevalent HPV genotype in anal samples of homosexual men that presented an altered cytology was HPV-51 (almost all in samples from HIV positive patients), followed by HPV-31 and HPV-66. Infection with only a HPV genotype is rare (7%). There is no difference in the frequency of LSIL, HSIL and HR-HPV between HIV negative and positive patients.

O367 High rate of adverse events and serious adverse events after antiretroviral therapy initiation in a resource-limited setting: preliminary data from a prospective study

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Background: The World Health Organization's commitment to the global scale-up of antiretroviral therapy (ART). Most antiretroviral drugs cause adverse effects (AEs). A prospective data regarding AEs among patients in resource-limited settings is limited. We hypothesize that AEs rate might be high in this setting where NNRTI-based regimen has been widely used for scale-up ART. We aimed to describe AEs among naïve HIV-infected patients whom ART were initiated in Thailand.

Methods: This study is a sub-study of Genotype Based Personalized Prescription of Nevirapine (GENPART) (NCT00986063) which mainly aimed to compare the incidences of nevirapine (NVP) associated rashes in patients who are initiated NVP guided by genetic tests and using standard of care approach. Patients were randomized to receive a fixed dose combination of stavudine or zidovudine plus lamivudine and NVP. The overall rate of AEs in all participants was prospectively monitored.

Results: Up to August 2010, 366 HIV-infected patients were enrolled and 141 (38%) patients experienced AEs. A total of 195 AEs were reported which 54 (28%) patients developed more than one AE. Of these, 42 (21%) events were serious adverse events (SAEs) which 28 (14.3%) events were hospitalization, and 4 (2%) events were death. Cutaneous reactions were reported in 73 (37%) events which one-third events were grade 3 and 4. Hepatitis was reported in 39 (20%) events. Most events were grade 1 and approximately half were symptomatic. Median (IQR) duration of AEs was 14 (7–29) days. Overall, 24 (12%) events were defined as definitely related to antiretroviral drugs. ART needed to be stopped and new regimens were initiated in 47 (24%) patients after AEs were improved. Over all, 67 (34%) events were ongoing, 55 (28%) events were improved, and however, 11 (6%) events were improved with sequelae, such as hyperpigmentation, cardiomegaly, and neuropathy.

Conclusion: A high proportion of naïve HIV-infected patients experienced SAEs in a resource-limited setting. Close monitoring and health education by health-care provider after ART initiation is crucial to prevent AEs and improve HIV care.

Quality control: is it needed?

S370 Quality control in molecular diagnostics

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Due to their high level of sensitivity and specificity, nucleic acid amplification tests (NAT's) have in many cases rapidly become the gold standard for diagnosis of infectious diseases and for monitoring treatment of patients with chronic or latent (viral) infections.

However, multicentre studies in the mid 1990's had revealed serious problems in specificity (false-positivity rates up to 40%) and sensitivity, as well as large variations in quantitative results and units used to express results. These problems have been addressed by a variety of approaches. For example, cross contamination of samples was addressed by various measures as spatial separation of the different steps in the procedure (reagent preparation, extraction, amplification and detection), the use of anti-contamination reagents (UNG system) and the development of real-time PCR assays allowing to avoid opening reaction tubes after amplification.

Standardisation was improved when diagnostic companies entered the field, and was further improved when International WHO Standards were developed, however at that time mainly for the detection of blood-borne viruses, *C. trachomatis* and *N. gonorrhoeae*. The lack of sensitivity due to sequence variation of the target gene was improved by more careful selection of (combinations of) primers and probes, and quantitation of viral loads was greatly helped by the introduction of real-time PCR. Similarly, more labs have recently started to use internal controls to monitor extraction and amplification efficacy.

However, the proof of the pudding of molecular diagnostic testing by laboratories is in external quality control (EQA) i.e. the blind testing of proficiency panels. For this purpose Quality Control for Molecular Diagnostics (QCMD) was established in 2001. Since the start, QCMD's EQA programs have confirmed the progress that has been made since the mid 1990's, but also identified remaining problem areas. These include: large differences (>1000-fold) in sensitivity between laboratories, persisting problems with false-positivity, albeit at a much lower level (<5%), and wide variation in quantitative results. These results stress that high quality molecular diagnosis can only be achieved by careful development and validation of assay protocols and meticulous execution of the assays.

S371 Quality control in MALDI-TOF

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The matrix-assisted laser desorption-ionization time-of-flight (MALDI-TOF) mass spectrometry is increasingly used in clinical diagnostic laboratory for microbial identification, since this new technology allows easy and cheap bacterial identification. Accuracy of the identification at species level is generally good and MALDI-TOF may even be used for typing given species.

MALDI-TOF results may however be impaired by problems arising during extraction, for instances (i) when testing encapsulated bacteria (*Streptococcus pneumoniae*, *Klebsiella pneumoniae*), (ii) when testing bacteria such as *Streptomyces*, that exhibit a particular cell wall that reduces the yield of protein extraction, (iii) when the extraction protocol is not properly conducted, or (iv) when the reagents used for extraction are outdated or impaired by inadequate storage. If problems i & ii, due to intrinsic bacterial properties, may only be circumvented by the development of specific extraction protocols, the two latter problems (iii & iv) might be prevented by an adequate quality program.

MALDI-TOF results may also be impaired by inadequate deposit of the sample on the microplate, by poor cleaning of the microplate between runs, by poor maintenance of the MALDI-TOF and by other technical factors. Moreover, despite adequate maintenance and correct procedures, some clade will repeatedly be misidentified due to poor content of some databases.

Thus, it appears critical to implement a quality control program. A quality control program is especially warranted when using MALDI-TOF for direct bacterial identification from positive blood culture bottles given the high impact of results on patients care.
The performance of the extraction step and of the MALDI-TOF mass spectrometer may be checked by routinely testing a few selected bacterial strains, which spectra are available in the database. This control should ideally be done in parallel with and without a specific extraction step. MALDI-TOF microplates need also to be checked before use for the absence of residual proteins trace. Moreover, frequency of maintenance of the MALDI-TOF should be increased if the apparatus is heavily used or located in a crowded/dusty area.
In conclusion, quality controls might help to improve the quality of proteins extraction, MALDI-TOF analysis and completeness of databases. This will further improve the accuracy and usefulness of MALDI-TOF.

Recent advances in the pathogenesis of cytomegalovirus (CMV) and Epstein-Barr virus (EBV)

S375 Recent advances in the pathogenesis of EBV infection

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Epstein-Barr virus (EBV) initiates productive (lytic) infection in the epithelial cells of the oropharynx upon oral transmission. On the contrary, in EBV infected B lymphocytes the viral genomes usually persist as latent, circular episomes, co-replicating with the cellular DNA. Latent EBV genemes are associated with a series of malignant tumors as well. These include lymphomas (Burkitt's lymphoma, Hodgkin's disease, T/NK-cell lymphoma, post-transplant lymphoproliferative disease, AIDS-associated lymphoma, X-linked lymphoproliferative syndrome), carcinomas (nasopharyngeal carcinoma, gastric carcinoma, carcinomas of major salivary glands, thymic carcinoma, mammary carcinoma), and a sarcoma (leiomyosarcoma). In tumor cells the expression of latent EBV genomes is highly restricted and cell type specific, due to the epigenetic control of the viral promoters. DNA methylation, histone modifications and binding of key cellular regulatory proteins contribute to the regulation of alternative promoters for transcripts encoding the nuclear antigens EBNA 1 to 6 and affect the activity of promoters for transcripts encoding transmembrane proteins (LMP1, LMP2A, LMP2B). In addition to viral oncoproteins, non-translated RNAs (EBER1 and 2, microRNAs) may also contribute to EBV-mediated tumorigenesis. The epigenetic marks left by cellular enzymes and macromolecular complexes on latent EBV genomes define distinct viral epigenotypes that differ in transcriptional activity in spite of having an identical (or nearly identical) DNA sequence. Whereas latent EBV genomes are regularly targeted by epigenetic control mechanisms, EBV encoded proteins may, in turn, affect the activity of a set of cellular promoters by interacting with the very same epigenetic regulatory machinery. EBNA2 interacts with histone acetyltransferases, and EBNALP (EBNA5) coactivates transcription by displacing histone deacetylase 4 from EBNA2-bound promoter sites. EBNA3C (EBNA6) seems to be associated both with histone acetylases and deacetylases, although in separate complexes. LMP1 affects both systems of epigenetic memory, DNA methylation and the Polycomb-Trithorax group of protein complexes. In epithelial cells LMP1 up-regulates DNA methyltransferases and in Hodgkin lymphoma cells it induces the Polycomb group protein Bmi-1. In addition, LMP1 modulates the levels of microRNAs (miR-146a, miR-155) implicated in tumorigenesis. These interactions may result in epigenetic dysregulation and disease development.

Mycobacterium tuberculosis

O382 Drug-resistant *Mycobacterium tuberculosis* in Taiwan, 2003–2008

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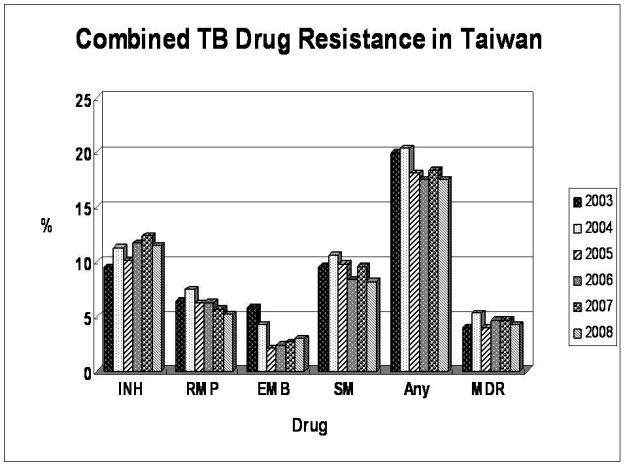
Objectives: Global surveillance of drug resistance has shown that a substantial proportion of tuberculosis patients are infected with drug-resistant *Mycobacterium tuberculosis* strains. The prevalence of drug resistance is a very important concern of the National Tuberculosis Program (NTP). To understand the extent of drug resistance in Taiwan, we initiated the Taiwan Surveillance of Drug Resistance in NTP in 2002.
Methods: The rates of drug resistance were analyzed according to a laboratory-based surveillance system. The system consists of nine contract laboratories distributed in different regions of Taiwan. Approximately, 50% of annually isolated *M. tuberculosis* was underwent antimicrobial drug susceptibility testing in the system. The external quality proficiency test was conducted by the national reference mycobacteriology laboratory using a *M. tuberculosis* panel provided by the World Health Organization supranational reference laboratory network. Since the clinical data were not available, only combined (primary plus acquired) drug-resistant rates were analyzed.
Results: In 2003–2008, the population in the survey ranged from 22,562,663 to 23,037,031. The confirmed tuberculosis cases and the incidence rate per 100,000 population were 15,042 and 66.7 in 2003, 16,784 and 74.1 in 2004, 16,472 and 72.5 in 2005, 15,378 and 67.4 in 2006, 14,480 and 63.2 in 2007, and 14,265 and 62.0 in 2008, respectively. The survey showed that the combined drug resistance rates were 9.5%-12.4% to isoniazid, 5.2%-7.5% to rifampin, 2.1%-5.8% to ethambutol, 8.2%-10.6% to streptomycin, 17.5%-20.4% to any drug, and 4.0%-5.3% to multiple drugs (Figure).
Conclusion: The incidence rates of drug-resistant tuberculosis remain relatively stable during the recent six years in Taiwan. However, this survey needs to be extended to analyze patient's clinical and epidemiologic data to reveal the reasons of drug resistance.



O383 Drug-resistant tuberculosis among foreign-born persons in Italy

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Objectives: Multidrug-resistant tuberculosis (MDR-TB) is caused by *Mycobacterium tuberculosis* strains resistant at least to isoniazid (I) and rifampin (R). In industrialized countries with high levels of immigration, MDR-TB among foreign-born persons is a serious problem but no information is known for Italy; the aim of this study was to address the extent of it in 2008–2009.

Methods: 29 laboratories were enrolled in 2007 in 18/20 Italian regions for proficiency testing (PT) of I, R, streptomycin (S), ethambutol (E) by the WHO Supranational Reference Laboratory of the Istituto Superiore di Sanità (ISS). MGIT960, BACTEC460 and proportion method in solid media were used for drug susceptibility testing (DST) in 22, 3 and 4 laboratories, respectively. DST data of patients born abroad (PBA), patients born in Italy (PBI), new cases (NC), previously treated cases (PTC) were annually sent to ISS.
Results: Efficiency (correct results/total results) of PT was high (95.5% for S, 97.6% for I, 95.7% for R, 96.2% for E). In 2008 and 2009 the TB cases in the PBA were 60.7 and 61.3% (1035/1704 in 2008 and 1528/2489 in 2009), due to immigration from 53 and 75 countries, respectively. The top-5 countries were Romania, Morocco, Peru, Pakistan, Senegal in 2008, and Romania, Morocco, India, Pakistan, Senegal in 2009. Monoresistance to I/R was 5.9/0.4% and 3.6/1.3% in PBA/PBI in 2008, and 4.8/0.5% and 2.7/0.5% in PBA/PBI, in 2009. As to MDR-TB, 89 and 92% of NC and 84 and 79% of PTC occurred in the PBA in 2008 and 2009, respectively; in the NC the top-5 countries ($\geqslant$2 cases) were Romania, Ukraine, Morocco, Moldavia, Peru in 2008 and Romania, China, Moldavia, Ukraine, Morocco in 2009. The highest rates of MDR-TB in NC were from Ukraine (19 and 11% in 2008) and Moldavia (8 and 13% in 2009). In 2009, in 27 MDR strains from the PBA, resistance (as determined by MGIT) to I, R, S, E, pyrazinamide, ethionamide, kanamycin, amikacin, capreomycin, ofloxacin, linezolid and moxifloxacin was 100, 100, 74, 26, 37, 33, 19, 19, 19, 11, 11 and 7%, respectively. A case of XDR-TB (extensively drug-resistant, i.e. MDR resistant to any fluoroquinolone and an injectable drug) was observed.
Conclusion: In 2008–2009 about 90% of the MDR-TB in the NC was due to foreign-born persons migrated in Italy from the former Soviet Union and other countries. Fluoroquinolones and linezolid were still quite active against these difficult-to-treat strains (Supported in part by the Italian CCM Projects).

O384 Six-year laboratory data report on drug-resistant tuberculosis in Lisbon health region, Portugal

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Objective: Multidrug-resistance (MDR) and extensive drug-resistance (XDR) pose a serious threat to tuberculosis (TB) management. In Portugal, the high TB incidence rates (28.7 cases/100 000 Pop in 2008) cause difficulties in the transmission control of this infectious disease. The country has high TB incidence rates in comparison with the other European Union countries. Furthermore, the high MDR- and XDR-TB rates in regions such as Lisbon Health Region hamper the TB management.
In this study we have retrospectively analyzed 3 025 *Mycobacterium tuberculosis* clinical isolates, recovered over a six-year period (2001–2006), in Lisbon Health Region.
Methods: The analysis was focused on first-line and second-line drug-resistance. The clinical strains were isolated in the Portuguese National Institute of Health – Dr. Ricardo Jorge which receives the majority of clinical specimens from Lisbon Health Region. Moreover, the clonality of a subset of isolates was assessed by 12-loci Mycobacterial Interspersed Repetitive Unit – Variable Number of Tandem Repeats (MIRU-VNTR) genotyping.
Results: We have found 22 different resistance profiles with MDR-TB rates ranging between 9.9–15.2% and, XDR-TB rates between 44.3–57.3% (excluding one year here considered as an outlier). Six MIRU-VNTR clusters were associated with MDR-TB and, of which three (Lisboa3, Lisboa4 and Q1) had XDR-TB isolates.
Conclusion: Through the present study we conclude that transmission of MDR- and XDR-TB is occurring mainly due to a limited number of genetic clusters, the majority belonging to Lisboa family. Implementation of genotyping in the diagnostic routine would most probably be useful in a timely detection of more serious types of resistance, like XDR-TB. A stronger and more efficient contact-tracing program would also bring some advantages to the halt transmission of primary drug-resistant TB.

O385 High-resolution melting analysis for rapid detection of rifampicin resistance in *Mycobacterium tuberculosis* clinical isolates

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Objectives: The worldwide emergence of multidrug resistant *Mycobacterium tuberculosis* (MDR-TB) has been reported in both developed and developing countries. Fast detection of rifampicin (RIF) resistance in *M. tuberculosis* is crucial for implementation of appropriate treatment and management of infected patients. The main objective was to elucidate the characteristics of the rpoB gene mutation in RIF-R *M. tuberculosis* strains.
Methods: Conventional methods results (Ziehl-Neelsen smear, Lowenstein-Jensen, MB/BACT cultures) were correlated with identification of *M. tuberculosis* specific sequences, by Real Time PCR technique (Primer Design TB Kit, LightScanner 32, Idaho Technology). The susceptibility tests were performed using CDC standard proportion method on LJ medium containing rifampicin (RIF) 40mg/L, isoniazid (INH) 0,2mg/L, ethambutol (EMB) 2 mg/L, ethionamide (ETH) 20 mg/L, streptomycin (SM) and kanamycin (K) 20 mg/L. Control was standard strain of *M. tuberculosis* H37Rv. The high-resolution melting (HRM) curve analysis, which detects single nucleotide polymorphisms, was used to identify rpoB mutations responsible for rifampicin (RIF) resistance. For the samples genotyping we used two-step reaction. First step is the amplification using custom Idaho primers and LightScanner Master Mix. The second step is HRM, and we used a 3′-blocked, unlabeled oligonucleotide probe (Idaho custom design) and the saturating dye LCGreen Plus to generate melting curves typical to the genotype under the probe.
Results: 525 nonrepetitive *M. tuberculosis* strains were enrolled in this study, between Jan. 2009 and Dec. 2010; all specimens were tested for drug susceptibility. 63 isolates from 525 (12%) were resistant to RIF (only RIF-R/2,1%; both RIF and INH-R/9,9%). From 63 MDR-TB, 16 strains were resistant to EMB, 22 to SM, 10 to K, 3 to ETH. We performed genotyping (HRM) for 35 MDR-TB strains: higher frequencies of mutation bearing sites found in codons 531 (21/35) and 526 (6/35) – total 77,1%. For 8 strains the mutations are under study, on other codons inside the 81 pb region of the rpoB gene.
Conclusions: This study demonstrates an association between the mutations of rpoB gene and their correlation with the predominant nucleotide change in codons 531 and 526. The mutations in codon 531 were more commonly identified in cases with high level RIF-resistance, in the MDR-TB isolates from our geografic area.

O386 Inhibiting the β-lactamase of *Mycobacterium tuberculosis* (Mtb) with novel Boronate-Transitional-State-Analogs (BATSAs)

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Introduction and Rationale: Currently, 4-drug regimens are the cornerstone of treatment against Mtb pulmonary infections. Against infections with multiple- (MDR) and extensively-drug-resistant (XDR) strains, therapeutic choices are limited and new options are sought. β-lactams and β-lactamase inhibitors are not routinely used to treat Mtb infections, since mycobacteria are considered to be resistant. BlaC, a β-lactamase expressed in Mtb, is identified as the major enzyme responsible for this resistance. BlaC which is capable of inactivating a broad range of penicillins and cephalosporins belongs to the class of β-lactamases which are usually susceptible to inhibitors such as clavulanate (Ambler Class A). The combination of carbapenems and clavulanate recently attracted attention as this combination was found to be very effective in sterilizing Mtb cultures, including extensive drug

resistant (XDR) strains. However, kinetic studies revealed that clavulanic acid, compared to its effect in other Class A enzymes, is not as potent an inhibitor. This poor inhibition may set the ground for development of inhibitor-resistance. Therefore, novel β-lactamase inhibitors that are effective against BlaC could play an important role in the treatment of MDR or XDR infections.

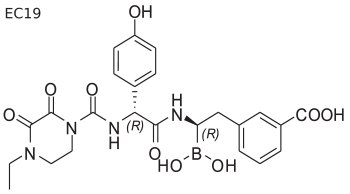
We hypothesized that boronate-based compounds, which sterically resemble the quarternary transition state of the β-lactam (Boronic-Acid-Transitional-State-Analogs, BATSAs) would inhibit Mtb β-lactamases with promising results. To this end, we tested a panel of 20 BATSA compounds in order to find candidates with excellent inhibitory properties against BlaC.

Methods: The blaC gene was cloned and expressed in *E. coli* BL21(DE3) and purified. Spectrometric assays were performed using Nitrocefin as a reporter substrate to determine the Ki of 20 BATSA-compounds.

Results: 5 compounds demonstrated Ki values of less than 5 microM:

	Ki (microM ±10%)
EC19	0.65
SM23	1.46
EC04	2.76
CO10	3.34
TaxTSI	4.13

Conclusion: From a collection of 20 compounds, we identified 5 novel BATSAs with effective inhibitory properties against Mtb BlaC. EC19, a cefoperazone analog, demonstrated sub-micromolar Ki. Comparing the structure of 6 cefoperazone analogs, we advance that a benzene-carboxylate moiety is crucial for effective inhibition. Systematic analysis of how structural variation influences binding gives insight into the role specific amino acids play in stabilisation of the transitional state.



Cellular and molecular immunology

O387 Characterising pandemic H1N1 cross-reactive influenza cytokine and T-lymphocyte responses induced by seasonal intranasal live-attenuated influenza vaccine

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Objectives: Re-assortment of influenza viruses leads to changes in surface glycoproteins, rendering the antibody responses directed at hemagglutinin largely ineffective. Generating cross protective responses has been the "holy grail" of influenza vaccine development. Various studies suggest that the use of a live-attnuated, mucosally administered vaccine may result in better induction of cellular immunity and thus a potential for cross protection., however the presence of blocking antibodies at the mucosa, may decrease the ability of live virus to generate the desired cellular immune response. We sought to determine the cross reactive responses induced by live attenuated influenza vaccine (LIV) in a vaccine naive population.

Material and Methods: Flumist LIV was purchased from MedImmune. The research was approved by local ethics boards. We enrolled 60 HIV negative Women from a commercial sex workers cohort in Nairobi, Kenya. Blood and mucosal samples were obtained prior to vaccine administration and after 1,7, 30 days and 4–6 month after enrolment. Plasma levels of cytokines were determined using 19 cytokine bead array (Millipore). PBMC's were stimulated ex-vivo with Influenza A/H1N1Brisbane (BRIS) corresponding to the H1N1 strain included in the seasonal formulation as well as with Influenza A/H1N1 Mexico 2009 (MEX) and analyzed using multi-parametric flow cytometry including markers of cell lineage, memory subsets (CCR7, CD45RA) and immune activation using CD38, HLA-DR, CCR5. Cytokine production was measured using intracellular staining. Proliferation and NK function were assessed using two separate panels. Levels of cytokines in the culture supernatant following stimulation were measured using 19 cytokine bead array (Millipore).

Results: Immune activation and cytokine production by CD4 and CD8 T lymphocytes increased in response to the vaccine component as well as cross reactive responses against MEX were demonstrated. The kinetics and levels of immune activation, cytokine production and proliferation in response to antigen specific stimuli will be presented.

Discussion: The study illustrates the ability of a LIV to induce cross-reactive influenza responses as measured by activation and cytokine production of CD4 and CD8 lymphocytes. It remains to be determined whether the cross reactive responses afford cross protection against a heterotypic influenza challenge.

O388 Mannose-binding lectin concentrations and infections in haemato-oncology patients: preliminary results

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Objectives: Mannose-binding lectin (MBL) is a secreted pattern recognition receptor capable of binding pathogens and activating the classical complement cascade. MBL deficiency is frequent due to genetic polymorphisms. We describe preliminary results of a study of serum MBL concentrations and infection in haemato-oncology patients.

Materials and Methods: Adults likely to be rendered neutropenic were recruited. MBL was assayed in baseline serum samples by ELISA. Patients were followed until death, discharge or four months after last admission for chemotherapy and/or transplantation. Microbiology results were recorded prospectively. Categorical and continuous variables were analysed by Fisher's exact and Wilcoxon Ranksum tests, respectively.

Results: Of the first 100 recruits, baseline sera were available for 95: age range 19–73y (median 52y; inter-quartile range 40–59y); 52 (54%) were female; follow-up time: 4–468d (212d;107–315d). Baseline MBL concentrations ranged between the ELISA limits of 5 and $\geqslant$4000 ng/mL (1600;390–3500ng/mL); one and 20 patients had 5 and $\geqslant$4000 ng/mL, respectively. Firstly, patients with baseline MBL $\leqslant$1500ng/mL (n = 47) were compared to those with MBL >1500ng/mL (n = 48): these groups did not differ with respect to age, sex and follow-up time, but a laboratory-confirmed bacterial or fungal infection was identified in 77% and 58%, respectively (P = 0.08), and in 75% and 50%, respectively, in the first 75d (P = 0.02). Time to first Enterobacteriaceae positive blood culture was shorter in the lower MBL group (median 8 vs. 21d; P = 0.033). Secondly, patients with baseline MBL <500ng/mL (n = 26) were compared to those with MBL $\geqslant$500ng/mL (n = 69): these groups did not differ with respect to age, sex and follow-up time, but median time to first positive blood culture (any organism) was shorter in the low MBL group (8 vs. 17d; P = 0.0275) as was median time to first Enterobacteriaceae positive blood culture (6 vs. 19d; P = 0.023).

Conclusions: This preliminary analysis suggests an association between a lower serum MBL concentration and increased infection risk. Analysis of the completed cohort may clarify whether there is a specific risk of infection with Enterobacteriaceae rather than all Gram-negatives, including others encountered in these patients (eg *Pseudomonas aeruginosa* and other 'non-fermenters').

O389 *Pseudomonas aeruginosa* vs. the innate immune system: understanding bacterial response to macrophage engulfment

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Objective: *Pseudomonas aeruginosa*, an emerging multi-drug resistant human pathogen, encounters innate immune system defences like the

release of reactive oxygen species (ROS) by macrophages upon host infection. Based on our previous work which demonstrated a role for soluble quinone oxidoreductase (QOR) enzymes in protecting a related bacterium (*Pseudomonas putida*) from oxidative stress, we hypothesized that *P. aeruginosa* would similarly utilize QORs to withstand ROS. However, when individual genes encoding QORs are deleted from *P. aeruginosa*, no apparent ROS sensitivity is seen. Our main objective now is to characterize the global genetic profile of macrophage-challenged *P. aeruginosa* thereby identifying genes promoting virulence of the bacterium by enabling it to resist host oxidative defences, including the oxidative burst of activated murine macrophages. In doing so, we hope to identify potential therapeutic targets for the treatment of *P. aeruginosa* infections.

Methods: In the process of characterizing the *P. aeruginosa* response to murine macrophages, we have developed a method for isolating internalized bacteria for analysis. Murine macrophages are infected with *P. aeruginosa*. Non-internalized bacteria are then killed by antibiotic application and washed away. The bacteria-containing macrophage is then allowed to mature up to 8 hours under normal culture conditions. The infected macrophages are then lysed by detergent. The internalized bacteria are isolated by a series of differential spins, analyzed by flow cytometry, confocal microscopy and by microarray.

Results: Preliminary results indicate that *P. aeruginosa* undergoes a potential phenotypic switch two hours post infection not previously seen by conventional survivability measurements (e.g. colony forming unit counts). Additionally, we have identified several New Zealand clinical isolates of *P. aeruginosa* that respond differently to macrophage interaction when compared to the laboratory strain.

Conclusions: The application of an in vivo approach to assessing bacterial response to infection may allow us to observe a more "realistic" infection scenario for *P. aeruginosa*. Initial results incorporating flow cytometry have allowed us to select ideal candidates for microarray analysis. This work will give us a global view of the genetic response *P. aeruginosa* undergoes when encountering the innate immune system, thus taking us one step closer to identify therapeutic targets to counter *P. aeruginosa* infections.

O390 Cytokine response of Th17 and oral lactobacilli in chronic periodontitis

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Objectives: Etiopathogenesis of chronic inflammation in periodontitis still remains unclear. Therefore, present study aimed at analysis of Th 17 cytokine response and of oral lactobacilli involvement in chronic periodontitis.

Methods: The studies were conducted on 24 patients (35–49 years of age) with chronic periodontitis. Clinical criteria (GI, Pl.I, CAL) permitted to distinguish the following research groups: 14 patients with moderate chronic periodontitis (group 1) and 10 patients with severe chronic periodontitis (group 2). The research material involved gingival fluid sampled with 1 ml syringe with a thin needle from three most deep periodontal pockets. The obtained gingival fluid was tested for presence of IL-17, TNF-α and bacteria of *Lactobacillus* genus, producing or not producing H2O2. Estimation of gingival fluid IL-17 and TNF-α levels were conducted by ELISA, using Quantikine Human IL-17 and Quantikine HS Human TNF-α Immunoassay kits (R&D Systems). In turn, *Lactobacillus* spp. were cultured on Rogosa agar and the cultured isolates obtained in anaerobic conditions were identified using API 50 CHL (bioMerieux). For detection of hydrogen peroxide production by the isolated *Lactobacillus* spp. TMB-Plus agar was applied.

Results: In both groups of patients periodontal pockets contained high concentrations of studied cytokines. In group 1 mean values of IL-17 and TNF-α amounted, respectively, to 19.66±6.1 pg/ml and 4.95±0.91 pg/ml. In turn, in group 2 mean values of IL-17 and TNF-α amounted to 34.7±6.65 pg/ml and 6.68±0.72 pg/ml, respectively, and proved to be higher than those obtained in group 1 ($p = 0.0028$ and $p = 0.0003$, respectively). In group 1 presence of *Lactobacillus* spp. producing H2O2 was disclosed in 10 (71.4%) patients, in group 2 such bacteria were present in 2 (20%) patients while in 3 (30%) patients *Lactobacillus* spp. bacteria were present which did nor produce H2O2.

Conclusion: Chronic inflammation of periodontal tissues is associated with cytokine response of Th 17 cells. Intensity of the response may determine progression of the pathological process, while oral lactobacilli which produce H2O2 may restrict development of infection with periodontal pathogens.

O391 Increased expression of CD11A and CD45 and TH2 predominant immune response in isolated coronary artery ectasia patients

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Objective: Aneurysm and ectasia have similar pathological pathways. TH2-associated cytokines are stimulated by aneurismal tissse and correspondingly lack mediators associated with TH1 response. We detected serum TNF-α and IL-18 levels which are strong TH1 stimulating cytokines and also investigated the expression of adhesion molecules CD11a, CD11b, CD18; and CD45 on leukocytes and anti *Chlamydia pneumoniae* and *Helicobacter pylori* serum antibodies in coronary artery ectasia (CAE) patients and normal coronary artery (NCA) controls.

Methods: 51 isolated CAE patients free of atherosclerosis, and 37 NCA controls were included in the study. Cell surface adhesion molecules were detected by flow cytometry using fluorescence conjugated monoclonal antibodies. Serum TNF-α, IL-18, *C. pneumoniae* IgG and IgM and *H. pylori* IgG levels were detected by ELISA method.

Results: The mean fluorescent intensities of CD11a on granulocytes, monocytes and lymphocytes (10.01±8.2 vs. 6.79±3.49, $p = 0.04$; 15.84±8.64 vs. 11.56±5.27, $p = 0.016$; 29.58±9.98 vs. 20.02±9.66, $p < 0.001$; respectively)and CD45 on granulocytes and monocytes (7.58±5.03 vs. 4.57±3.05, $p = 0.003$ and 18.73±12.38 vs. 10.74±7.38, $p = 0.004$ respectively) were significantly higher in CAE patients, when compared with NCA group. TNF-α levels were significantly lower in patient group (18.76±7.07 vs. 24.29±8.46; $p < 0.001$) and IL-18 levels were lower in patient group without significance, when compared with controls (519.47±221.35 vs. 621.65±287.76; $p = 0.063$). The percentage of granulocytes was higher in CAE group when compared with control (NCA) group (65.52±14.91 vs. 52.28±15.37; $p = 0.002$). *C. pneumoniae* serum IgG levels were significantly higher in patient group when compared with controls (81.62±48.53 vs. 63.79±33.83; $p = 0.045$). In CAE patients, TNF-α levels were significantly correlated with CD45+ granulocyte (0.525, $p < 0.001$), CD45+ monocyte (0.469, $p = 0.001$) and lymphocyte (0.376, $p = 0.013$) mean fluorescent intensity levels.

Conclusion: The decreased levels of TNF-α and IL-18 may indicate TH2 predominant and TH1 lacking immunity in CAE patients, similar as in aortic aneurysm patients. Increased levels of cell surface adhesion molecules in CAE may be an indicator of activation of leukocytes for adherence and transmigration through the vessels for the initiation of inflammation.

Under your skin: skin manifestations of parasitic diseases

S398 Toxocariasis in patients with skin disorders

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Toxocariasis is a disease consequent to visceral migration of dogs' and cats' ascaris larvae, *Toxocara canis* or *T. cati*. People become infected accidentally eating *Toxocara* infected eggs or eating row tissue (cow/lamb liver). Tissue migrating larvae are trapped in eosinophilic granulomas, mostly in liver and lungs, but any organ including eye or CNS can be involved. Clinical forms: Visceral Larva Migrans, Ocular Larva Migrans, Covert Toxocariasis and asymptomatic

patients. VLM is considered when following signs are present: hepato-splenomegaly, diarrhea, digestive disorders, dry cough, wheezing, fever, limphadenopaty, anorexia, malnourishment, skin disorders, pallor, asthenia, anorexia, cardiac and neurological signs. Positive serology and hipereosinophylia are the most relevant markers of infection activity. Skin disorders are present in about 24% of symptomatic cases. Urticaria and angioedema are quite often associated with allergic reactions, like asthma, rhinitis, conjunctivitis. About 17–65% of cases with chronic urticaria are confirmed with VLM, or have a positive serology for *Toxocara* with good evolution after antiparasitic treatment. Facial oedema and even laringial angioedema, requiring emergency interventions, can be present, as well as palpebral and lips edema. Pruritus is very common in Toxocariasis; sometime scratched skin is associated (all skin lesions are very itching). Acute or chronic pruritus, sometimes generalized or localized either on sole and palms, or on thorax, sacrum or nodular pruritus can be noticed. Skin rash (papular or purpuric), polymorphic eritema, hypodermal lesions and even Fiessinger-Leroy-Reiter syndrome can be associated. Eczema, dishydrotic or nodular lesions occur on palms and soles. Atopic eczema can be aggravated by Toxocariasis. *Toxocara* spp. is considered now etiologic factor for Wells syndrome (eosinophilic cellulites), together with other parasites, insect bites, infections or hematological disorders. In atopic patients, co-infection with *Toxocara* spp. amplifies the symptoms; sero-prevalence of Toxocariasis is higher in asthmatic patients than in normal population. Total and specific IgE anti-*Toxocara* are increased in RAST positive patients. CT associates skin manifestations and allergies together with asthenia, bronchospasm, hepatomegaly, digestive complaints, limphadenopaty, myalgia, asthenia. OLM is almost always an independent condition, being rarely consequent to VLM. Any ocular segment can be involved.

S399 Maggot debridement therapy

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Maggot therapy is successfully used for the treatment of wound infections. The underlying mechanisms of action of maggot therapy are unknown, but could provide information for a novel treatment modality against infection, which is important in these times of increasing antibiotic resistance. Therefore, in this research the effect of living maggots on planktonic cells was investigated. Furthermore, the influence of maggot excretions/secretions (ES) on planktonic cells, on bacterial biofilms and on complement activation was tested.

Sterile tubes were filled with living maggots in a bacterial suspension and every two hours samples were cultured and compared with controls. A turbidimetric assay was performed to test the susceptibility of six bacterial species to ES. Bacterial biofilms were formed in vitro on polyethylene, stainless steel and titanium and ES were added to test their influence. The effect of ES on complement activation was investigated in healthy donor sera and in pre- and postoperatively gained sera from trauma patients. Different immunoassays, that are also clinically used to determine complement deficiencies in patients, were performed in absence or presence of maggot ES.

The results show that living maggots as well as their ES stimulate the bacterial growth of *S. aureus*, *E. faecalis*, CNS, *S. pyogenes* and *K. oxytoca* (all p-values $\leqslant 0.0002$). The strongest biofilms in vitro were formed by *S. aureus*, *S. epidermidis* and *P. aeruginosa*. ES were added to these biofilms and reduced these on all biomaterials. The maximal biofilm inhibition by ES was seen on polyethylene: 82% for *P. aeruginosa* ($p < 0.0001$), 61% for *S. aureus* ($p < 0.0001$) and 92% for *S. epidermidis* ($p < 0.0001$). Furthermore, ES reduced complement activation in sera from healthy and postoperatively immune-activated sera up to 99.9% ($p < 0.0001$), via all three pathways of complement activation.

This study shows that nor living maggots, neither maggot ES have direct antibacterial properties. However, ES do reduce biofilms and complement activation. The biofilm reduction and the immunosuppressive effect of maggot ES may explain part of the improved wound healing caused by maggot therapy. Furthermore, the biofilm- and complement-inhibitor(s) present in maggot ES could provide novel treatment modalities for various diseases, e.g. (chronic) infections in trauma patients. Future research focuses on the identification and isolation of the effective substance(s) in ES of the *Lucilia sericata* larvae.

Infective endocarditis: lights and shadows

S404 The changing epidemiology

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Epidemiology of endocarditis has considerably changed in the last decades as a consequence of medical progress, although geographical differences still exist. The median age of incidence in developed countries has grown from 40 years in the fifties to 70 years at present. Risk factors have changed accordingly. Rheumatic valve disease and drug addiction are still more frequent in developing countries and in younger patients, whereas in the western world nosocomial acquisition, immunosuppression, dialysis, diabetes mellitus and cancer predominates in the elderly population. In these countries the proportion of responsible organisms have changed as well. Instead of viridans Streptococci that were the most common etiological agent in the past, *Staphylococcus aureus* represents nowadays the leading pathogen, and community-acquired methicillin-resistant *Staphylococcus aureus* is emerging. Similarly, the increase of invasive procedures in elderly patients and the growing number of cardiac device implants has caused an increase of infections by skin bacteria such as coagulase-negative Staphylococci and *Propionibacterium acnes*, that in the past were considered as contaminants. The increase in population age has also caused an increase of infections due to bacteria that are commensals of intestinal and genitourinary flora such as *Streptococcus gallolyticus*, particularly in mediterranean countries, and Enterococci. Furthermore, we are facing the return of Gram negative bacteria and a worrying increase of fungi of nosocomial origin. Lastly, the introduction of new serological and molecular diagnostic tests have highlighted the major role of difficult to grow organisms, such as *Coxiella*, *Bartonella*, and *Tropheryma whipplei* in cases that were considered in the past culture-negative endocarditis. Hospital-acquired organisms harbour antibiotic resistant genes that cause problems in treatment. Therefore, despite the progresses in diagnosis and surgical treatment, mortality from endocarditis remains high.

S406 Culture-negative endocarditis: a diagnostic conundrum

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Blood culture negative endocarditis (BCNE), i.e. endocarditis in which no causative microorganism can be grown in a culture taken from a blood sample using usual laboratory methods, acount for 2.5 to 31% of all cases of endocarditis. This variation in incidence among hospitals may be explained by several factors including: i) differences in the diagnostic criteria used; ii) variations in early use of antibiotics prior to blood sampling; iii) involvement of unknown pathogens; iv) specific epidemiological factors, as for fastidious zoonotic agents and v) non-infectious aetiologies. Among fastidious microorganisms, *Coxiella burnetii*, the agent of Q fever, and *Bartonella* sp. appear to be the most common. Other bacteria, including *Brucella* sp., *Abiotrophia* sp., *Tropheryma whipplei*, *Legionella* sp., and mycobacteria are less frequent. Since 2001, we have used a systematic protocol that included serology assays, detection of auto-antibodies, PCR from blood and, when available, culture and PCR from valvular specimens. To this protocol were added in 2009 the detection of antibodies to pork in patients who had a porcine valvular bioprosthesis. Over 10 years, we studied 1,334 patients with blood culture negative endocarditis, including 1,230 classified as possible or definite using the Duke criteria. Our strategy enabled us to identify an aetiological agent in 775 patients, including *C. burnetii* in 54.2% of cases, *Bartonella* sp. in 19.2%, and other fastidious bacteria in 4.1% (*T. whipplei* 2.6%). Overall, fastidious bacteria accounted for 77.5% of all diagnoses. In addition to infectious

causes, we detected 10 cases of marantic endocarditis, 12 cases of Libmann-Sacks endocarditis, 4 other auto-immune diseases, and one case of relapsing BCNE in whom the only possible cause was allergy to pork. We suggest that investigation of BCNE should systematically include *C. burnetii* and *Bartonella* sp. serologies, broad range PCR assays and histological examination when valvular specimens are available, and detection of auto-antibodies.

Virology (non-HIV/non-hepatitis)

O408 Upsurge of human enterovirus 68 infections in patients with severe respiratory tract infections

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Objectives: Human enterovirus 68 (EV68) is member of human enterovirus species D. It is unique among enteroviruses because it has properties of both enteroviruses and human rhinoviruses (HRV). EV68 is found to be highly similar to HRV87 and is almost exclusively associated with respiratory disease. Reports of EV68 infection are uncommon; however, during the second half of 2010 we detected an increased amount of patients with EV68 infection in association with serious respiratory disease. In order to determine if changes in the virus could account for the upsurge of EV68 infections, we characterized the clinical isolates and compared these with isolates found in 2009. We furthermore related these findings to clinical data.
Methods: EV68 clinical isolates were identified by sequencing of the VP4/VP2 genomic region. Further characterization of the isolates was done by partial sequencing of the VP1 capsid gene (728 base pairs). Sequences were aligned with Clustal W 2.0, and phylogenetic trees were constructed by the neighbour-joining method using MEGA 4.0. Clinical data were systematically collected.
Results: From August till November 2010, 22 patients were identified with EV68 infection (out of 111 patient samples collected for respiratory viral detection). Clinical isolates of 4 patients collected in 2009 were added. Sequence data showed that the majority of the isolates detected in 2010 were phylogenetically distinct from those found in 2009. The 2010 isolates contained a 3 nucleotide deletion in the VP1 genomic region as compared with the isolates of 2009. Hospital admission was necessary in 22 out of 26 patients. Median age of patients with EV68 infection was 6 years (range 0–72). 84% of the patients had a chronic underlying illness. Detection of EV68 was associated most notably with lower respiratory tract infections (pneumonia, exacerbation asthma, wheezing). Two patients required mechanical ventilation because of infection with EV68. All but one patient had only EV68 detected as possible viral cause of illness. No bacterial cause of infection was identified. All patients recovered.
Conclusions: In the second half of 2010 an upsurge of EV68 detection in patients with severe respiratory tract infections was observed. Genetic changes in the virus might be associated with these observations.

O409 Occurrence and characteristic of viral agents in respiratory tract infection in Nigerian children

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Acute respiratory infections (ARI) are the most common cause of death in children in developing countries and little information is available on risk factors for mortality among African children presenting with symptoms compatible with acute respiratory.
Objectives: The study was designed to identify viral agents responsible for respiratory infections among young children in Nigeria, to define the incidence of respiratory viral agents in children suffering from ART, and to identify the role of newly discovered viruses among West African children.
Methods: Using conventional real time RT-PCR, we discovered that all major respiratory viral pathogens are well represented in specimens of the children.
Results: Out of 246 nasal and throat specimens collected from ARI patients, 77 percent contained the genome of at least one viral agent, as shown by molecular techniques. Human rhinoviruses (HRVs) was the most prevalent present in 87 (36%) of cases, followed by parainfluenza virus (PIV) type 3, detected in 50 (20.3%), human enterovirus, 35 (14.2%); PIV type 2, 21 (8.5%); PIV type 1, 15 (6.1%); adenovirus, 12 (4.9%); influenza virus C, 12 (4.9%); human metapneumovirus (hPMV), 7 (2.8%); human bocavirus (HBoV), 6 (2.4%); influenza virus A, 3 (1.2%); influenza virus B, 2 (0.8%); respiratory syncytial virus (RSV), 1 (0.4%). HRV strains were subjected to partial genomic sequencing in the VP4/VP2 capsid protein coding region followed by phylogenetic analysis. We disclosed the genetic type of 17 of these strains.
Conclusion: Our study underlines the importance of viruses in the pathogenesis of respiratory infections of children in Nigeria. These results are among the first genetic typing results of HRV in the African continent and confirm the global circulation of the novel HRV-C strains.

O410 Clinical impact of HSV-1 detection in the lower respiratory tract from hospitalised adult patients

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Objectives: HSV types 1 and 2 are highly prevalent alfa-herpesviruses, that may reactivate from latency in favouring conditions. HSV (particularly, but not exclusively HSV-1) has been detected in respiratory specimens from different categories of patients, as well as in up to 5% of asymptomatic adults. Occasionally, HSV has been associated to pneumonia in immunocompromised and critically ill patients. Given the availability of an effective antiviral agent (i.e. acyclovir), it is relevant to evaluate the potential clinical impact of HSV in the lower respiratory tract. Herein, the occurrence and clinical role of HSV in bronchoalveolar lavage (BAL) specimens from hospitalized adult patients were studied.
Methods: In a one-year period 265 BAL specimens from 203 patients (129M/74F, mean age 56.2 years; 85 transplant recipients, 27 other immunocompromised patients, and 91 immunocompetent individuals) were tested for HSV-1 and -2 by real-time PCR (R-gene™, Argene). A diagnostic panel including community-acquired respiratory viruses and other herpesviruses, bacteria, and fungi was also evaluated. Demographic and clinical features, including mortality within 28 days, were evaluated.
Results: HSV-1 resulted positive in 75/265 (28.3%) specimens from 66/203 (32.5%) patients, with no difference according to age-group, immune status, transplant status or other clinical features. Two specimens were positive to HSV-2. Viral load was 652970 genome copies/ml BAL (mean) and $<1.5\times10^4$ for HSV-1 and -2, respectively, and tended to be higher in patients with a discharge diagnosis of pneumonia. Co-infections with other respiratory pathogens were detected in 16.6% of specimens. HSV-1 resulted significantly more frequent in mechanically ventilated vs non-ventilated patients ($p < 0.01$). Mortality within 28 days occurred in 21 cases, 12 of which HSV-1 positive (p = n.s.). Acyclovir treatment resulted efficacious in the remaining patients (HSV-negativity confirmed at subsequent sampling in 73% of cases).
Conclusion: HSV-1 DNA is frequently detected in different categories of patients, without distinction according to immunological status, transplant status, or other clinical features. Critically ill patients (i.e. mechanically ventilated) appear to be a category at particular risk of lower respiratory tract involvement by HSV. The availability of an efficacious treatment suggests the need to include HSV in the diagnostic panel for respiratory pathogens.

O411 PMOplus™ antisense oligomers protect nonhuman primates against Ebolavirus and Marburgvirus

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Objectives: Zaire ebolavirus (ZEBOV) and marburgvirus (MARV) are highly virulent emerging RNA viruses of the family Filoviridae and are causative agents of viral hemorrhagic fever (VHF). Currently, no antiviral therapeutics or vaccines are licensed for treatment or prevention of ebolavirus or marburgvirus infections. The postexposure therapeutic efficacy of a new class of phosphorodiamidate morpholino oligomers (designated PMOplus), containing positively charged piperazine moieities, was explored using nonhuman primate (NHP) models of ZEBOV and MARV infection.
Methods: Infected with a lethal dose of ZEBOV, NHPs were treated using i.v. injection with either one of four doses of AVI-6002 [a PMOplus combination therapeutic specific to ZEBOV viral protein (VP) 24 and VP35], a negative-control PMOplus agent, or PBS beginning 30–60 min following infection and continuing through day 14. NHPs infected with a lethal dose of MARV were treated i.v. with either one of three doses of AVI-6003 [a PMOplus combination targeting MARV nucleoprotein (NP) and VP24], a negative-control PMOplus agent, or PBS according to same postexposure regimen used for the EBOV investigation. Both studies were conducted using randomized, single-blind experimental methods and all PMOplus treatments contained 4–5 animals. Animal health was monitored at least twice daily for 28 days, and blood was sampled periodically during the study to monitor viremia and clinical pathology parameters. Additionally, viral genomic material obtained from infected animals was sequenced to evaluate potential development of resistance to the therapies.
Results: While control NHPs succumbed to ZEBOV infection by day 8, 60% of the animals treated with either 28 or 40 mg/kg AVI-6002 survived to study termination. Treatment of 30 mg/kg AVI-6003 completely protected NHPs against lethal MARV challenge and protected 60% of animals when delivered at 7.5 and 15 mg/kg doses. AVI-6002 and AVI-6003 ameliorated VHF disease signs, reduced circulating levels of hepatocellular markers of liver damage, and generally decreased viremia in surviving animals. Preliminary viral genomic sequence analyses suggest that neither ZEBOV nor MARV readily adapted to the antiviral selective pressure of AVI-6002 or AVI-6003, respectively.
Conclusions: These results establish PMOplus as effective therapeutic agents against multiple highly virulent human pathogens and support the further development of PMOplus therapies for use in humans.

O412 The role of WU and KI viruses: pathogens or passengers?

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Objectives: In 2007, two new human Polyomaviruses, KI polyomavirus (KIV) and WU polyomavirus (WUV), were discovered. Genetically these viruses are related to JCV and BKV, but cluster into a separate lineage. In contrast to JCV and BKV, WUV and KIV have predominantly been found in respiratory tract secretions from pediatric patients. Information on their prevalence and disease associations are still ongoing. It has been suggested that these viruses more likely reactivate in immunocompromised patients and might be responsible for disease.
The objective of our study was to determine the prevalence of WUV and KIV infections in respiratory samples and to collect and analyse the clinical information of patients tested positive within the University Medical Center Groningen. This is a tertiary referral hospital for both adults and children and has a large solid organ transplant program.
Methods: Respiratory samples collected from September 2007 to August 2009, were tested using RT-PCR methods. In total 1964 respiratory samples from 1126 patients were tested, regardless of age, underlying disease or immune status. Subsequently, clinical information of the patients tested positive for either WUV or KIV were retrospectively collected.
Results: 60 (3.05%) samples tested positive for KIV and 71 samples (3.62%) tested positive for WUV.
No significant seasonal distribution was found for WUV and KIV.
Co-infections with other respiratory pathogens were found in 35 (58%) of the KIV and 47 (66.2%) of the WUV positive samples. Three samples were found positive for both WUV and KIV.
In total, 57 patients tested positive for WUV, 6 of these patients were adults and all adults were co-infected with other respiratory pathogens. WUV mainly infected young children without severe respiratory symptoms. In contrast, 18 of the 40 patients tested positive for WIV were adults. Half of these adults were found without a viral co-infection and 72% of these adults were severely immuno-compromised.
Conclusions: High percentages of co-infections with other respiratory pathogens complicated the interpretation of the clinical features in these patients. However, interestingly, the majority of adults only positive for KIV were immuno-compromised patients. These results indeed are suggestive that KIV and to a lesser extent WUV may reactivate in solid organ transplant and stem cell transplant patients.

O413 Asymptomatic Crimean-Congo haemorrhagic fever infection in endemic regions

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Objectives: Crimean-Congo haemorrhagic fever (CCHF) is a disease generally transmitted to humans by infected tick bites or through contact with blood and tissues from viremic animals, and causes viral haemorrhagic fever. The virus may cause asymptomatic disease in animals, though its asymptomatic course in humans is unknown.
This study was intended to investigate the seroprevalence of Crimean-Congo haemorrhagic fever virus (CCHFV) in patients confirmed as having undergone CCHF and in volunteers in the same high-risk environment, and to determine whether the disease occurred asymptomatically, together with the risk factors involved.
Methods: CCHFV IgG from serum from friends/relatives of patients monitored with a diagnosis of CCHF at our clinic in 2004–2008 and sharing the same epidemiological characteristics was examined using the ELISA technique at the Refik Saydam Institute of Hygiene virology laboratory.

Table 1: Risk factors for CCHF undergone asymptomatically

Risk Factors	IgG (+) n=85	IgG (-) n=540	P	OR	95%CI
Age	47.6±16.3	41.5±18.6	0.005		
Sex (F/M)	52/33	315/225	0.621	1.13	0.69-1.85
Farming	68	393	0.146	1.59	0.87-2.95
Animal husbandry	60	306	0.021	1.84	1.09-3.11
Contact with animals	76	424	0.029	2.31	1.08-5.10
Animal slaughtering	22	123	0.635	1.18	0.67-2.05
Sharing a house with a CCHF-positive patient	34	238	0.481	0.85	0.52-1.38
Close contact with a CCHF patient	21	169	0.267	0.72	0.41-1.25
Tick contact	70	310	0.00002	3.45	1.87-6.46
Tick bite	18	103	0.770	1.13	0.62-2.06
Removing ticks by hand	59	258	0.0003	2.48	1.48-4.18
Living in rural areas	79	412	0.001	4.05	1.65-10.56

Results: Serum specimens were taken from 625 volunteers and analyzed using ELISA and specific IgG antibodies. IgG positivity was determined in 85 volunteers (13.6%). None of the volunteers with IgG positivity had a history of symptomatic infection. Age (P = 0.005), working in animal husbandry (P = 0.021: OR 1.84), contact with animals (P = 0.029; OR 2.31), contact with ticks (P = 0.00002; OR 3.45), removing ticks by hand (P = 0.0003; OR 2.48) and living in rural areas (P = 0.001; OR 4.05) were determined as statistically significant risk factors in these individuals who had undergone the disease asymptomatically. Living in the same house as a patient did not constitute an additional risk

(P = 0.481), but living in the same environment and under the same conditions did represent a major risk factor (Table 1).
Conclusion: Our study shows that CCHF can be undergone asymptomatically. Living in an area in which CCHF is endemic and contact with ticks was established as the most important risk factors.

O414 Prevalence of the newly described human enterovirus 109 in infants with acute respiratory disease in Italy

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Objectives: A novel human Enterovirus (HEV) type within the species HEV-C, named EV109, was discovered and characterized from a case of acute respiratory illness in a Nicaraguan child in September 2010; EV109 isolates were then detected in 1.6% of respiratory samples of children with influenza like illness (ILI) in Managua, Nicaragua. Given the very recent discovery, no studies have been still performed to evaluate epidemiological features nor the distribution of EV109 in the different countries all over the world. In addition, due to the wide variety of enterovirus-associated diseases the full spectrum of EV109 disease association have to be evaluated.
In the present study, the presence and the role of EV109 were examined retrospectively in infants with acute respiratory disease (ARD) hospitalized in a pediatric department in northern Italy.
Methods: From June 2005 to September 2006 a total of 149 nasopharingeal aspirates were collected from patients and tested for the presence of EV109 by reverse transcription-PCR (RT-PCR). The presence of Respiratory Syncytial viruses (RSV), human Metapneumoviruses (hMPV), human Bocaviruses (hBoV), human Coronaviruses (hCoV), Adenoviruses, Influenza A and B viruses, Parainfluenza viruses (PIV1–3) and human Rhinoviruses was also assayed by PCR and clinical symptoms were evaluated.
Results: Four out of 149 samples (2,68%) tested positive for EV109 genomic sequences. Positive specimens were found in infants with a median age of 3,5 months (range 2–6 months) and occurred in winter (3 cases) or in late autumn (one case). Infants positive for EV109 infection had bronchiolitis (3 cases) or asthmatic bronchitis (one case). Single EV109 infection was detected in only one infants, whereas the remaining three cases were co-infected with RSV (2 cases) or hCoV HKU1 (one case).
Conclusions: Overall, results obtained in this study, suggests that EV109, in hospitalized infants with ARD in Italy, have a circulation slightly higher than that observed in children with ILI in Nicaragua. Furthermore, given the presence, in the majority of EV109-positive infants, of other viruses, including RSV, larger studies are needed to best investigate the pathogenetic role of the novel human enterovirus both in single infections and co-infections.

O415 Different rates of spontaneous clearance of HPV infections in anal and cervical lesions

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Objectives: HPV is a risk factor for the onset of both cervical and anal carcinoma. The aim of the study is the estimate of the persistence and clearance of the different genotypes of HPV in male anal and female cervical samples during a one-year follow-up period.
Methods: We analyzed 440 cervical samples from 220 female patients (CF group) and 46 anal samples from 23 male immunodeficient patients (AM group). For each patient, two samples were collected after an interval of one year. 52 female and 11 male patients underwent a biopsy within the follow-up period. The remaining 172 female and 12 male patients did not undergo a biopsy. The samples were tested for the presence of HPV-DNA and genotyped by a PCR-based molecular method.
Results: The genotype prevalence results considerably different between the two groups. In AM group, the most frequent genotypes are, in order of frequency, HPV 31, 11, 16, 6, 52, while in CF group the most frequent ones are HPV 53, 16, 31, 66, 58. The persistence rate results higher in AM group than in CF group (51.1% vs. 37.5%). The biopsy does not seem to play any role in regression or persistence (not significant differences between biopsy and non-biopsy groups). Interestingly, some genotypes show different rates of persistence compared to the others. While HPV 6, 11, 16, 33, 53 show a higher persistence rate in AM group, HPV-31 shows a higher rate of spontaneous clearance in AM group (80.0%) than in CF group (64.7%). In the AM group, the difference between the persistence rate of genotype 11 (88.9%) and 31 (20.0%) is statistically significant ($p < 0.01$).
Conclusion: Our data point a different rate of spontaneous clearance and persistence of HPV genotypes between two groups of patients that differ for the immunitary status (immunodeficient vs. immunocompetent) and for the site of infection (anal vs. cervical). The high rate of spontaneous clearance of HPV-31 in anal samples from immunodeficient patients in comparison with cervical samples is opposite of the low rate of clearance of the other genotypes. It can reflect a different mechanism of persistence or of response of the immune system or both. Interestingly, the genotype that seems to persist in a higher proportion of cases is HPV-11, a low-risk type, instead of HPV-31, a high-risk type. Further evaluations are needed to assess whether these differences are primarily due to the different type of mucosal tissue or to the different degree of immune response.

O416 Rhinovirus-induced basic fibroblast growth factor release from bronchial epithelial cells mediates airway remodelling features

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Objectives: Human rhinoviruses (RV), major precipitants of asthma and chronic obstructive pulmonary disease (COPD) exacerbations, induce lower airway inflammation and mediate angiogenesis. On the other hand, asthma is characterized by remodeling of the bronchial tree, expressed as epithelial shedding, exaggerated number of goblet cells, thickening of the basement membrane, hypertrophy and hyperplasia of smooth muscle, and increased collagen deposition. The objective of our study was to assess whether RV may also contribute to the fibrotic component of airway remodeling.
Methods: Levels of basic fibroblast growth factor (bFGF) mRNA and protein were measured following RV infection of both primary and continuous bronchial epithelial cell lines. The profibrotic effect of epithelial products was assessed by tritiated thymidine uptake of primary fibroblasts and matrix metalloproteinase (MMP) activity assays. Moreover, epithelial cells were exposed to supernatants from cultured peripheral blood mononuclear cells, obtained from adult healthy donors or atopic asthmatic subjects and subsequently infected by RV, and bFGF release was estimated. bFGF was also measured in respiratory secretions from atopic asthmatic children before and during virologically confirmed RV-induced asthma exacerbations.
Results: Rhinovirus epithelial infection stimulated mRNA expression and release of bFGF, the latter being positively correlated with cell death under conditions promoting RV-induced cytotoxicity. Supernatants from infected cultures induced lung fibroblast proliferation, which was inhibited by the addition of an anti-bFGF antibody, and demonstrated increased MMP activity. Rhinovirus-mediated bFGF release was significantly higher in an in vitro simulation of atopic asthmatic environment and, importantly, during RV-associated asthma exacerbations.
Conclusion: Rhinovirus infection induces bFGF release by bronchial epithelium, and stimulates stroma cell proliferation contributing to airway remodeling in asthma. Repeated RV infections may promote asthma persistence, particularly in the context of atopy; prevention of such infections may influence the natural history of asthma.

O417 Outcome of pregnant patients post-VZIG

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Objectives: Varicella immunoglobulin (VZIG) is the current standard of care in the UK for pregnant patients in contact with varicella zoster virus (VZV), provided it can be administered within 10 days of contact. This study investigated the outcome of pregnant patients exposed to varicella in 2009.
Methods: Pregnant patients tested for VZV immunity were identified from laboratory records. Patients' general practitioners were contacted by telephone or letter for clinical outcome data, including breakthrough infection, neonatal, and pregnancy outcome.
Results: Pregnant women tested for VZV immunity totalled 887; 157 were non-immune; 108 received VZIG, costing £114000. Twenty patients (18%) received VZIG on day 9 or 10 post-exposure; 17 presented beyond the 10-day cut-off, including 7 with active varicella, and did not receive VZIG.
Post VZIG, 4/66 patients (6%) miscarried; 4/66 patients (6%) developed varicella, with 2 neonates requiring VZIG.
VZV exposure via their own child occurred in 47/114 patients (41%); and in 12 (11%) through occupational exposure.
Multiple VZIG dose were issued to 6/108 patients (6%), 2 in previous pregnancies, comprising 3 nursery workers/teachers and 2 healthcare workers.
There were no cases of neonatal varicella or varicella syndrome.
Conclusions: Although preliminary, these data confirm that VZIG use does not afford complete protection amongst non-immune pregnant women.
These include late presentation, breakthrough infection and subsequent VZIG administration to neonates. Moreover the impact of VZV and VZIG exposure upon miscarriage rates has not been fully characterised, as miscarriages may not be investigated for infective causes. VZIG is a human blood product with potential associated risks, yet 6 patients required multiple doses.
VZIG is a high cost intervention; half of VZIG issued could have been prevented by a targeted vaccination strategy of "at-risk" occupations or post-partum vaccination of primigravidas. VZIG requirement in the UK could be minimized by introducing VZV vaccination, at the time of HPV vaccination, for females with no remembered history of varicella.
VZIG versus a vaccination strategy should be re-visited on the basis of clinical governance, finance and impending VZIG shortage.

Pneumococcal infections and vaccine

O418 High rates of multidrug-resistant *Streptococcus pneumoniae* in Europe. Pneumococcal activity profile from GLOBAL 6 surveillance (2009–2010)

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Objective: It is important to take into account anticipated resistance among target pathogens when selecting an empiric therapy for the treatment of community acquired pneumonia (CAP), because the selection of an appropriate initial agent is critical with respect to beneficial clinical outcome. Local and regional surveillance data can be used to understand resistance patterns within hospitals and across countries, as resistance rates are known to vary based on geography and patient population. This study reports current resistance among *S. pneumoniae*, a prevalent CAP pathogen, as determined by GLOBAL surveillance conducted across Europe in 2009–2010.
Methods: As part of GLOBAL 6, non-duplicate clinical isolates of *S. pneumoniae* were collected from Belgium, France, Germany, Italy, Spain, and the United Kingdom from 2009–2010. Isolates were centrally tested for susceptibility by broth microdilution (CLSI M7/M100). Multidrug-resistant *Streptococcus pneumoniae* (MDRSP) were defined as those resistant to $\geqslant$2 of penicillin (oral), azithromycin, cefuroxime, trimethoprim-sulfamethoxazole, and tetracycline.
Results: The current activity profile of evaluated agents against *S. pneumoniae* in Europe is shown in the table below. Of evaluated agents, SP were most susceptible to levofloxacin (99.1%), and sitafloxacin had the lowest MIC_{50}/MIC_{90}. Overall, 27% of SP in Europe were multi-drug resistant (MDRSP), though rates varied by country (36–38% in France, Italy, and Spain; 12–13% in Germany and the UK). 97% of MDR-SP were susceptible to levofloxacin, 87.9% were susceptible to ceftriaxone, 83.8% were susceptible to amoxicillin/clavulanate, 42.4% were susceptible to cefuroxime, 21.0% were susceptible to penicillin (oral), and 20.4% were susceptible to azithromycin.
Conclusions: MDRSP were prevalent in Europe, though variable by country. MDRSP were highly susceptible to fluroquinolones relative to other evaluated agents. Given the limited activity of many of the commonly utilized respiratory agents against MDRSP, the rates of MDR-SP among several European countries are concerning. It is important to consider an initial therapy that will cover these resistant phenotypes when treating CAP in these countries.

Organism	Drug	MIC 50 (mg/L)	MIC 90 (mg/L)	%S	%R
S. pneumoniae (n=1,162)	Amoxicillin/ Clavulanate	0.03	2	95.6	3.1
	Azithromycin	0.12	>4	73.3	26.7
	Ceftriaxone	0.03	1	96.7	0.3
	Cefuroxime/ Axetil	≤0.12	4	82.6	15.7
	Levofloxacin	0.5	1	99.1	0.8
	Penicillin (oral)	≤0.03	2	73.4	11.1
	Sitafloxacin	0.03	0.06	-[a]	-
	Tetracycline	0.25	>4	75.9	23.0
	Trimeth/ Sulfa	0.25	>2	77.8	14.1

[a]CLSI breakpoints not available for interpretation

O419 Antimicrobial resistance of *S. pneumoniae* in Russia: ten-year (1999–2009) prospective study PEHASus

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Objectives: To investigate the level and phenotypes of antimicrobial resistance of *Streptococcus pneumoniae* in different regions of Russia and to evaluate their dynamics in period from 1999 to 2009.
Methods: This study was conducted in Central, North-Western, Southern, Volga, Urals, Siberia, Far-Eastern regions of Russia from 1999 to 2009. Identification of the strains was done on the basis of colony morphology, Gram stain, optochin susceptibility and bile solubility tests. Susceptibility to 13 antimicrobials was performed using cation-adjusted Mueller-Hinton broth (BBL, USA) with 2–5% lysed horse blood, inoculum 0,5 MacFarland. Microtiter plates were incubated for 24 h at 35ºC and 5% CO_2. Breakpoints were those of Clinical and Laboratory Standards Institute (CLSI).
Results: A total of 2,419 of non-duplicate clinical *S. pneumoniae* were included to the study in ten-year period (1999–2009). The majority of strains in 1999–2003, in 2004–2005 and in 2006–2009 were isolated form respiratory samples (sputum, BAL, sinus aspirate, middle ear and pleural fluid) – 87.4%, 86.7% and 88.0%, respectively.
The susceptibility testing results are presented in the Table.

	I,%	R,%	I,%	R,%	I,%	R,%
Antimicrobial	1999-2003 (n=791)		2004-2005 (n=913)		2006-2009 (n=715)	
Penicillin G	7.8	1.9	6.9	1.2	9.1	2.1
Amoxicillin/clavulanate	0	0	0	0,3	0.4	0
Ceftriaxone	1.4	0.4	0.9	1.1	0.4	0.6
Azithromycin	0.5	7.6	0.2	6.2	0.9	6.4
Clarithromycin	0.5	7.5	0.3	6.1	1.6	5.7
Spiramycin	1.0	1.0	0.9	3.6	1.0	5.3
Clindamycin	0.1	2.8	0	3.6	0.2	4.3
Levofloxacin	0	0	0	0.1	0	0
Moxifloxacin	0	0	0.1	0	0	0
Tetracycline	2.4	24.9	4.8	24.8	3.1	21.5
Co-trimoxazole	26.3	5.4	29.1	11.8	22.4	16.6
Chloramphenicol	0	7.7	0	5.9	0	7.1
Vancomycin	0	0	0	0	0	0

Conclusions: Thus β-lactams (penicillin G, amoxicillin/clavulanate), macrolides, lincosamides, respiratory fluoroquinolones retained the good

in vitro activity against *S. pneumoniae*. High resistance to tetracycline and co-trimoxazole dictates the necessity to limit their usage for treatment of pneumococcal infections. During the 10-years period, no substantial changes occurred in the level of resistance, indicating the circulation of relatively susceptible pneumococcal population.

O420 Decrease in antibiotic resistance of *Streptococcus pneumoniae* between 2003 and 2009 in France and changes in serotype distribution: ongoing survey of the French Pneumococcus Network

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Background: The French regional pneumococcal observatories (ORP) network was created in 1995, it participates to the close monitoring of the trends in antimicrobial resistance and serotype distribution with the National Reference Centre for Pneumococci (NRCP) and the Institut de Veille Sanitaire (InVS). The aim of this survey was to assess the antibiotic resistance and the distribution of vaccine and non-vaccine serotypes in invasive pneumococcal disease (IPD) in adults and children as well in otitis in children in France in 2009.
Methods: Antimicrobial susceptibility testing was performed on 5,194 isolates of *S. pneumoniae* recovered from cerebrospinal fluid (CSF), blood, middle ear fluid (MEF) and pleural fluid during the year 2009 by the 23 ORP. MICs of penicillin (P), amoxicillin (AMX) and cefotaxime (CTX) were determined by the agar dilution method and interpreted according to the Antibiotics Committee of the French Society of Microbiology breakpoints. Serotyping was performed at the NRCP with serotype-specific antisera, by latex agglutination test.
Results: Results of susceptibility between 2003 and 2009 to P are presented in Table 1. The pneumococci with decreased susceptibility to penicillin G (PDSP) decreased significantly in all types of samples except for MEF's isolates in children between 2007 and 2009. In the global population, the percentage of I+R (2003 vs 2009) decreased significantly for AMX (30.3% versus 9.6%) and for CTX (18.2% versus 10.5%). Strains highly resistant (MIC >2 mg/L) remained rare: 0.4%, 1.1% and 0.2% for P, AMX and CTX respectively. The most frequent capsular types in CSF were 3, 7F, 19A, 6C, 23B for adults and 7F, 19A, 3, 33F and 15B for children. Serotypes included in the PCV-7 and PCV-13 vaccine accounted in children for 4% and 51% respectively and in adults for 12% and 48% respectively. By contrast, the serotype 19A remained highly predominant (80%) among MEF isolates.
Conclusions: We observed a significant decrease of PDSP between 2003 and 2009 (50.2 to 33.0%). The decrease coincided with the introduction of PCV7 and with a general reduction in levels of antibiotic consumption in France. This continuous survey is necessary to underline modification in serotype distribution in France after PVC13 introduction (June 2010).

Table 1: % I+R to P between 2003 and 2009

		Number of strains	Percentage I+R to P			
			Blood	CSF	MEF	All
Children	2003	1922	45.5	44.1	68.8	62.5
	2005	1853	33.7	38.0	63.3	54.3
	2007	1439	27.8	33.7	60.2	50.1
	2009	1497	23.7	29.2	63.0	48.3
Adults	2003	3492	43.2	45.5		43.3
	2005	4303	38.7	38.0		40.4
	2007	3230	32.5	34.6		32.7
	2009	3696	27.2	26.1		26.8

O421 Invasive pneumococcal disease: serotypes and antimicrobial susceptibility in adult patients from Madrid

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Objectives: After the introduction of the pneumococcal seven valent conjugate vaccine (7-VCV) in the systematic immunization schedule for children in November 2006, changes in serotypes distribution and in the resistance antimicrobial patterns of *Streptococcus pneumoniae* have been observed in Madrid. These changes could affect no only paediatric isolates. The aim of this study was to describe the distribution of serotypes, its antimicrobial susceptibility profiles and the relation with vaccines in pneumococcal invasive strains isolated from adult patients attending a public hospital in Madrid during a three year period.
Methods: From July 2007 to June 2010, all pneumococci isolated in our laboratory from blood cultures were serotyped by latex agglutination (Pneumotest Latex) and Quellung reaction (antisera from the Statens Serum Institut, Denmark). Antimicrobial susceptibility testing to penicillin (PEN), cefotaxime (CT), erythromycin (ERY) and levofloxacin (LEV) was performed by the E-test method (Biomèrieux, France). The susceptibility break points considered were according to the Clinical Laboratory Standard Institute guideless (CLSI).
Results: A total of 68 isolates from 67 adult patients were studied (one patient had 2 recurrent infections). The coverage of 7-VCV and thirteen valent conjugate vaccine (13-VCV) were respectively of 16.4% and 55.2%. Twenty-two strains (32.8%) were serotypes covered only by the 23 valent vaccine (23-VPV) but not by conjugate vaccines. Ten serotypes hampered 71.6% of the isolates: serotype 3 (11.9%), 22F (11.9%), 19A (7.5%), 7F (7.5%), 1 (6.0%), 8 (6.0%), 6A (6.0%), 4 (6.0%), 12F (4.5%) and 14 (14.5%). PEN MICs (mg/L) were: $\leqslant$0.06 (82.1%); 0.12–1 (14.9%), and $\geqslant$2 and <8 (3.0%). Only one strain (1.5%), belonging to the serotype 6B, showed intermediate susceptibility to PEN according the 2008 CLSI criteria (4 mg/L). Three strains were intermediate susceptible to CT (serotypes 6B, 14 and 19A). None strain showed resistance to LEV. Resistance to ERY was 19.4%. Four out of 13 ERY resistant strains (30.8%) belong to serotype 19A.
Conclusion: The 13-VCV covered most of the serotypes found in our hospital in adult patients. No resistance to PEN, CT or LEV was found. Serotype, 19A is a cause of clinical and epidemiological concern by its resistance to ERY. Among non conjugate vaccine strains serotypes 22F, 8 and 12F seems to be in increased.

O422 Activity of ceftaroline against serotyped *Streptococcus pneumoniae* isolates from Europe (2007–2008)

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Objective: Ceftaroline (CPT) fosamil, the prodrug of the active compound CPT, is a broad-spectrum, parenteral cephalosporin recently approved in the USA for the treatment of community-acquired bacterial pneumonia (CABP) and acute bacterial skin and skin structure infections. The aim of this study was to determine CPT activity against *Streptococcus pneumoniae* (SP) causing CABP from Eastern Europe (Czech Republic, Greece, Hungary, Poland, Russia and Turkey) and Western Europe (Austria, Belgium, Denmark, France, Germany, Ireland, Italy, the Netherlands, Portugal, Spain, Sweden and the UK).
Methods: 70 European centres submitted 803 SP isolates causing CABP during 2007–2008. These were re-identified and serotyped (using Latex and Quellung reaction). MICs for CPT and comparators were determined by CLSI broth microdilution at a central laboratory. Data were analysed by region, age group and serotype (where $N > 30$).
Results: Summary CPT MIC data are shown in the Table [cefotaxime (TAX), penicillin G (PEN) and erythromycin (ERY) data are shown as a reference]. CPT was on average 4-fold more active than TAX and 6-fold more active than PEN. Overall, SP were ~97% susceptible to TAX or PEN and 76% susceptible to ERY. Although no differences in CPT activity based on age or geography were observed (data not shown),

serotypes 3, 1, 7 and 11 were more susceptible to CPT (and the reference antibiotics) than the population as a whole (All SP).
Conclusions: These data from a large collection of European SP isolates with varying serotypes confirm the excellent activity of CPT against SP causing CABP.

Table. MICs by geography, age and serotype

Demographic (N)		MIC mg/L			
		MIN	50%	90%	MAX
All SP (803)	CPT	<=0.001	0.008	0.12	0.5
	TAX	<=0.004	0.015	1	8
	PEN	<=0.008	0.015	1	8
	ERY	<=0.015	0.06	>=64	>=64
Serotype 1 (50)	CPT	0.002	0.004	0.008	0.008
	TAX	<=0.004	0.015	0.015	0.03
	PEN	<=0.008	0.015	0.015	0.03
	ERY	<=0.015	0.03	0.06	>=64
Serotype 11 (34)	CPT	0.002	0.008	0.008	0.12
	TAX	<=0.004	0.015	0.03	2
	PEN	<=0.008	0.015	0.03	4
	ERY	0.03	0.06	8	16
Serotype 3 (80)	CPT	<=0.001	0.004	0.008	0.008
	TAX	<=0.004	0.015	0.015	0.015
	PEN	<=0.008	0.015	0.015	0.03
	ERY	<=0.015	0.03	0.06	>=64
Serotype 7 (46)	CPT	0.002	0.008	0.008	0.015
	TAX	<=0.004	0.015	0.03	0.03
	PEN	<=0.008	0.015	0.03	0.03
	ERY	<=0.015	0.06	0.06	>=64

O423 The associated mortality of invasive pneumococcal disease – Why are we not vaccinating?

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Invasive pneumococcal disease (IPD) has an all cause mortality rate of 5–35% in the developed world. Vaccination with 23-valent pneumococcal polysaccharide (PPV 23) is recommended for many chronic conditions including include HIV infection, malignancy, alcohol abuse, hepatitis C infection, cigarette smoking, asthma and chronic obstructive pulmonary diseases. Uptake of the PPV23 in specific risk groups in Ireland is unknown. In Ireland 7-valent pneumococcal conjugate vaccine was introduced into routine childhood vaccination schedule in 2008. We describe the epidemiology, clinical characteristics, serotype distribution, vaccination rates, outcome of IPD cases from 2006 to 2010 diagnosed in a tertiary referral hospital in Ireland.
Electronic patient record and patient case notes were examined for baseline demographics, clinical characteristics, risk factors, mortality and vaccination status.
There were 122 episodes in 116 patients during the study period. Table one describes demographics and risk factors for each patient. One hundred and twenty of the 122 total (98.4%) had one or more risk factors for which PPV23 vaccination is recommended. Eighty six patients (70.5%) had two or more risk factors. Only 2 patients (1.6%) had no risk factor. However, only 11 (9%) of the 122 patients had received PPV23 prior to IPD infection. Seven of these (77.8%) were HIV-1 seropositive with average CD4 count of 301 (5–591). Serotypes were known for 6 of these isolates, 4/6 were in PPV 23 and there was no difference between infecting serotypes in this group and the group overall. Forty nine of 76 known serotypes (64.5%) were contained in the PPV23 vaccine, 37/76 (48.7%) contained in CPV 7 and 47/76 (61.8%) in CPV 13. The most commonly infected serotypes were 4,7F, 9A, 18C, 19A and 23F. Twenty six of 122 patients died during this hospital admission, giving an overall mortality rate of 21.5%. IPD has a significant associated mortality. Health care providers need to be aware of importance of vaccination. Patient education and SMS text reminders may help adherence. Dedicated HIV vaccination services increase uptake in this at risk cohort. Expansion of vaccination services to include drug treatment centres may facilitate uptake. Vaccination at lower CD4 counts should be considered for those unlikely to immune reconstitute secondary to non adherence to highly active antiretroviral therapy.

Baseline demographic	Number of patients (%) N=116
Age in yrs (mean/median)	58 (51.5)
Sex (Male)	69 (59.5%)
Ethnicity	
Irish	112 (96.5%)
African	4 (3.5%)
Risk Factors	Number of patients (%) N=122 episodes
Age >65 yrs	47 (38.5%)
HIV infection	36 (29.5%)
Chronic liver diseases	40 (32.8%)
Alcohol excess	36 (29.5%)
Hepatitis C infection	37 (30.3%)
Illicit drug use	41 (33.6%)
Diabetes mellitus	20 (16.4%)
Malignancy	23 (18.9%)
Cigarette smoker	58 (47.5%)
Chronic obstructive pulmonary disease	29 (23.8%)
Asthma	2 (1.7%)
End stage renal disease	2 (1.7%)

O424 Epidemiology of community-acquired pneumonia requiring hospitalisation after introduction of the conjugated pneumococcal vaccine in Switzerland

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Objective: To compare the epidemiology of hCAP before (years 2002 to 2005 = PreVY) and after (years 2007 to 2008 =PostVY) the introduction of PCV7 in Switzerland in 2006. PCV7 is recommended on a voluntary base to all infants at the age of 2 and 4 months with a booster dose at 12 months of age. Estimated vaccine coverage was 80% in 2010.
Methods: National hospitalization data were obtained from the Federal Institute of Statistics for the years 1998 to 2008 including the primary diagnosis (first-listed; International Classification of Diseases (ICD-10)), up to 7 additional diagnoses, and other parameters characterising hospitalisation. hCAP was defined by a primary diagnosis of pneumonia or meningitis/septicaemia plus a code for pneumonia. Pneumococcal CAP (SpnCAP) was defined as hCAP with a pneumococcal disease code. Hospitalization rates for hCAP and SpnCAP were calculated by segmented regression analysis.
Results: There were 67′723 hCAP in the PreVY and 33′759 in the PostVY with an annual average of 16′931 (PreVY) and 16′880 (PostVY) hCAP. SpnCAP was coded in 5.02% of hCAP in PreVY versus 7.47% of hCAP in PostVY. Annual hCAP rates varied by age group. The highest rates were seen among infants and elderly people, but rates did not change significantly after the introduction of PCV7: <2 years olds 369 hCAP per 105 population during PreVY versus 352 hCAP per 105 population during PostVY ($p = 0.69$) and >80 years olds 1509 hCAP per 105′ during PreVY versus 1594 hCAP per 105 during PostVY ($p = 0.64$). Males predominated (56.5%) among hCAP cases. Average duration of hospital stay was 12.4 days (PreVY) versus 11.0 (PostVY) days. Case fatality rate was 7.8% (PreVY) vs. 7.3% (PostVY) ($p = 0.006$) overall with most (87%) fatal cases occurring in the elderly. Admission to intensive care treatment was needed in 6.5% (PreVY) vs. 7.9% (PostVY) of CAP ($p < 0.001$).
Conclusion: Hospitalisation rates for community acquired pneumonia did not change two years after the introduction of PCV7 in Switzerland

despite high vaccine up-take among infants. However, a slight increase in severe hCAP requiring ICU admission among the elderly was observed. Serotype redistribution might account for a lack of herd immunity and trend towards more severe hCPA among the elderly.

O425 Immunogenicity and safety of a 13-valent pneumococcal conjugate vaccine in adults 70 years of age and older previously vaccinated with 23-valent pneumococcal polysaccharide vaccine

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Objectives: To compare the immunogenicity of 13-valent pneumococcal conjugate vaccine (PCV13) with 23-valent pneumococcal polysaccharide vaccine (PPSV23) in adults 70 years of age and older previously vaccinated with a single dose of PPSV23 at least five years prior to enrollment.
Methods: We performed a randomized, double-blind, comparative trial of PCV13 vs. PPSV23 in 938 adults 70 years of age and older who had previously received PPSV23. At one year after the enrollment vaccination (PCV13 or PPSV23), all subjects received a dose of PCV13. Serotype-specific opsonophagocytic antibody (OPA) titers were measured in blood samples obtained prior to and at one month after each vaccination.
Results: At one month after the enrollment vaccination, the OPA geometric mean titers (GMTs) were significantly higher in the PCV13 group compared with the PPSV23 group for 10 of the 12 serotypes common to both vaccines, non-inferior for the other 2 common serotypes (3 and 14), and significantly higher for 6A (not contained in PPSV23) (Table). In analyses stratified by age, OPA GMTs were lower in subjects 80 years of age and older but the responses to PCV13 were greater than to PPSV23 in all age groups.

Comparison of Pneumococcal OPA GMTs, PCV13 Relative to PPSV23 in Subjects Previously Immunized with PPSV23

	PCV13 (n=402-426)	PPSV23 (n=395-445)	Vaccine Comparison	
Serotype	GMT	GMT	GMT Ratio	95%CI
1	81	55	1.5	(1.17 , 1.88)
3	55	49	1.1	(0.91 , 1.35)
4	545	203	2.7	(1.93 , 3.74)
5	72	36	2.0	(1.55 , 2.63)
6B	1261	417	3.0	(2.21 , 4.13)
7F	245	160	1.5	(1.07 , 2.18)
9V	181	90	2.0	(1.36 , 2.97)
14	280	285	1.0	(0.73 , 1.33)
18C	907	481	1.9	(1.42 , 2.50)
19A	354	200	1.8	(1.43 , 2.20)
19F	333	214	1.6	(1.17 , 2.06)
23F	158	43	3.7	(2.69 , 5.09)
6A	903	94	9.6	(7.00 , 13.26)

In both study groups, OPA titers declined in the year following the enrollment vaccination, and increased in response to the PCV13 given at one year, but the responses to the dose of PCV13 given at one year were generally higher in the group that received PCV13 at enrollment compared with the group that received PPSV23 at enrollment. The OPA GMTs following the one year PCV13 were significantly higher in the PCV13/PCV13 group than in the PPSV23/PCV13 group for 12 of the 13 serotypes. In the PCV13/PCV13 group, the responses to the second PCV13 administration were similar to the post dose one responses.
After the enrollment vaccination, local reactions were more common in the PPSV23 group (64%) than the PCV13 group (57%) ($p = 0.03$). After the PCV13 dose given at one year, the frequency and severity of local reactions were similar between PCV13/PCV13 and the PPSV23/PCV13 groups.
Conclusion: In adults 70 years of age and older previously vaccinated with PPSV23, PCV13 was at least as immunogenic for all common serotypes and was significantly more immunogenic than PPSV23 for 10 of those 12 serotypes. The OPA GMTs following the dose of PCV13 at one year indicate that a prior dose of PPSV23, but not PCV13, diminishes the response to a subsequent dose of PCV13.

O426 Immunogenicity and safety of a 13-valent pneumococcal conjugate vaccine in pneumococcal vaccine naïve adults, 50–64 years of age

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Objectives: To compare the immunogenicity of a single dose of 13-valent pneumococcal conjugate vaccine (PCV13) with 23-valent pneumococcal polysaccharide vaccine (PPSV23) in adults 60–64 years and to compare the immunogenicity of PCV13 in adults 50–59 years with that in adults 60–64 years.
Methods: We performed a randomized, double-blind, comparative trial of PCV13 vs. PPSV23 in 835 pneumococcal vaccine naïve adults aged 60–64 years. An additional group of 404 adults aged 50–59 years received open-label PCV13. For all subjects, serotype-specific opsonophagocytic antibody (OPA) titers were measured at baseline and at one month and one year after vaccination.
Results: Among adults 60–64 years, the one month postvaccination OPA geometric mean titers (GMTs) in the PCV13 group were significantly higher than in the PPSV23 group for eight of the 12 serotypes (serotypes 1, 4, 6B, 7F, 9V, 18C, 19A, 23F) common to both vaccines, significantly higher for 6A (not contained in PPSV23), and were comparable for the other four common serotypes (Table).
In comparisons of the response to PCV13 in the younger and older age groups, the OPA GMTs at one month after vaccination were significantly higher in subjects 50–59 years than in subjects 60–64 years for nine serotypes and were comparable for the other four serotypes. In both age groups OPA titers declined from one month to one year after PCV13 administration but remained higher than baseline titers.
PCV13 was well tolerated in both age groups. Among subjects 60–64 years, any local reaction was reported by 82% of subjects in the PCV13 group and by 76% of subjects in the PPSV23 group ($p = 0.05$).
Conclusion: These data support a potential benefit of PCV13 in pneumococcal vaccine naïve older adults.

Comparison of Pneumococcal OPA GMTs 1 Month After Vaccination With PCV13 and PPSV23 in Subjects Aged 60-64 Years

	PCV13 (n=359-404)	PPSV23 (n=367-402)	Vaccine Comparison	
Serotype	GMT	GMT	GMT Ratio	95%CI
1	146	104	1.4	(1.10, 1.78)
3	93	85	1.1	(0.90, 1.32)
4	2062	1295	1.6	(1.19, 2.13)
5	199	162	1.2	(0.93, 1.62)
6B	1984	788	2.5	(1.82, 3.48)
7F	1120	405	2.8	(1.98, 3.87)
9V	1164	407	2.9	(2.00, 4.08)
14	612	692	0.9	(0.64, 1.21)
18C	1726	925	1.9	(1.39, 2.51)
19A	682	352	1.9	(1.56, 2.41)
19F	517	539	1.0	(0.72, 1.28)
23F	375	72	5.2	(3.67, 7.33)
6A	2593	213	12.1	(8.63, 17.08)

O427 Pneumococcal vaccination and invasive pneumococcal disease in East Yorkshire, UK

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Background: Vaccination with pneumococcal polysaccharide vaccine (PPV) has been recommended to persons ⩾65 years in the United Kingdom (UK) since 2005, although in Hull and the East Riding of Yorkshire (ERoY) since 2002. Controversy remains concerning the effectiveness of PPV in older adults. Pneumococcal conjugate vaccine (PCV) has been recommended routinely in UK children since 2006.
Objectives: To explore the epidemiology of invasive pneumococcal disease (IPD) in Hull & ERoY and to determine the impact of vaccination at the population level.
Methods: Microbiology databases were interrogated to identify pneumococcal isolates retrieved from sterile sites from 1st January 2002 to 31st December 2009 inclusive, from all (seven) hospitals that routinely receive residents of Hull & ERoY. Demographic details of cases, information concerning the isolate and pneumococcal serotype were recorded. Only individuals resident in Hull & ERoY were included. One isolate per patient episode was selected for calculation of incidence rates. Information on vaccination uptake for both PPV and PCV were retrieved from data held nationally (Department of Health) and or locally (Health Protection Unit).
Results: 727 isolates (91.5% blood cultures) concerning 660 individuals (355 male, median age 67 years) were included. The number of cases of IPD increased almost year on year (65 in 2002, 101 in 2009) and incidence rates of IPD appeared to increase (11.8 to 16.7 per 100,000 overall and 39.7 to 44.5 per 100,000 in those ⩾65 years, 2002–9) despite increased PPV vaccination uptake over the 8 year study period (49% to 70%) and high uptake of PCV (98%, 2009), [Figure 1]. There was no increase in blood culture sampling or significant change in the diagnostic methodology used. The burden of IPD was highest in the elderly and very young and was unequally distributed geographically across the region. A rise in the proportion of cases of IPD caused by non-vaccine pneumococcal serotypes was noted following PCV introduction whilst those due to PCV serotypes have fallen.
Conclusions: The introduction and improved uptake of PPV has not resulted in a reduction in the rates of IPD at the population level. Determinants for the continuing burden of IPD and its unequal geographical distribution should be investigated. Ongoing surveillance of pneumococcal serotypes is imperative to inform PCV vaccine formulation. Policy makers may wish to reconsider recommendations on the use of PPV.

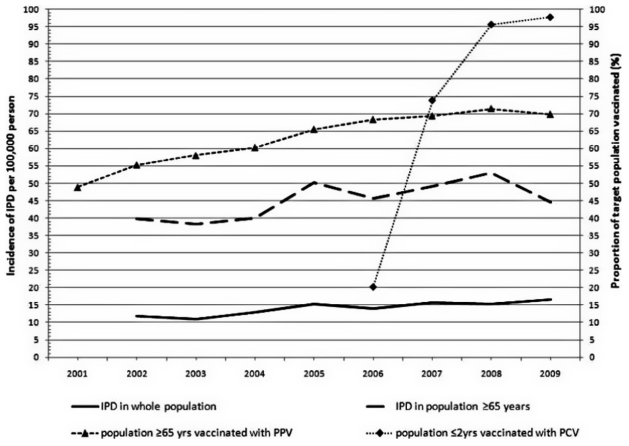

Figure 1. Pneumococcal vaccination and invasive pneumococcal disease 2002–2009.

Decision points in the management of invasive *Candida* infections: navigating the choices for optimal patient care

S428 Susceptibility, breakpoints, and resistance to antifungals among *Candida* species – where are we in 2011?

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Candidaemia and other invasive *Candida* infections are the most common invasive fungal infections in the hospital setting, and are associated with a crude mortality rate of 30–40% or more. In the EPIC II study of ICU infections, *Candida* spp were the third most common pathogens behind *Staphylococcus aureus* and *Pseudomonas* spp, and accounted for almost 90% of diagnosed fungal infections.
An epidemiological shift from *C. albicans* to other *Candida* spp has been discussed widely in recent years, with each *Candida* species having distinct antifungal susceptibility profiles. *C. krusei* is intrinsically resistant and *C. glabrata* shows reduced susceptibility to fluconazole; a recent Danish nationwide survey also detected decreased fluconazole susceptibility (MIC of >4 μg/ml) in *C. albicans* (0.6% of isolates), *C. dubliniensis* (3.1%), *C. parapsilosis* (6.0%), and *C. tropicalis* (6.7%). Between 2004–2009, the proportion of *Candida* blood isolates showing full susceptibility to fluconazole decreased from 79.7% to 68.9%. In another recent study, 19% of 243 candidaemia episodes at two tertiary centres in the US involved isolates with decreased fluconazole susceptibility, one third of which were from normally-susceptible species displaying acquired resistance. Although echinocandin-resistant strains of *Candida* have not been isolated from echinocandin-naïve patients, they have been documented in some patients after relatively short periods of treatment. Such acquired resistance has been associated with mutations in the FKS gene.
In 2008, the CLSI proposed a single breakpoint (susceptible: ⩽2 μg/ml) for all 3 echinocandins for *Candida* spp. However as these breakpoints do not reliably identify isolates with resistance mechanisms associated with treatment failures revised compound and species specific breakpoints have recently been approved. Recent data suggest that the revised breakpoints (lower than the original CLSI breakpoint) help differentiate *Candida* isolates with FKS mutations from those which remain fully susceptible. Revised and specific CLSI breakpoints have also been developed for fluconazole ensuring harmonisation with the EUCAST breakpoints and a better classification of isolates of *C. glabrata*, many of which were susceptible using the former CLSI breakpoint even though clinical response rates to fluconazole were lower than to those observed for other species. In addition to the EUCAST and CLSI broth dilution methods for susceptibility testing of *Candida* species, other approaches (Etest®, agar dilution, disk diffusion) are available, which can differ from the reference methods in their detection of resistance to echinocandins.

S429 Early, effective treatment of *Candida* infections – implications for patient, physician and hospital

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The importance of early antifungal therapy is well established, with delays associated with increased mortality in patients with candidaemia. Treatment is often initiated before a definitive diagnosis is obtained, and is subsequently modified on the basis of blood culture results. In many centres, fluconazole remains the treatment of choice for initial treatment. Given the shifts in the epidemiology of *Candida* species, there is an increased risk for infections caused by fluconazole-insensitive isolates. The identification of such an isolate in patients receiving fluconazole usually necessitates a change in treatment (often switching to another class of antifungal), thus delaying the onset of effective treatment. An alternative to this "step-up" approach is to start therapy with a broad-spectrum antifungal agent, such as an echinocandin, and step down to fluconazole, if appropriate, once the isolate has been characterised.

To investigate the potential utility of an approach involving initial treatment with micafungin with the option of stepping down to fluconazole, a model of systemic *Candida* infections was developed. The model included epidemiological, susceptibility, and cost data from the UK, and assumed that all patients had blood cultures and susceptibility testing undertaken within 24 hours of the first suspicion of invasive candidiasis, with the results available on day 3. For patients receiving step-up treatment (starting with fluconazole), all those with fluconazole-insensitive isolates were taken to be switched to micafungin on day 3, while those receiving the step-down approach (starting with micafungin) were modelled as switched to fluconazole on day 3 if fluconazole-sensitive isolates were identified.
Results from the initial model showed an incremental cost-effectiveness ratio (ICER) per quality-adjusted life year (QALY) of between £17,500 and £27,400, depending on the projected duration of survival for patients surviving an episode of candidaemia. Based on the specific variables included in the model's base case, the step-down approach appears to be cost-effective, as the ICER per QALY is below the estimated threshold used to guide such assessments in the UK. The model also indicated that when compared with the step-up approach, in addition to the cost-effectiveness of step-down therapy, there was also an impact on mortality, with one extra life being saved for every 100 patients treated.

Impact of molecular diagnostics on public health and patient care

S431-S434 Impact of molecular diagnostics on public health and patient care

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Molecular diagnostics play a pivotal role in the rapid detection of infectious agents, enabling effective treatment and patient management strategies to be initiated promptly. For both healthcare-associated and community-based infections, rapid detection can limit exposure of patients to contagious illnesses, thus reducing transmission. However, two major limitations of molecular diagnostics as currently utilized are not reporting results in a clinically actionable timeframe due to batch processing and the inability to perform the tests where they are needed most, such as in developing countries. This session will focus on four areas in which rapid, decentralized molecular detection may have a profound impact on improving public health and patient care. First, the rapid detection of multidrug resistant strains of tuberculosis using rifampin resistance as a surrogate marker in less than two hours may, according to the World Health Organization, revolutionize the treatment of tuberculosis, especially in developing countries. Second, in the hospital setting, two decades of using insensitive tests for *Clostridium difficile* has led to dramatic increases in the prevalence of these infections. New molecular amplification tests that provide identification of *C. difficile* in as little as 30 minutes, with both high sensitivity and specificity, are optimizing both patient management and infection control efforts in healthcare settings. Third, the availability of highly accurate molecular tests on demand for detecting sexually transmitted diseases, such as chlamydia and gonorrhea, has the potential of improving public health efforts to control the spread of disease. Finally, the rapid and accurate identification of the etiologic agents of sepsis, both bacterial and fungal, using novel molecular technologies will likely improve therapy and outcomes for the most critically ill patients. The impact of rapid diagnostics on improving health, especially in developing countries, is just now beginning to be realized.

Bridging the gap of innovation – what we all could do

S449 Antibacterial pipelines – what to expect in the future

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Antibacterial resistance is rising dramatically in various community and hospital specific bacteria creating a critical need for new antibiotics without cross-resistance to existing drugs. Yet, antibacterial pipelines fail to reflect this medical urgency and novel compounds focused on new targets or with new mode of action, especially in Gram-negative bacteria, cannot be expected in the near future. Most novel compounds with activity against Gram-negative bacteria are in research or early development phase. Hence, resistance will continue to outrun our antibacterial development efforts. Driven by concern regarding this rapidly worsening global health crisis and the confounding lack of robust drug pipelines, international recognition of the problem and several multinational campaigns have initiated broad discussion and put action plans in place. These campaigns create a ray of hope as innovative models of research and development may translate into promising pipelines during the next decade. Until then we can only address the problem of multi-drug resistant bacteria with a multifaceted set of solutions based on currently available tools.

Infectious disease with liver involvement – an update

S454 Hepatitis B treatment. Current guideliness in Latin America and variability in access to treatment

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The most effective approach to prevent chronic HBV infection and its sequlae is to implement appropriate immunization programs. Over the past 15 years numerous immunization campaigns have been developed with varying degrees of success in Latin America. Further, there is already a significant number of people with chronic hepatitis B acquired in the past for which the National Health Systems must provide diagnostic testing and the best possible treatment. About 400 million people worldwide are chronically infected with HBV. Approximately 2% of the Latin American population is chronically infected with HBV. The highest prevalence is observed in the Northeast of Brazil, Venezuela and Colombia, and the lowest prevalence is in Chile and Uruguay. The globally prevalent serotypes are A, D and F. Genotypes A and B are associated with high rates of response to pegylated interferon. There is as yet no solid evidence regarding the relationship of "genotype to treatment response", and, thus, determination of genotype has not yet been incorporated into therapeutic decision making. Since 2009, the role of liver biopsy has been widely discussed in Latin America. Currently, the value of liver biopsy for treatment decisions is primarily restricted to patients who do not clearly meet criteria based on HBV DNA levels, ALT and, in some special cases, related to age (>40 years old). The Brazilian Guidelines for Treatment of Chronic Hepatitis B are more specific in regard to the indications for liver biopsy.
Treatment guidelines in most cases have been based on the recommendations of national scientific societies (Infectious Diseases, Hepatology) but in other countries have been initiated at the national government level (Chile, Argentina, Brazil). Throughout the region, the drugs of choice are entecavir, adefovir, telvibudina tenofovir and pegylated interferon, but access to such treatment is asymmetrical in the area. Not all drugs are registered and available in all countries of the region, which does not allow a common and comparable assessment of treatment outcomes. Tenofovir still has some limitations to its widespread use. Most countries still consider Tenofovir the drug of choice for HIV-HBV co-infection, but they have not destablished programs for the treatment of non-coinfected chronic HBV patients.

Antimicrobial-resistant bacteria in food and potable water: a recipe for disaster?

S458 Antibiotic-resistant bacteria in food: from farm to fork

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In the last few years links between humans and food, mainly of animal origin, with regard to some antimicrobial resistance of human clinical relevance have been reported. One of the new concerns is the increasing occurrence of bacterial strains producing extended-spectrum β-lactamases (ESBL) and/or acquired AmpC β-lactamases causing difficult to treat severe infections in humans and their possible link to food or food producing animals. Bacterial strains producing these β-lactamases are resistant to third and fourth generation cephalosporins, antimicrobials considered to be critically or highly important medicines for humans. The genes encoding resistance to these cephalosporins are transferrable and often linked to other resistance genes. Use of third and fourth cephalosporins selects for resistance, but cross and co-selection by other antimicrobials is also likely to influence prevalence of resistance. The increasing occurrence of ESBL and/or AmpC producers in animals for food producing and food is highlighted and discussed in this review with respect to the circulation of these resistance traits and potential risk factors that favours its emergence and spread.

S459 Antibiotic-resistant bacteria in potable water: tapping into the problem

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The microbial flora of the gastrointestinal tract is an important environment for the emergence and spread of antimicrobial resistance. Consumption of water by humans and animals represents a continuing opportunity for the addition of new elements to the diversity of microorganisms and mobile genetic elements in this habitat. Antimicrobial resistance in Enterobacteriaceae, in particular in *E. coli* is a particular concern because of their importance in both extra-intestinal infection (urinary tract and invasive infection) and intestinal infection. However criteria for potable water include *E. coli* and coliform bacteria (Enterobacteriaceae) not detectable in a 100ml sample therefore potable water by definition should not have antimicrobial resistant Enterobacteriaceae at readily detectable levels. However water consumed by human and food animals in many areas of the world including parts of Europe may fail to meet this requirement with resulting opportunities for introduction of new viable Enterobacteriaceae with potential transferable antimicrobial resistance mechanisms into the gastrointestinal tract. Bacteria other than Enterobacteriaceae are present normally in potable water and may introduce new resistance elements. Even in the absence of viable bacteria potable water may contain and introduce new resistance determinants to the genetic diversity of the gastrointestinal tract. The extent of contamination of drinking water sources and derived potable water with antimicrobial resistant bacteria and antimicrobial resistance determinants is an indicator of the extent to which antimicrobial use has altered the microbial biodiversity of aquatic environments. It is prudent to consider ways to limit such impacts both from a direct human health perspective (in particular with respect to novel resistance determinants) but also with a view to minimising unintended ecological disturbance. As drinking water distribution systems now frequently serve much greater populations than in the past there may be greater potential for potable water to contribute to mixing of microorganisms or microbial genetic material in the gastrointestinal tract although there is little evidence to support this and it may be impractical to eliminate it. The fundamental disturbance of this aspect of microbial ecology that must be controlled is almost certainly intense selective pressure related to the antimicrobial use rather than changes in water distribution.

Genetic predisposition and the outcome of infection: time to include genetic polymorphisms in routine diagnostic work-up?

S464 Genetic dissection of host resistance to *Mycobacterium tuberculosis*

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Combinations of biomarkers can provide insights into host resistance against tuberculosis (TB). A tailored biosignature composed of carefully selected essential markers will be required. Globally more than 2 billion individuals are infected with *Mycobacterium tuberculosis* (Mtb). Of these, 10% will remain latently infected without clinical signs and 10% will develop disease later. Hence, comparison between these two groups can provide insights into the mechanisms that discriminate between active TB and latent infection. Moreover, biosignatures:

- will help in better understanding biology of infection, immunity and pathogenesis;
- will allow monitoring of vaccine and drug trials;
- can predict risk of disease reactivation in latently infected individuals.

We have studied gene expression profiles in latently infected and non-infected individuals and compared them with patients with active TB. We found that a handful of biomarkers suffices for robust distinction between the three groups. These include Fc-γ receptor, guanylate-binding protein, granzyme A and defensin-α. In a more sophisticated approach, we defined biosignatures in selected T cells. We found that members of the JAK/STAT pathway involved in T cell regulation provided robust biomarkers for discrimination of patients with active TB, individuals with latent infection and non-infected healthy controls. Additional analysis focused on metabolomics. Of 370 different metabolites, ca. 20 were sufficient for robust discrimination between the three groups of study participants. Hence, biosignatures (i) provide deeper insights into mechanisms underlying host resistance and (ii) serve as a platform for the development of novel intervention measures against TB.

Studying infectious diseases in vivo

O466 Experimental leptospiral infection in transgenic/mutant mice: attenuated nephritis in inducible nitric oxide synthase knock-out C57BL/6 mice and pulmonary haemorrhages in SCID CB17 and recombinase activating gene Rag-1 knock-out C57BL/6 mice

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Objectives: (1) To investigate the frequency of pulmonary hemorrhage in mice unable to differentiate functional B and T lymphocytes; in the light of the hypothesis that pulmonary hemorrhages in leptospirosis are related to immunopathogenesis/auto-antibodies, and previous inconsistent data on the frequency of this complication in SCID mice of the C3H and C3H/HeJ backgrounds; and (2) To explore the effect of the genetic deficiency of inducible nitric oxide synthase (iNOS) on the frequency/severity of interstitial nephritis in vivo, in the light of previous in vitro data suggesting that leptospiral products induce renal tubular cells to express pro-inflammatory genes such as iNOS.
Methods: We studied the outcome of infection by the virulent *Leptospira interrogans* serogroup Icterohaemorrhagiae Cop strain. Animals used: iNOS-knock out (KO) mice (B6.129 P2-Nos) and the control wild-type C57BL/6 (B6) strain, in three experiments (7–15 per group) by intraperitoneal inocula (10^3 and 10^6); C57BL/6 129S(Cg)-Rag1 (Rag-1 KO) and CB17 SCID, and the respective B6 and BALB/c controls, in three experiments (5–15 per group) by intraperitoneal inocula (10^6 and 10^7).

Results: iNOS KO and B6 survived with no signs of disease. Leptospiral load counted in kidney samples, anti-*Leptospira* agglutinating antibody titres and specific anti-*Leptospira* IgG (by ELISA) did not differ between wildtype and transgenic infected animals. Some degree of interstitial nephritis was observed in 87% and 73% of B6 mice and 57% and 25% of iNOS-KO mice infected with a low or high inoculum, respectively. The frequency of severe nephritis was also lower in iNOS KO mice. All SCID and Rag-1 KO animals died of acute disease (median of days to death: 7 and 9 at 10^7, 7 and 10 at 10^6 inoculum, respectively) while B6 and BALB/c survived. The frequency of pulmonary haemorrhages was 10/12 (83%) and 30/30 (100%) in SCID and 4/7 (57%)and 16/17 (94%) in Rag-1 KO mice, at 10^7 and 10^6 inocula, respectively, and not detected in wild-type controls.
Conclusions: The lack of a functional iNOS gene in murine models has minimal effect on the outcome of leptospiral infection, except for a reduced susceptibility for the development of interstitial nephritis in iNOS-deficient mice. In addition, the absence of functional B and T lymphocytes does not preclude the occurrence of pulmonary haemorrhages. This is strong evidence against the hypothesis that pulmonary hemorrhages in leptospirosis are related to autoimmune mechanisms.

O467 Bacterial translocation is the major driver to death in experimental multiple organ dysfunction

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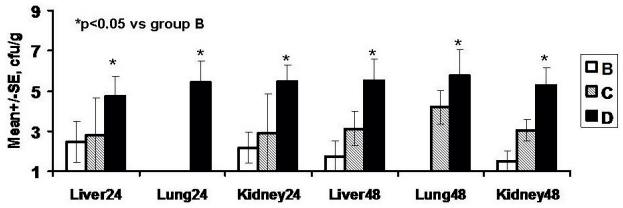
Objectives: The role of bacterial translocation in multiple organ dysfunctions (MODS) is debatable. To investigate this, a model of sterile MODS is needed i.e. MODS induced after challenge by microbial ligands and not by live bacteria.
Methods: MODS was induced in 176 male C57B6 mice (male C57Bl6) by the sequential ip injection of lipopolysaccharide (LPS 40 mcg) followed after 6 days by zymosan (ZM). Mice were divided into four groups: A sham; B LPS; C LPS + 0.6 mcg/g ZM; and D LPS + 1.2 mcg/g ZM. Survival was recorded. Mice were sacrificed at 24 and 48 hours for quantitative tissue cultures; splenocytes were isolated and stimulated with LPS for the release of IL-10 and IL-17; cytokines in serum and supernatants were measured by ELISA. Mice challenged with LPS + 1.2 ZM were treated im with placebo/ertapenem 250 mg/kg bid for five days. Survival was recorded; tissue bacterial growth was measured upon sacrifice at 48 hrs.
Results: Median survival of group B was 240 hrs; of group C 240 hrs; and of group D 36 hrs (p: 0.002). Total tissue recovery of enterobacteriaceae and enterococci is shown in the figure. Mean respective splenocyte apoptosis of groups A, B, C and D was 25.4, 45.6, 19.1, and 55.0% (pNS) at 24 hrs; and 17.4, 16.4, 11.6 and 17.8% at 48 hrs (pNS). At 48 hrs mean respective serum IL-6 was 20, 240.9, 158.5 and 468.7 pg/ml (pNS). Mean respective release of IL-10 was 24.0, 66.6, 253.4 and 241.6 pg/ml (pNS) at 24 hrs; and 15.4, 48.2, 121.0 and 65.3 pg/ml at 48hrs (pNS). Mean respective release of IL-17 was 15.0, 15.8, 24.9 and 19.3 pg/ml at 24 hrs (pNS); and 15.0, 15.2, 13.3 and 13.3 pg/ml at 48 hrs (pNS). Median survival after treatment with ertapenem was 7 days compared with 2 days of placebo (log-rank: 5.174, p: 0.023). Mean log10 bacteria in liver, lung, spleen and kidney was 5.51, 5.75, 5.27 and 5.30 in placebo-treated mice respectively; they became 2.22, 1.40, 1.45 and 1.38 in ertapenem-treated mice ($p < 0.0001$ vs placebo).



Conclusions: Bacterial translocation is early implicated even when MODS is induced by sterile ligands. Major changes of the immune response do not occur. Findings imply a need for appropriate adaptation of antimicrobials upon early signs of MODS.

O468 A model of tick-borne encephalitis in rats infected with Langat virus

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Objectives: Tick borne encephalitis virus (TBEV) and Langat virus (LGT) are closely related viruses within the mammalian group of tick-borne flaviviruses that cause menigoencephalitis in humans. The lethality rate of TBEV infections in Europe can reach 6.3% and causes permanent neurofunctional sequelae in up to 45% of the survivors. LGT, a naturally attenuated member of the TBEV-complex, has been previously used as a live vaccine against TBEV but its use has been abandoned since it caused meningoencephalitis with permanent neurological deficits in 0.005% of the vaccinees. The pathogenesis of TBEV and the mechanisms of brain injury underlying the neurofunctional sequelae of TBE are poorly understood and currently no effective therapy is available. Here we established a rat model of TBE using LGT strain TP21, with the aim to extend the understanding of the disease mechanisms and to develop novel treatment strategies.
Methods: Eleven-days old Wistar rats were inoculated intracisternally with 25 ul of saline (controls; n = 11) or with 25 ul saline containing increasing concentration of LGT ranging from 10^2(n = 4), 10^3 (n = 4), 10^4 (n = 4) to 10^5 (n = 8) focus forming units (ffu). After injection animals were monitored for clinical symptoms of disease by assessing appearance, roaming, feeding and weight over 10 d. Neurofunction was scored at 2, 4, 7 and 9 d after infection by assessing performance in behavioural tests i.e. rotating rod and open field. At predetermined time i.e. at 4, 7 and 9 d after infection animals were sacrificed, brains removed and analysed for histopathology.
Results: Over 90% (7/8) of rats infected with the highest concentration of LGTV (10^5 ffu) showed symptoms of menigoencephalitis including gait disturbances, hypokinesia, and reduced weight gain or weight loss. Hind leg paralysis and loss of balance was evident in 4/8 infected animals at 4 d after infection. On the rotating rod, infected animals showed a significant ($p < 0.05$) higher frequency of falls within 120 sec. compared to controls at 4 d after infection. Brains of infected animals exhibited characteristic histopathologic features of TBE including subarachnoid pleocytosis, perivascular cuffs, glial nodules and neuronal rarefaction.
Conclusions: The newly established rat model of tick-borne flavivirus-menigoencephalitis provides the opportunity to investigate mechanisms of disease and to develop therapeutic strategies for TBE where currently no effective treatment is available.

O469 Dengue fever, Dengue haemorrhagic fever, Dengue shock syndrome modelled in the ferret, *Mustela putorius* furo

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Objectives: Dengue virus is a mosquito-borne flavivirus responsible for human Dengue fever, Dengue hemorrhagic fever, and Dengue shock syndrome. Dengue infection in humans occurs in an estimated 50–100 million cases annually with 22,000–26,000 deaths attributed to Dengue infection. Dengue viruses exist in nature in four antigenically distinct serotypes with primary infection with any serotype responsible for Dengue fever. While murine and non-human primate models of Dengue virus infection exist, there are limitations to each model for understanding the role of innate, humoral, and cellular immune responses to primary and secondary infection by Dengue viruses. Our laboratory is focused on understanding the role of virus-host interactions in the pathogenesis of Dengue virus infection. Our laboratory utilizes the ferret (*Mustela putorius* furo) as a model of acute respiratory (influenza and coronavirus) infection and has been focused on the development of

the ferret as a model of Dengue fever, Dengue hemorrhagic fever, and Dengue shock syndrome.
Methods: IP Inoculation of ferrets with Dengue virus type 2 (DV2) led to primary Dengue infection with clinical signs of Dengue fever. Animals were assessed for clinical signs (fever, weight loss, activity changes) daily for 10 days, and blood or tissues harvested for virology, immunology, and pathology studies. Animals were administered a secondary Dengue challenge with Dengue virus type 1 (DV1) in an attempt to model Dengue hemorrhagic fever/Dengue shock syndrome using the above-described parameters. Additionally, vascular leak was assessed using Evan's blue dye (EBD) administration followed by quantification of EBD in tissues, gross and histopathological examination of tissues.
Results: Dengue virus antigen was detected in peripheral blood mononuclear cells, viral genome detected in serum and multiple tissues for up to 10 days post challenge. Humoral responses to Dengue viruses were detected early in infection with neutralizing antibody detected to the challenge virus serotype, but not to heterologous serotypes. Re-challenge of of ferrets after primary infection led to demonstrated evidence of vascular leak modeling Dengue shock syndrome.
Conclusions: Taken together, these results support the ferret as a model of Dengue fever, Dengue hemorrhagic fever, and Dengue shock syndrome. This novel model of Dengue infection may have utility in understanding Dengue pathogenesis and in therapeutic and vaccine development.

O470 Brain inflammation in a new mouse model for meningococcal meningitis

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Objectives: Meningococcal disease is a leading cause of meningitis worldwide, and brain edema is among the serious consequences of meningococcal meningitis. Aquaporin-4 (AQP4) is a water-channel that is highly expressed in the brain, predominantly in astrocyte endfeet and that is involved in the development of brain edema. Few models for bacterial meningitis exist, and here we present a new mouse model for the induction of meningitis by direct inoculation of bacteria into the subarachnoid space.
Methods: Mice from three strains (CD1, C57BL/6 and Aqp4 null mice in C57BL/6 genetic background) underwent intrathecal injection of either *Neisseria meningitidis* serogroup B (the meningococcus, Mc), *Streptococcus pneumoniae* (the pneumococcus, Pc) or purified Mc lipo-oligosaccharide (LOS). The degree of inflammation and aquaporin expression in the brain was quantified both morphologically and by assessing the gene expression of relevant markers quantitative real-time PCR.
Results: The Mc infection that developed was histologically characterized by acute inflammation primarily with meningeal infiltration of neutrophil granulocytes and an absence of parenchymal abscess formation, as well as ultrastructurally by pronounced edema. In Mc mice, the Tnf-a expression levels were increased after 9 h, predominantly in deep brain tissues. After 30 h, the inflammation was mostly confined to the brain surface. In Pc-injected animals, Tnf-a expression was increased in the brain surface at 9 and 30 h, and the inflammation was histologically more severe. In LOS-injected mice, Tnf-a levels were increased both in the brain surface and core brain. Locomotion was more severely reduced in mice inoculated with Mc than in those inoculated with Pc or LOS. Aqp4 null mice with meningitis were more active in locomotion than wildtype mice with meningitis, consistent with a clinically more favorable picture.
Conclusion: This is the first study of intrathecal inoculation of Mc in different mouse strains and monitoring Aqp4 null mice in the context of Mc meningitis. Our meningitis model shares many of the features characteristic of human meningitis and is the first to allow for a direct comparison between the two major bacterial pathogens, Mc and Pc. The model reveals that the two bacterial species differ significantly in regard to histological inflammation and involvement of different brain structures. Aquaporins are implicated in the pathogenesis of both types of meningitis.

O471 Demyelination in murine cerebral malaria is reversed by neuroprotective erythropoietin treatment

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Objectives: Cerebral malaria (CM) is the most severe complication of malaria, causing high risk of mortality and neurological sequelae in survivors. The development of an adjunct neuroprotective treatment requires improved knowledge about the underlying pathophysiology. Post-mortem investigations of fatal human cases suggest that demyelination may play an important role in CM pathogenesis. We have previously reported that erythropoietin (EPO) significantly improves survival in experimental murine CM. We tested the hypotheses that, a. experimental CM is associated with marked demyelination of cerebral white matter and, b. EPO treatment reverses this demyelination.
Methods: C57BL/6 mice were infected with *Plasmodium berghei* ANKA, which leads to CM approximately one week post infection (p.i.), or mock infected as controls. EPO treatment or vehicle was administered on day 4–7 p.i. The four experimental groups – CM, CM EPO, Control, EPO control – were terminated on day 8 when CM mice were terminal. Clinical signs of CM were assessed by body temperature plus behavioural and coordination tests. Brains were perfusion- and immersion fixed and processed for immunhistochemistry (for myelin basic protein and cell markers) by light microscopy and electron microscopy plus stereology. All microscopies were done blindly. The national board for animal studies approved all experiments.
Results: EPO treatment protected clinically against CM. In CM considerable patchy demyelination was seen in the white matter, particularly in the cerebellum, concurring with the occurrence of coordination impairment. There were multiple infarcts in the corpus callosum, which was selected for ultrastructural stereological assessments. The thickness of myelin was not decreased in CM, but a significant number of axons had degenerated myelin sheaths (Figure). A considerable number of activated microglia infiltrated the areas of demyelination and phagocytosis of myelin debris was seen. EPO treatment significantly reduced demyelination and microglia activation.
Conclusion: We demonstrate considerable myelin damage in experimental CM. This is reversed by neuroprotective treatment with EPO.

O472 Grafted neuronal stem/progenitor cells differentiate and functionally integrate at the site of pneumococcal-induced hippocampal brain injury in vitro

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Objectives: Survivors of bacterial meningitis (BM) frequently suffer long-term sequelae, including learning and memory deficits. The neurofunctional deficits are associated with the occurrence of apoptotic cells in the subgranular zone of the hippocampal dentate gyrus in experimental BM. Here we screened neural precursor cells (NPCs) for their potential to migrate, differentiate and integrate into organotypic hippocampal slice cultures (OHCs) injured by challenge with live *Streptococcus pneumoniae*.
Methods: Green fluorescence protein-expressing NPCs from the fetal hippocampus and subventricular zone, and from adult bone marrow were grafted into the hilus region of the dentate gyrus of injured slices and controls. The migration and differentiation of grafted cells was assessed by immunohistochemistry. Patch clamp and multielectrode array (MEAs) recordings were used to document the functional integration of the grafted cells.
Results: Seven days after engraftment histomorphologic analysis revealed migration of grafted NPCs from the site of injection to the molecular layer of the dentate gyrus in control slices. In contrast, when grafted in slices challenged with bacteria, NPCs migrated to the site of hippocampal damage in the granular layer of the dentate gyrus. Bone

marrow-derived stem cells failed to migrate and differentiate. Whole cell patch-clamp and MEA recordings of grafted NPCs demonstrated that grafted cells developed properties of mature neurons and became functionally integrated into the host neuronal network.
Conclusions: In OHCs, grafted NPCs migrated to the pneumococcal induced area of hippocampal damage and differentiated into neurons that functionally integrated into the hippocampal network. The transplantation of neurosphere derived NPCs may hold promise for regenerative therapies aimed at repair of apoptotic brain damage in the hippocampus after BM.

O473 Host response during *Pseudomonas aeruginosa* adaptation to cystic fibrosis lung

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Objectives: Cystic fibrosis (CF) lung disease is characterized by transient airway *P. aeruginosa* infections and excessive neutrophil-dominated inflammation early in life followed by permanent chronic infection that causes persistent respiratory symptoms and a decline in lung functions. Here we aimed to dissect the host response to *P. aeruginosa* patho-adaptive strains during acute and chronic infection.
Methods: We analysed the pathogenicity of sequential clonal strains isolated from a CF patient during a period of up to 7.5 years in a multihost system including four different models, namely, *Caenorhabditis elegans*, *Galleria mellonella*, *Drosophila melanogaster* and mouse. In addition, the epithelial bronchial cells of CF origin IB3–1, their wt-like isogenic cells C38 and macrophage-like cells THP-1 were used to sustain the ability to provoke inflammation or damage during early or late phases of *P. aeruginosa* infection.
Results: *P. aeruginosa* strains at the onset of infection are more pathogenic than late isolates from the same patient when tested in *C. elegans*, *G. mellonella* and *D. melanogaster*. In murine model of acute infection, the early *P. aeruginosa* strain induced higher mortality than late clonal strains. Although attenuated in mortality, *P. aeruginosa* late isolates retained their capacity to persist in mouse models of chronic infection. H&E, PAS-staining and Tunnel assay of lung tissue sections showed that early strain induced pronounced leukocytes recruitment indicating strong inflammatory response while late strains increased numbers of mucin-positive goblet cells and apoptotic cells, a typical hallmark of damage in the airway chronic diseases.
To establish the "pathological drift", IB3–1 cells were infected with early and late *P. aeruginosa* clonal strains from CF patients and subjected to microarray analysis. Results indicated a decreased inflammatory response, including down-regulation of leukocyte receptors and adhesion molecules, and an increased damage mediated by tissue remodelling, due to late strains compared to early strains.
Conclusion: Our findings suggest that during long-term infection *P. aeruginosa* revises its interaction with CF host by activating alternative pathways including evasion of the immune response, non-inflammatory cell death and those relevant for tissue damage and remodelling process to ultimately result in chronic disease and decline in lung functions.

O474 In vitro and in vivo reduced fitness and virulence in ciprofloxacin-resistant *Acinetobacter baumannii*

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Objectives: Antimicrobial resistance confers a biological fitness cost on bacteria that may be manifested as a decreased growth rate, and reduced virulence. However, there are limited experimental data on the relative fitness and virulence of antimicrobial resistant *A. baumannii*. The aim of this study is to assess the fitness cost caused by ciprofloxacin resistant *A. baumannii*, as well as its virulence relative to the susceptible parental wild type (wt) strain in in vitro and in vivo murine peritoneal sepsis (MPS) model.
Methods: Two *A. baumannii* strains: 77wt (susceptible to ciprofloxacin) and its derivative 77R (resistant to ciprofloxacin) were used. Biofilm formation was determined using crystal violet assay. Cellular viability of human lung epithelial cells (A549) was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Live/Dead®, and lactate dehydrogenase (LDH) assays after addition of 77wt or 77R (10^8 cfu/mL) for 24 h. Moreover, mortality and competition fitness between both strains was monitored in vitro and in vivo in a MPS model during 24 h.
Results: The acquisition of ciprofloxacin resistance by 77R strain reduces the formation of biofilm observed with the 77wt strain by 50%. A549 cells infected with 77R strain showed lower decrease in the cell death and release of LDH (86.53±2.44% and 5.98±1.81%) than 77wt strain (66.23±4.01% and 48.38±3.5%), respectively. Additionally, the assessment of A549 cells survival by Live/Dead® showed less dead cells, stained with ethidium homodimer-1 producing a bright red fluorescence, in A549 cells infected with 77R strain than with 77wt strain. Furthermore, resistance to ciprofloxacin acquired by 77R strain decreases the mortality of animals in MPS from 100% (observed with 77wt strain) to 20% at initial inoculum of 8.3 log cfu/mL. This resistance to ciprofloxacin slow the growth of 77R strain in vitro and in vivo MPS model in competition with the 77wt strain by 1.7 and 2.02 log at 24 h, respectively, compared to 77wt strain alone; thus, the in vitro and in vivo competition index were 0.02 and 0.03, respectively.
Conclusion: Our findings reveal the presence of an in vitro and in vivo fitness cost and reduced virulence of ciprofloxacin resistance in *A. baumannii*. The understanding of the gap between antibiotic resistance, biological fitness and virulence of *A. baumannii* will be useful to improve the antibiotic therapy against *A. baumannii*.

O475 Relationship between the gut microbiota and obesity in children and adolescents

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Objectives: Obesity is considered as one of the most important public health problems of our times. The last few decades the prevalence of obesity, especially among children and adolescents, has increased dramatically worldwide. The aim of our study was to determine whether the composition of the gut microbiota is related to obesity in childhood.
Methods: A cross-sectional study was set-up to examine the gut microbiota using faecal samples from 22 obese children and 33 non-obese children aged 6–16 years. The microbial composition in the faecal samples was analyzed by quantitative plating for *Staphylococcus* spp., *Bacteroides fragilis* group, *Clostridium* spp., *Lactobacillus* spp. and for *Bifidobacterium* spp.; matrix-assisted laser-desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) for identification of species of the *Bacteroides fragilis* group and quantitative real-time polymerase chain reaction (qRT-PCR) to determine the number of *Staphylococcus* spp., *Bacteroides-Prevotella-Porphyromonas* group, *Clostridium coccoides-Eubacterium rectale* group, *Clostridium leptum* subgroup, *Lactobacillus* spp., and *Bifidobacterium* spp. For statistical analysis, the BMI z-score was used as dependent variable thereby correcting for age and gender. A P-value of <0.05 was considered statistically significant.
Results: Both quantitative plating and qRT-PCR showed that the faecal concentration of the *Bacteroides fragilis* group in obese children was significantly lower than in non-obese children ($P = 0.017$ and 0.018, respectively). Additionally, MALDI-TOF MS analysis demonstrated that obese children were colonized more frequently with *B. fragilis* than non-obese children (19.18% and 7.33%, $P = 0.039$) whereas colonization with *B. vulgatus* was significantly higher in non-obese children compared to obese children (8.14% and 18.91%, $P = 0.016$). Furthermore, *B. fragilis* was significantly positively correlated to the BMI z-score ($P = 0.03$). Higher colonization with *B. fragilis* could therefore be associated with an increase in weight. The microbiota of obese children was also associated with a higher Firmicutes/*Bacteroides* ratio ($P = 0.02$).
Conclusions: Significant differences were found in the composition of the fecal microbiota of obese and non-obese children. These results indicate that changes in the gut microbiota during childhood and

adolescence could lead to the development of obesity and that the gut microbiota could be an additional risk factor for obese-prone children.

Antifungals: in vitro and in vivo activity, pharmacokinetics and resistance

O476 EUCAST susceptibility testing of *Candida* species to echinocandins: improved separation between wild type isolates and fks mutants by supplementation of BSA to the test medium

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Background: MICs of the three echinocandins against isolates with fks mutations from clinical failure cases are higher than those relative to wild type isolates, but the ranges of these susceptible and mutant populations either overlap or are just separated by 1–2 dilution steps. A recent preliminary study (Garcia-Effron, ICAAC M-352, 2009) reported that the addition of BSA to the growth medium leads to a better separation between wild type and mutant isolates using the CLSI method. We here investigated if this was also true for the EUCAST method.
Methods: MICs were determined for anidulafungin, caspofungin and micafungin by the EUCAST method without (EDef7.1) and with addition of 50 mg/mL BSA. A total of 94 clinical isolates including (no. of wt/no. of mutants) *C. albicans* (10/10), *C. glabrata* (9/11), *C. dubliniensis* (1/1), *C. krusei* (13/3), *C. parapsilosis* (19), and *C. tropicalis* (15/4) isolates. Three isolates harbour mutations outside the resistance hot spots and are regarded as wild type concerning echinocandin susceptibility because of their normal kinetic inhibition properties.
Results: The addition of BSA to the growth medium resulted in higher MICs for all isolates and all three compounds (Table). The increase was greatest for anidulafungin and micafungin, and notably greater for fks mutants compared to WT isolates. Among WT isolates the greatest increase was observed for *C. parapsilosis* which intrinsically harbours a "hot spot mutation".
Conclusion: Addition of BSA to the EUCAST growth medium enhances the MIC differences between fks hot spot mutants and wild-type isolates and thus increases the ability of the susceptibility test to differentiate between susceptible isolates and those harbouring resistance mutations.

Table. Increase in MIC_{50} (in 2-fold dilution steps) for WT isolates and *fks* mutants using EUCAST supplemented with BSA compared to the EUCAST EDef 7.1.

	Anidulafungin		Caspofungin		Micafungin	
	WT	*fks* mutant	WT	*fks* mutant	WT	*fks* mutant
C. albicans	≥2	6	0	≥6	≥5	8
C. dubliniensis	4	≥7	2	≥4	≥7	≥6
C. glabrata	5	≥7	2	5	5	≥9
C. krusei	≥6	6	2	4	≥9	≥7
C. parapsilosis	≥6		4		≥8	
C. tropicalis	≥4	7	1	≥6	≥7	≥8

O477 Comparison of in vitro fungicidal activities of echinocandins against *C. albicans* in different peritoneal dialysis fluids

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Objectives: Continuous ambulatory peritoneal dialysis used in the treatment of patients with end-stage renal failure is often complicated by peritonitis. The current guidelines advocate the use of intermittent intraperitoneal (IP) antimicrobial management for peritoneal dialysis (PD)-associated fungal peritonitis. IP administration results in very high antimicrobial concentrations at the site of infection. Peritoneal dialyses fluids (PDFs) affect inhibitory efficacy on the microorganisms' growth, which may compromise the affectivity of some antibiotics. However, there is no data about activity of antifungal agents in PDFs. The purpose of this study was to investigate in vitro the fungicidal effectiveness of three echinocandins in diverse PDFs.
Methods: The fungicidal efficacy of caspofungin, anidulafungin and micafungin against *C. albicans* ATCC 90029 was studied in the PDFs: Dianeal PD4® (glucose 1.36%, 2.27%, 3.86%), Physioneal 40® (glucose 1.36%, 2.27%, 3.86%), Extraneal® (7.5% icodextrin), and Nutrineal PD4® (1.1% amino acid) using time-kill curves. The protein concentration of the PDFs was adjusted to 2 g/l with human serum albumin (HSA) and the pH adjusted to 7.4 with NaOH, corresponding to conditions of used PDFs after a 4–6 h intraperitoneal dwell. Sabouraud buillon (SAB) was used as a control broth. Ten milliliter of diverse PDFs and SAB containing yeast inoculum of approximately 106 CFU/ml was incubated for 2 h at 37 ºC. Following incubation, the echinocandins at concentrations: 1×MIC, 4×MIC, 8×MIC were added. Samples were taken at 0, 2, 4, 6, 8, 10 and 24 h and the number of CFU/ml was determined.
Results: No difference in fungicidal activity between different echinocandins was shown. However, echinocandins were significant less active in PDFs then in control broth ($p < 0.01$) In SAB all three echinocandins at the concentration of 1×MIC attained 100% reduction of viable cells. In contrast, at concentration of 1×MIC echinocandins achieved no fungicidal activity (reduction <1 log10 cfu/ml). At concentration of 4–8×MIC the highest decrease was detected in icodextrin containing PDF Extraneal®, however for 1.5 log10 cfu/ml only.
Conclusion: Based on these in vitro data, we conclude that PD fluids using in clinical settings could impact the activity of echinocandins.

O478 Prospective surveillance of azole-resistance in *Aspergillus fumigatus* in the Netherlands

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Objectives: Antifungal azoles are the cornerstone of the management of *Aspergillus* diseases. However, acquired azole resistance of aspergilli is increasingly reported, which compromises the use of this class of antifungals. The prevalence and spread of azole resistance in clinical *A. fumigatus* isolates is currently unknown.
Methods: We performed a prospective multicentre surveillance study to determine the prevalence of azole-resistance in *Aspergillus* species. Between June 2007 and January 2009 medical microbiology laboratories of 7 Dutch University Medical Centres screened all clinical *Aspergillus* isolates for resistance to itraconazole (ITZ) using Sabouraud agar-slants supplemented with 4 mg/l of ITZ. Phenotypic susceptibility profile and the mechanisms of resistance were determined for resistant isolates. Patient characteristics were also collected.
Results: In total 2,062 *Aspergillus* isolates from 1,385 patients were screened of which 87% were identified as *A. fumigatus*. 82 isolates were ITZ-resistant, of which 79.8% was also resistant to voriconazole and 16.7% to posaconazole. In 90.2% of ITZ-resistant *A. fumigatus* isolates a L98H substitution combined with a 34 base pair duplication in the Cyp51A-gene promoter region was found. The overall prevalence of ITZ-resistance in *A. fumigatus* was 5.3% (range 0.8 to 9.5%). Patients with a hematologic/oncologic disease were more likely to harbour an azole-resistant isolate compared to patients groups with other underlying diseases ($p = 0.02$). 64% of patients in whom a resistant isolate was recovered were azole-naïve and the mortality-rate of patients with azole-resistant invasive aspergillosis was 88%.
Conclusion: Multi-azole-resistance in *A. fumigatus* is wide-spread in the Netherlands and is associated with a poor outcome in patients with invasive aspergillosis.

O479 In vitro secondary azole resistance in *A. fumigatus* isolates does not necessarily involve mutations in the cyp51A gene

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Background: Most *Aspergillus fumigatus* isolates showing phenotypic antifungal azole resistance have mutations at codons 54, 98, 138, 220, or 448 of the cyp51A gene. Secondary azole resistance in *A. fumigatus* isolates has been described in patients taking long-term itraconazole (ITRA) therapy. We searched for the presence of cyp51A mutations in *A. fumigatus* isolates after prolonged in vitro exposure to increasing ITRA concentrations.
Methods: We selected 20 *A. fumigatus* sensu stricto isolates without mutations at codons 54, 98, 138, 220, or 448 in cyp51A. ITRA, voriconazole (VORI), and posaconazole (POSA) MICs were determined using the CLSI M38-A procedure (MICinitial). Isolates were grown on Sabouraud dextrose agar, and adjusted conidia inocula (3x107 c.f.u./mL) were prepared in water. Twenty microliters of each suspension was stroked on plates containing ITRA at concentrations one-fold below the MICinitial. The plates were incubated for 7 days at 35ºC. When fungi grew, another adjusted suspension was prepared and propagated to a different plate containing the next two-fold ITRA concentration (to a maximum of 16 μg/mL). From the plate containing the highest ITRA concentration allowing fungal growth, a conidia suspension was prepared to calculate the MIC of the three azoles (MICfinal). The cyp51A sequence was obtained and STRAf genotyping of each isolate performed before and after exposure to ITRA to demonstrate that the strain was the same, ie, it had not been contaminated by other isolates.
Results: The percentages of isolates able to grow on plates containing different concentrations of ITRA were 95% (2 μg/mL), 75% (4 μg/mL), 70% (8 μg/mL), and 50% (16 μg/mL). Antifungal susceptibility of the isolates before and after ITRA exposure is shown in the table.
The MICfinal of the three azoles obtained by the CLSI M38-A procedure was significantly higher than the MICinitial ($P \leqslant 0.001$). No mutations were found in the cyp51A gene at codons 54, 98, 138, 220, and 448 of isolates growing at the highest concentration of ITRA. STRAf demonstrated that the genotype did not change before or after exposure to ITRA.
Conclusions: Our results indicate that prolonged exposure of *A. fumigatus* isolates to increasing ITRA concentrations leads to a loss of susceptibility to ITRA, VORI, and POSA. Secondary phenotypic resistance to azoles does not necessary involve the presence of mutations at codons 54, 98, 138, 220, or 448 in the cyp51A gene.

	ITRA			VORI			POSA		
	Range	Geometric mean	MIC_{90}	Range	Geometric mean	MIC_{90}	Range	Geometric mean	MIC_{90}
$MIC_{initial}$	1-2	1.40	2	0.5-2	1.05	2	0.5-1	0.63	1
MIC_{final}	1-32	15.90	32	1-8	3.42	8	0.5-2	1.29	2

O480 Multidrug resistance transporter genes play a potential role in *Aspergillus fumigatus* azole resistance

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Objectives: Mutation of the CYP51A gene locus is a common azole mediated mechanism of resistance in *Aspergillus fumigatus*. Recent data suggests that mechanisms other than these mutations contribute to resistance and treatment failure. Efflux pump mediated resistance has been described is in pathogenic fungi, therefore, the aim of this study was to examine the expression and function of multidrug resistance (MDR) efflux pumps on azole resistance.
Methods: Expression of AfMDR1–4 genes was assessed in vitro on three phases of filamentous growth (8, 12 and 24 h) by qPCR analysis using three cystic fibrosis *A. fumigatus* strains. In addition, qPCR analysis was performed on lung samples (day 1 to 5) obtained from an animal model of invasive aspergillosis±posaconazole treatment. AfMDR1–4 were then knocked out by fusion PCR, which were then characterised biochemically and using molecular techniques.
Results: AfMDR1–4 were differentially expressed in vitro, with AfMDR2 showing the highest levels of constitutive expression for all phases of growth, followed by AfMDR1, AfMDR4 and AfMDR3. AfMDR1 and AfMDR4 showed an increasing trend over time towards up-regulation, whereas AfMDR3 showed a transient up-regulation. Within the animal model AfMDR1 was preferentially expressed, followed by AfMDR2–4. AfMDR3–4 exhibited apparent up-regulation following posaconazole treatment. Deleting these genes improved sensitivity towards the azoles.
Conclusion: This study has shown that each of the AfMDR efflux pumps have defined roles, and which are also expressed within an infection model. Azole treatment appeared to stimulate *A. fumigatus* by altering the expression of efflux pumps, which may have implications to refractory azole therapy. Deletion of these genes impacted azole sensitivity, implying that these pumps have a possible role as a resistance mechanism.

O481 Azole-resistance in *Aspergillus fumigatus*: collateral damage of fungicide use?

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Objectives: Since the year 1998 the emerge of multi-azole-resistance in *A. fumigatus* isolates has been reported in clinical isolates containing the TR/L98H mutations in the cyp51A gene. Is has been postulated that the TR/L98H mutations have been introduced in *A. fumigatus* in the environment due to the agricultural use of azole fungicides.
Methods: All licensed azole fungicides between 1970 and 2010 were purchased and tested for in vitro activity against azole susceptible and azole resistant TR/L98H mutated *A. fumigatus* isolates. Docking studies were performed by using a CYP51A homology model to determine similarities in docking of medical and fungicide azole compounds. Microsatellite typing was used for genotyping the TR/L98H isolates and subsequently used for evolutionary study to determine the origin of the TR/L98H mutations in the *A. fumigatus* population.
Results: Five out of all 30 licensed Dutch azole fungicides show cross-resistance against the TR/L98H isolates, from which four fungicides also showed the highest similarity in docking-studies when compared with the medical azoles. The four agricultural fungicides that show cross-resistance have been introduced between 1990–1996. Analysis of microsatellite data showed that the TR/L98H mutated isolates have been introduced in the Dutch *A. fumigatus* population around the year 1997 (95% CI:1993.7–1999.7).
Conclusion: By providing the link between fungicide use and TR/L98H multi-azole resistance *A. fumigatus* a route of azole resistance development is given which could not be explained by the medically known resistance development within patients. Wind-dispersion of fungal spores and high levels of azole compounds will provide perfect conditions for further spread of TR/L98H azole resistant *A. fumigatus* isolates. As already observed, global spread of azole resistance in *A. fumigatus* can be anticipated.

O482 Caspofungin exposure in patients with haematological malignancies is a risk factor for fungaemia due to decreased susceptible *Candida* spp.: a case-control study in Paris

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Objectives: *Candida* spp. bloodstream infections (BSI) are associated with poor outcomes in haematological patients. Caspofungin is among recommended first line treatments in invasive candidiasis especially in patients preexposed to fluconazole and as empiric therapy in febrile neutropenic patients. There are increasing reported infections

due to resistant isolates in patients receiving caspofungin therapy. We here aimed at identifying and quantifying factors associated with BSI due to *Candida* spp. with intrinsically (*C. parapsilosis*, *P. guilliermondii*) or acquired (*C. albicans*, *C. glabrata*, *C. lipolytica*) reduced susceptibility to caspofungin (CRSC) in adults suffering from haematological malignancies.
Methods: We used data from an ongoing surveillance program in the Paris area to build a nested matched case-control study considering *Candida* spp. BSI in patients ⩾17 y-o suffering from haematological malignancies from October 2002 to February 2010 and using EUCAST method (AM3 medium) with a MIC cut-off of 0.5 mg/L. Fifty-one cases were matched to 102 controls on hospital and 1 year-time criteria. Cases were patients infected with CRSC and controls were those infected with *Candida* spp. susceptible to caspofungin. Univariate and multivariate analysis were performed and associations were estimated by odds ratio (OR) with 95% confidence interval (CI) using conditional logistic regression.
Results: In univariate analysis exposure to caspofungin within the past 30 days preceding fungemia was associated with CRSC (OR = 6.31, CI 95% [2.06–19.33]) as was age ⩽65 years (OR = 3.81, CI 95% [1.51–9.57]). In multivariate analysis, exposure to caspofungin within the past 30 days preceding fungemia remained associated with CRSC (OR = 5.25, CI 95%[1.68–16.35]), as was age ⩽65 years (OR = 3.27, CI 95% [1.26–8.50]).
Conclusion: Recent exposure to caspofungin increases the occurrence of *Candida* spp. BSI with reduced susceptibility to caspofungin. This study adds evidence to previous report on the influence of recent preexposure to caspofungin on the epidemiology of *Candida* spp. BSI.

O483 Efficacy of anidulafungin and caspofungin against a caspofungin-resistant isolate of *Candida albicans* in a murine model

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Objectives: Echinocandin resistance in *Candida* is unusual, and rarely there are differences between echinocandins. Our aim was to assess in vivo efficacy of caspofungin (CAS) and anidulafungin (ANID) against a CAS-resistant isolate.
Methods: Our large culture collection was screened for an isolate with discrepant echinocandin susceptibility, and only one was found, #03–248. CD-1 mice were infected iv with various inocula of CAS-resistant, 03–248, or susceptible, 98–144, strains of *C. albicans*. Treatment with CAS or ANID ip, QD, began 4 days later. One day after 7 days of therapy, CFU in the kidneys of survivors was determined.
Results: 100% inhibition/⩾80% inhibition (CLSI)/MFC of 03–248 was 25/6.25/50 to CAS and 6.25/0.78/6.25 to ANID. After an inoculum of 3.8×10^5 of 03–248, no dose (0.1, 5 or 20 mg/kg) of CAS or ANID prolonged survival nor were there differences among ANID treatment groups. A replicate study with 4.4×10^5 03–248 gave similar results. In a 3rd study using 03–248, inoculum 1.2×10^7, CAS or ANID at 10 mg/kg prolonged survival versus controls (P ⩽ 0.04); 20 mg/kg doses trended to significance (P >0.05 <0.08). Mice carried significant burdens of 03–248; no treatment was superior to controls nor were there significant differences among dosages or comparing drugs. Against the CAS-susceptible 98–144 (MIC/MFC ⩽0.1/⩽0.3, CAS and ANID), ANID at 20 mg/kg significantly reduced kidney CFU of 98–144 versus controls (P = 0.0001), similar to our published data on CAS-susceptible isolates.
Conclusions: In vitro susceptibility predicts in vivo outcome. Resistance to CAD>ANID does not translate to in vivo efficacy by ANID; in vitro resistance to CAS predicted in vivo resistance for both. Resistance to CAS or ANID in vivo does not depend on the severity of infection, ANID and CAS demonstrated the lack of efficacy against 03–248 in disease differing from moderate to severe. These data suggest resistance in vitro to any echinocandin should caution the use of any echinocandin in therapy, regardless of in vitro differences.

O484 In vitro posaconazole pharmacokinetics

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Objectives: Infections due to *Aspergillus fumigatus* (AF) remain a critical concern after allogeneic stem cell transplants and in patients with hematologic malignancy. Posaconazole prophylaxis has proven highly effective in preventing these infections, despite relatively low serum concentrations. We have shown that posaconazole concentrates over several hundred fold within the membranes of pulmonary epithelial cells, and that this level of posaconazole can inhibit AF growth and protect host cells from injury. The objectives of this study were to define the pharmacokinetics of posaconazole within pulmonary epithelial cells, and to determine the level of posaconazole required to inhibit fungal growth.
Methods: A549 pulmonary epithelial cells were exposed to different concentrations of posaconazole and then analyzed at varying times after the drug was removed. Decay kinetics of posaconazole were determined through sequential harvesting of cells after drug exposure and analyzed using HPLC. Concentrations of posaconazole required for fungal inhibition, posaconazole exposed cells were infected at fixed time intervals after drug removal. To monitor fungal growth, serial samples of culture supernatants were taken and analysed for galactomannan (GM) content using the *Aspergillus* EIA Platelia assay.
Results: A549 cells exposed to 2 μg/ml of posaconazole inhibited the growth of AF when immediately infected. However, when the cells were infected >6h after drug removal, AF growth was not inhibited. GM content analysis confirmed the visual breakthrough: A549 cells infected >6h after loading with 1 and 2 μg/ml posaconazole, GM content progressively increased over time. In contrast, A549 cells exposed to 4 and 8 μg/ml were able to inhibit fungal growth when infected at time points up to 48 hours post drug exposure. HPLC analysis suggests a two phase decay model: a rapid initial decay to approximately 25% of the initial intracellular concentration within the initial 6 hours, followed by a plateau. As expected, observed fungal growth correlates with the decrease in drug levels.
Conclusion: These results demonstrate that posaconazole exhibits unique pharmacokinetics that differ from the reported pharmacokinetics of the serum compartment. These data support a re-examination of the approach to therapeutic drug monitoring during antifungal prophylaxis with posaconazole, and may provide an explanation for the observed lack of correlation between efficacy and serum levels of this agent.

O485 Pharmacokinetics and pharmacodynamics of micafungin, anidulafungin and caspofungin for disseminated candidiasis due to *Candida glabrata*

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Objectives: Echinocandin agents are increasingly considered first line agents for the treatment of disseminated candidiasis. *Candida glabrata* is the second most common cause of disseminated candidiasis and is frequently resistant to triazoles. Currently there are limited data to guide the optimal echinocandin dosage for neutropenic hosts infected with *C. glabrata*. Here we use a neutropenic murine model of disseminated candidiasis to study the pharmacokinetic and pharmacodynamic relationships of the echinocandins against this medically important opportunistic pathogen.
Methods: The MICs of micafungin, anidulafungin and caspofungin against *C. glabrata* (ATCC 2001) were 0.06, 0.03, and 0.5mg/L respectively (using CLSI methodology). Each drug was administered 5 hours post-inoculation and every subsequent 24 hours at 0.1, 1, 5 and 20mg/kg intraperitoneally. Echinocandin concentrations in plasma and kidney were determined between 0–96 hr using HPLC. The fungal burden in the kidney was simultaneously quantified. A pharmacokinetic-pharmacodynamic mathematical model was used to link drug concentrations with the antifungal effect, and to define the drug exposure that produced near maximal fungicidal activity.

Results: In untreated mice there was a progressive rise in kidney fungal burden throughout the experimental period. The pharmacokinetics of each agent were linear. For each agent, the rate of killing was relatively slow with progressive antifungal effect with higher dosages. Near maximal antifungal effect of micafungin and caspofungin was observed following a dosage of approximately 5mg/kg, whereas dosages >5mg/kg of anidulafungin were required to achieve this same endpoint. For micafungin, these drug exposures are higher than those associated with currently licensed human regimens.
Conclusion: The echinocandins exhibit fungicidal activity against *C. glabrata*. Higher echinocandin dosages may be required to achieve near maximal antifungal effect in profoundly neutropenic hosts infected with this organism.

Travel medicine: tropical and parasitic diseases

O486 *Dientamoeba fragilis* DNA detected in eggs of *Enterobius vermicularis* by conventional and real-time PCR

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Objectives: For more than 50 years it has been hypothesised that *Enterobius vermicularis* (EV) can serve as a vector for *Dientamoeba fragilis* (DF), an intestinal protozoan parasite with suspected pathogenic potential. While some authors have been able to show higher than expected co-incidence of EV and DF in clinical samples, not all studies have been able to do so, and successful culture of DF trophozoites from the eggs of EV has yet to be demonstrated. The objective of this study was to show DF specific DNA in eggs of EV, harvested from a patients co-infected with both species.
Methods: Cellophane tape- and faecal samples were collected from the patient simultaneously, and positive identification of EV was made using microscopy and of DF by real-time PCR. An intact EV female was recovered from the Cellophane tape sample, and eggs harvested by compressing the worm in a solution of 0,5% NaClO (hypochlorite). The eggs were then both centrifuged (1500 xg, 5 min.) and washed with DNA-free H20 three times. Thirty visibly intact eggs were individually transferred into PCR tubes and treated with proteinase K, followed by heat-inactivation (100ºC, 15 min.). Eggs 1–10 were tested with real-time PCR for DF, eggs 11–20 with conventional PCR for EV, and eggs 21–30 with conventional PCR for DF. Amplified PCR-products were sequenced.
Results: 2 of eggs 1–10 were tested positive for DF (Ct-values of 30 and 32), eggs 11–20 all showed positive bands for EV, and eggs 21–30 showed 1 positive band for DF. An internal positive control was used for the real-time PCR, and all PCR's had 5 negative controls, using water from the last washing step. Results were confirmed in repeated testing, often with more eggs tested positive for DF (at most 6 out of 10 with real-time PCR).
Conclusion: We have shown that DF-specific DNA sequences can be amplified from DNA extracted from EV eggs that have been surface-sterilised with hypochlorite. Not all eggs were found to be positive for DF though. These findings have important implications for our understanding of the transmission of *D. fragilis* and therefore also for control and public health measures, as well as antimicrobial interventions related to both of these common intestinal parasites.

O487 Long-term sonographic follow-up of inactive echinococcal cysts of the liver

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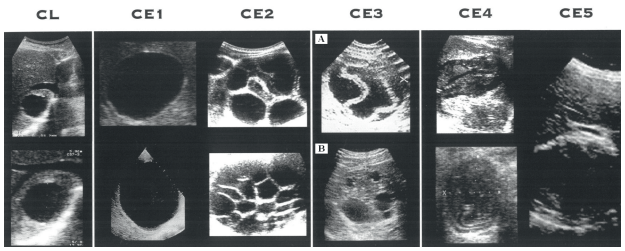
Objectives: Ultrasound is the ideal tool to diagnose, guide percutaneous treatments, and monitor abdominal cystic echinococcosis. The sonographic classification proposed by WHO Informal Working Group on Echinococcosis (WHO-IWGE) allows the distinction in active, transitional and inactive cysts, thus facilitating selection of treatment modalities (Fig. 1). As for uncomplicated, inactive cysts (CE4, CE5), recent expert opinion recommends they should be left untreated and monitored, but no data exist on the safety and effectiveness of this approach. To fill this gap, we report our experience with long-term sonographic monitoring of inactive cysts.
Methods: Records of patients who presented at our clinic and were diagnosed with inactive echinococcal cysts of the liver were searched. Inclusion criteria for the retrospective evaluation were:
– presence of cysts exclusively in inactive stage at the time of diagnosis;
– follow-up with abdominal ultrasound performed every 6 or 12 months;
– minimal length of follow up: 24 months.
For each patient demographic details, number and location of the cyst within the liver, complications, if any, were obtained.
Results: From March 1994 to March 2010, 68 patients with exclusively inactive liver cysts were seen in our clinic, but 38 were excluded because their follow-up period was shorter than 24 months. Of the 30 patients who met the inclusion criteria, 14 were males and 16 females (mean age at time of diagnosis 48, range: 14–86), harbouring a total of 38 cysts. Twenty-two patients harboured 1 cyst each, 8 patients harboured 2 cysts each. 29 cysts were located in the right lobe, 2 in the left lobe and 7 in the fourth segment. Nineteen patients were diagnosed with inactive cysts at our clinic, while 11 were diagnosed in other centres. The mean follow-up period was 79 months (range: 25–453) and in 29 patients (97%) the cysts remained in inactive stage (reactivation occurred only in 1 patient during follow-up).
Conclusion: The idea of leaving certain cyst types untreated is the consequence of the observation that a proportion of cysts become completely inactive, without any treatment, and cysts that reached this stage are likely to remain inactive. Our experience confirms that watch and wait is a viable management option for inactive liver cysts.



O488 The United Kingdom National External Quality Assessment Service (UKNEQAS) for parasitology: will the participants never learn?

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Objectives: In 2003 we examined performance in UKNEQAS Parasitology for evidence of improved standards in parasite diagnosis in clinical specimens and found that although there was an overall improvement, the study still highlighted problem areas in both faecal and blood parasitology. (1)
Methods: Performance of Blood and Faecal Parasitology Schemes was analysed from 2003 to2011 in particular those areas of concern that were established in the 2003 study. The problem areas identified in the faecal parasitology scheme were difficulty in recognising small protozoan cysts, differentiating vegetable matter from parasites and detecting ova and cysts when more than one species is present. In the blood scheme, participants had problems in identifying mixed malarial infections, distinguishing between P ovale and P vivax, and estimating the percentage parasitaemia.
Results: In this study, it was noted that although performance continues to improve for the detection of one parasite present in a specimen, it has not improved in the aforementioned problem areas despite the teaching sheets targeting these areas.
Conclusion: This study showed that participants continue to have problems in faecal parasitology with recognizing small protozoan cysts, differentiating vegetable matter from parasites and detecting ova and

cysts when there is more than one species present. In Blood parasitology participants continue to have difficulty in differentiating the malaria species, estimating the percentage parasitaemia and identifying mixed malaria infection. This is despite the educational aspect of the UK NEQAS Parasitology Schemes.

O489 Discordance with World Health Organization 2009 admission criteria in outpatient confirmed adult Dengue patients in Singapore

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Objectives: World Health Organisation (WHO) 2009 dengue guidelines classified non-severe dengue with and without warning signs and severe dengue as alternatives to WHO 1997 criteria of dengue fever, dengue haemorrhagic fever (DHF) and dengue shock syndrome. Hospitalisation is recommended for patients with warning signs or severe dengue. We aim to assess their utility in our prospective adult dengue study.
Methods: Adults with undifferentiated febrile illness referred to Communicable Disease Centre were recruited prospectively. Dengue polymerase chain reaction and non-structural protein-1 antigen testing was performed. Positive cases (n = 62) were managed daily until recovery. We analysed cases requiring only outpatient care.
Results: Males comprised 84% with a mean age of 33.7 years (range 20–54 years); 98% of subjects had no co-morbidities. Cases were followed up for a mean of 3.2 days (range 1–5 days). All fulfilled WHO 2009 clinical definition of probable dengue, while 88% fulfilled WHO 1997 definition. None had DHF. Warning signs (WHO 2009) in order of frequency at presentation were: lethargy (98%), mucosal bleed (30%), abdominal pain/tenderness (13%), persistent vomiting >3/day (3%), with no incidence of clinical fluid accumulation, hepatomegaly or haematocrit 20% above baseline with platelet <50000/mm3. All had at least one warning sign; if lethargy was excluded this dropped to 38%. There was one case of severe dengue with transaminases >1000 units/L. Of two cases with possible severe bleeding, one with a history of melaena was not corroborated on examination or investigation, and one with menorrhagia was well-controlled on progesterone. All cases had complete resolution of symptoms, signs and laboratory parameters at convalescent follow-up on day 21–30.
Conclusions: In our cohort of outpatient confirmed adult dengue patients, at least 38% with one or more warning signs would have been hospitalised under WHO 2009 guidelines. Three cases of severe dengue were appropriately managed as outpatients. None was identified as DHF by WHO 1997 criteria. We propose that current WHO 2009 guidelines may lead to over-hospitalisation and suggest further refinement to optimise scarce clinical resources.

O490 Adventure tourism and schistosomiasis: serology and clinical findings in a group of Danish students after white-water rafting in Uganda

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Objectives: Returning travellers with schistosomiasis are known to present few if any symptoms, especially if the infection is light. In July 2009 a group of Danish students travelled to Uganda, where they participated in white-water rafting and swimming on the river Nile (Jinja). Upon return a single person sought medical attention for symptoms developed (macroscopic haematuria), and was found to have eosinophilia as well as schistosoma-antibodies. Subsequent testing of the entire group revealed a large number of sero-positives, all with no previous history of schistosomiasis. The objective of this study was to describe serology and clinical findings in a group of returning travellers, with possible exposure to Schistosomiasis.
Methods: From each patient ⩾2 serum-samples were collected, with ⩾1 sample collected later than 3 months after exposure. Serology was performed using IFAT for Gut Associated Antigen (GAA) and Membrane Bound Antigen (MBA). 24-hour urine-samples were collected from 33 out of 40 patients and examined using microscopy. A patient-response questionnaire was sent to the group to address exposure, travel behaviour and patient experienced symptoms. Medical records were retrieved to access laboratory results, physician noted symptoms and clinical findings.
Results: 36/40 patients were exposed to fresh water (rafting and/or swimming), 14/36 developed a positive serology (positive GAA and/or MBA) in the follow-up period (1 year), and 4/14 sero-converted later than 2 months. 3/14 developed a positive MBA before GAA, and 1 MBA-positive patient never developed GAA. No schistosoma eggs were found in the urine samples, and 4/14 had eosinophilia. In the questionnaire 12/14 reported symptoms upon return, mainly GI-related symptoms interpreted by patients as travel diarrhoea, and 5/14 reported fever. Medical records showed that only 1/14 (index patient) had multiple symptoms (⩾3, non-GI) clinically consistent with Katayama fever, and physical examination of all patients were with normal findings. Higher degrees of exposure (swimming) showed a trend towards higher risk of sero-conversion. All sero-positive patients were offered and received treatment with praziquantel 40 mg/kg.
Conclusion: Even brief exposure to fresh water in schistosoma-endemic regions can carry a high risk of infection, in some cases with late development of antibodies, and overall few or misinterpreted symptoms.

O491 Multilocus sequence typing of *Blastocystis* sp. subtype 3

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Objectives: *Blastocystis* is possibly the most common non-fungal eukaryotic organism found in the human intestinal tract. Its clinical significance is not settled. It is possible that variation in the clinical outcome of *Blastocystis* carriage is linked to differences in genetic make-up of the parasite, which, based on SSU rDNA analysis, comprises at least 9 subtypes (STs) in humans. Aims of this study included the development of a MLST assay for the most common subtype of *Blastocystis*, ST3, and its application to the analysis of ST3 strains from humans and non-human primates.
Methods: Three genomes from mitochondrion-like organelles of ST3 strains were sequenced and aligned to identify candidate MLST loci. Five suitable loci were chosen and 82 ST3 strains, 26 of which were from patients with irritable bowel syndrome (IBS) and 13 of which were from non-human primates, were analysed. Concatenated sequences were analysed using phylogenetic methods and allele frequency distribution was analysed using the Arlequin software.
Results: The MLST assay had a discriminatory index of 0.996 and was able to differentiate 70 sequence types (SQTs) among 82 samples. Phylogenetic analysis showed that concatenated sequences segregated into two clades supported by high bootstrap values. One clade contained 7 non-human primate strains and 1 from a monkey handler. The other clade comprised all other human strains and 6 non-human primate strains. Significant differences in allele distribution were associated not only with host species, but to some extent also with differences in clinical status. Hence, the nad4 locus allele frequency distribution differed significantly between random patients and patients with IBS.
Conclusion: A robust and highly discriminatory MLST for ST3 was developed. Extensive genetic diversity in the MLO genome is not reflected at the SSU rDNA level. At least two major clades exist within ST3, and one of them appears to be restricted mainly to samples of non-human origin. Evidence of zoonotic transmission was uncovered. Future studies should aim at developing MLST assays for the other common STs in humans, identifying differences in alleles that may be useful for predicting both patterns of transmission and potential links to intestinal symptoms.

O492 Anti-*Wolbachia* antibodies and acute lymphadenopathy in lymphatic filariasis

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Background: Lymphatic filariasis (LF) afflicts 120 million people. While the most profound images of LF are elephantiasis and hydrocoele, acute disease in the form of lymphadenopathy, malaise and fever collectively termed adenolymphangitis (ADL) causes significant economic disenfranchisement and suffering in resource poor areas. The pathogenesis of acute LF is not well understood but the endosymbiont *Wolbachia* has been implicated, and has been shown to activate toll-like receptors. This study aims to examine adaptive immunity to *Wolbachia* and whether this affects development of acute disease.
Methods: Two studies were formulated using a pool of banked sera from a previous Mass Drug Administration (MDA) study in Papua New Guinea from 1993–1998. The first study looked at anti-WSP levels in individuals with a recent (within 30 days) ADL event using a case control design with age and sex-matched endemic controls. The second study was a retrospective cohort which looked at anti-WSP levels in longitudinal sera over a 6-year period with ADL as the primary outcome. ELISA using recombinant *Wolbachia* surface protein (WSP) was used to detect anti-*Wolbachia* antibodies. ELISA to detect anti-filaria antibody against crude *Brugia* antigen (BmA) was also performed.
Results: Antibody levels against WSP for the ADL group in Study 1 were significantly lower than the non-ADL group ($p = 0.0006$). Anti-BmA levels were not significantly different. In Study 2, anti-WSP levels in the pretreatment year were significantly lower ($p = 0.0016$) for those who developed ADL within the six year period. Odds ratio of developing ADL was 3.31 ($p = 0.011$) for an anti-WSP level lower than the median of the ADL group in the pretreatment year. Anti-WSP levels in succeeding sera in the subsequent treatment years were not significantly different between groups. Antibody levels for the entire cohort significantly increased from the pretreatment year to the post-treatment year ($p = 0.0363$). Anti-BmA levels were not significantly different between groups for all blood draw years.
Conclusion: Individuals exposed to LF with low anti-*Wolbachia* WSP antibody titers have a significantly higher risk of developing acute lymphadenopathy. This relationship holds whether the ADL event is recent or over six years. MDA results in a significantly higher anti-WSP antibody titer over time. These findings support an association between the pathogenesis of acute LF and adaptive immune response to *Wolbachia* endosymbionts.

O493 Protection against diarrhoea and fever without localising signs associated with asymptomatic *Giardia lamblia* infection is lost with multi-nutrient supplementation: a prospective study among rural Tanzanian children

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Objective: Asymptomatic infections with *Giardia lamblia* are common among children in developing countries, but the role of giardiasis as cause of diarrhea in such settings has been questioned. Impaired linear growth and cognition have been associated with giardiasis, presumably mediated by malabsorption of nutrients. In a prospective cohort study, we aim to compare rates of diarrhea in pre-school children with and without *Giardia* infection. In addition, we assessed how micronutrient supplementation influenced the relationship between *Giardia* and diarrhoea rates, and to what extent *Giardia* modifies the effect of supplementation on nutritional status.
Methods: Data were collected in the context of a randomized placebo-controlled trial with 2x2 factorial design assessing the effects of multi-nutrients (with or without zinc) on morbidity. Children ($N = 612$, aged 6–59 months and height-for-age z-score $\leqslant -1.5$ SD) from a poor rural area were followed for at least 7.4 months after enrolment. Outcome measures were episodes of diarrhea and fever without localizing signs, as detected by clinic-based surveillance. G lamblia was detected in stool samples by enzyme-linked immunosorbent assay. Multivariate Cox regression analysis was used to compare disease rates between groups, and to assess interaction effects.
Results: Asymptomatic *Giardia* infection was associated with a substantial protection against diarrhea (HR 0.32; 0.15–0.66) and fever (HR 0.56; 0.36–0.87), but only so among children who dit not receive multinutrients; no such protection was observed among children who received multi-nutrients (p-values for interaction between *G. lamblia* and multi-nutrients 0.03 for both outcomes, after adjustment for age, HAZ scores and distance to the dispensary.
Conclusions: Although causality af the *Giardia*-associated reduction in morbidity cannot be established, the data also show that multi-nutrient supplements neutralise this protection and are thus likely to influence the proliferation or virulence of G lamblia or associated intestinal pathogens.

O494 The occurrence of *Demodex* spp. in blepharitis patients and healthy people a 3-year observational study

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Objectives: Two species of *Demodex*: *D. folliculorum* and *D. brevis* are supposed to be correlated with human skin and ocular diseases. However the clinical significance of *Demodex* spp. infection remains still controversial, partially because single parasites can be found in asymptomatic subjects. The aim of the study was to estimate the prevalence of *Demodex* infection in patients with blepharitis and healthy population in correlation to patients' characteristics.
Methods: The study was carried out in years 2007–2010. The enrolled patients were divided in two groups: the first consisted of 544 patients (362 women and 182 men, aged from 5–88 years) with blepharitis,. The control group was composed from 100 healthy individuals (78 women and 22 men, aged from 17 to 88 years) without history of ocular pathologies. A sample of 6–10 eyelashes was taken aseptically from each eye of examined person and later studied under a light microscope. The host factors, such as: sex, age, history of facial rosacea, ocular symptoms like: itching, redness, blurred vision and pain in eyes were documented. The determination of 4–5 *Demodex* spp. was accepted as positive.
Results: *Demodex* spp. was found in 58,45% (318/544) of patients in first group and in 24% (24/100) of controls. The difference was statistically significant, indicating that incidence of ocular *Demodex* infestation was much higher in first group compared to controls ($p = 0{,}001$, OR= 0,006). The overall prevalence was 51,10% (342/644) in all examined subjects. Among patients with blepharitis and demodicosis, 8,8% (28/318) had history of facial rosacea. The presence of *Demodex* infection increased with age in both groups, whereas the highest prevalence was noted in subjects >65 years 75,7% in first group and 43,3% in controls, respectively. A significant correlation for *Demodex* infection was found between males and females (104/204 vs 241/440, $p < 0{,}05$).
Conclusion: The prevalence of ocular demodicosis is significantly correlated with blepharitis and increases with age. The incidence of *Demodex* infection is higher in women than in men. Considering long and difficult treatment of chronic demodectic blepharitis, there is need to develop the clear diagnostic standards for recognizing *Demodex* infection of the eye.

O495 Mitochondrial phylogenetic analysis of Portuguese samples of *Echinococcus granulosus*

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Introduction and Objectives: Hydatidosis is a worldwide zoonosis caused by the larval stage of the tapeworm *Echinococcus granulosus*. The taxonomy and phylogeny of the genus *Echinococcus* has been a controversial issue for several years but with the use of molecular tools has helped the characterization of this parasite. As a result, a new

classification has been proposed, and is now widely accepted. The aim of this work was to determine the range of genetic variability within, and between, Portuguese *E. granulosus* isolates.
Methods: The Portuguese isolates obtained from sheep, goat and cattle were characterized using mitochondrial COI, ATP6, CYTB, NDI 12S and 16S partial gene sequencing. The sequences were aligned and compared with those present in the GenBank using a molecular phylogenetic approach.
Results: Preliminary results showed that the Portuguese isolates were *E. granulosus* sensu stricto (G1-G3 cluster). In addition, phylogenetic analysis of *E. granulosus* isolates, using the partial nucleotide sequence of several mitochondrial markers was performed using neighbour-joining (Kimura 2-parameter correction) and Bayesian analyses.
Conclusion: The findings showed some degree of variance within single isolates and a significant degree of variance between the cluster G1-G3, where our isolates were, and the other *Echinococcus* sp. Despite the variance found among Portuguese isolates, they were all localized within one robust cluster.
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C. difficile news

O496 Differential risk of *Clostridium difficile* infection with Proton pump inhibitor use by level of antibiotic exposure

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Objectives: *Clostridium difficile* Infection [CDI] is a major cause of hospital-acquired diarrhea worldwide. The objectives of this study were to determine the risk of CDI associated with the use of acid suppressive agents (proton pump inhibitors [PPI] and Histamine-2 receptor [H2] blockers), and to determine whether this risk varies by number or type of antibiotic (high or low CDI risk) received during hospitalization.
Methods: Retrospective cohort study of hospitalizations among adult, non-psychiatric patients at a tertiary academic teaching hospital in Rochester, New York, US during which two or more days of antibiotics were prescribed. Data on pharmacologic exposures and outcomes were obtained from pharmacy and microbiology records. Multivariable marginal Cox proportional hazards models with time-varying exposures were used to examine time to the development of CDI while controlling for the effects of potential confounders.
Results: 10,154 hospitalizations and 241 cases of CDI, defined as detection of *C. difficile* toxin in a diarrheal stool sample within 60 days of discharge, were identified. PPI and H2 blockers were prescribed during 6,322 (62%) and 1,083 (11%) of hospitalizations, respectively. PPI use was associated with an increased risk of CDI (adjusted hazard ratio [HR] 5.5, 95% confidence interval [CI]=3.0–10.3) independent of number and class of antibiotics and other medications received. Among hospitalizations during which one, two, three or four, and five or more antibiotics were prescribed, the adjusted HRs for PPI use were 20.6 (CI=8.8–48.1), 6.4 (CI=3.0–13.4), 4.9 (CI=2.3–10.7), and 2.9 (CI=1.3–6.4), respectively (p for interaction <0.01). Among hospitalizations during which patients received high risk antibiotics, PPI use was associated with a 3.7-fold (CI=1.6–8.6) increase in risk of CDI, but among those that received low risk antibiotics that estimate increased to 50 (CI=18.9–132.0) (p for interaction <0.0001). H2 blockers were not associated with increased risk of CDI (HR 1.7, 95% CI=0.7–4.1) and there was no evidence of interaction.
Conclusions: The prescription of PPI is common among patients receiving antibiotics during hospitalization. The greater risk of CDI in relation to PPI among hospitalizations during which fewer or low-risk antibiotics were prescribed suggests a potentially clinically relevant interaction between antibiotics and PPI. Further study is needed to elucidate possible mechanisms for the observed effect.

O497 The changing epidemiology before, during and after an outbreak of CD027 in Denmark

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Objectives: To describe the changing epidemiology before, during and after the first large outbreak of *Clostridium difficile* infection with PCR ribotype 027 (CD027) in Denmark.
Methods: All stool samples from patients with diarrhea from October 1, 2008 to November 30, 2010 from four hospitals located north of Copenhagen were examined for *C. difficile*. Detection was done by culture and production of toxin A and B using ImmunoCard (Meridian), and from May 2009 by a real-time PCR directly on stool samples, demonstrating both the tcdC gene and deletions of 18 bp and 39 bp. The antibiotic susceptibility to 6 antibiotics was tested by agar diffusion using discs (Oxoid). *C. difficile* isolates resistant to moxifloxacin and/or deletion positive by PCR were further characterized by determining the genes for toxin A, toxin B and the binary toxin and by PCR ribotyping. Recurrence was defined as the detection of *C. difficile* a minimum of 4 weeks later than the first positive sample.
Interventions: National, regional and local surveillance of CD027; focus on hand washing prior to disinfection; on-line registration of all isolated patients; daily disinfection of rooms with chlorine-containing agent was introduced; focus on reduced use of fluoroquinolones; and localisation of where the patient was infected.
Results:
- 593 cases (first episodes) of diarrhea due to *C. difficile* were found, of which 94% were detected in hospitalized patients. In total, 36% of isolates were deletion negative, 55% were deletion positive (35% CD027, 4% CD078, 2% CD66, 1% other PCR ribotypes and 13% were unculturable, wherefore PCR ribotyping could not be done), and 9% were not primarily examined with PCR
- 38% of patients with CD027 had one or more recurrences versus 18% of patients with deletion negative CD, $p < 0.00005$. No significant difference was found between the number of recurrences caused by CD027 (38%), CD078 (27%) and CD66 (33%)
- The percentage that CD027 constituted of all CD cases per month reached a peak May 2009, and declined hereafter

Conclusion:
- The epidemiology of *C. difficile* changed during the study period. When CD027 declined, the number of deletion negative isolates began to increase and there also was a tendency towards a rise in the number of cases with CD078 and CD066 (both deletion positive)
- Significantly more recurrences were found among the deletion positive isolates
- Surveillance ought to comprise all toxin-producing *C. difficile*, even in an outbreak situation

O498 Community versus healthcare-associated *Clostridium difficile* infection: a surveillance-based study

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Context: C difficile disease is mainly considered as a nosocomial disease associated with broad-spectrum antibiotics. Since early 2003, both the incidence and the severity of CDI appear to have increased. Recently, C difficile infection (CDI) has been reported outside health care institutions in persons previously thought to be at low risk.
Objective: To characterize epidemiological of patients with CDI regarding the infection presumed acquired in the community or in the hospital.
Design, Setting, and Patients: Between November 2006 through August, 2010, a prospective surveillance study of CDI was conducted in French University Hospital. A case of healthcare-associated CDI (HCA-CDI) is defined as one in which symptom onset occurs more than 48 h after admission to, or within 4 weeks of discharge from, a healthcare facility. A case of community-associated CDI (CA-CDI) is defined as

one in which symptom onset occurs within 48 h of admission and over 4 weeks following discharge from a healthcare facility

Results: Of 218 cases of CDI, 65 (29.8%) were CA-CDI and 153 (70.2%) were HCA-CDI. The mean age of patients was 66 and 65.3 years in CA and HCA cases respectively (P = 0.8) and 7.8% of CA cases were under 20 years. In CA cases, women were more frequent (66.7% vs 48.4% in HCA-CDI, P = 0.02). CA group had lower rate of recent antimicrobial exposure (47.5% vs 81.3% in HCA cases, P < 0.001) whilst showing higher rate of severity compared to HCA group (41% and 9.4% respectively, P < 0.001). Colitis related to CDI was observed in 6 cases (9.2%) of CA-CDI and in 9 cases (5.9%) of HCA-CDI.

Conclusions: We found that approximately 30% of all CDI cases were CA and most of half was not exposed to antimicrobial drugs, a classical risk factor of CDI. Monitoring and active surveillance of *C. difficile* infection is needed continually improve our understanding of the changing epidemiology of the disease.

O499 Investigation of outcome in cases of *Clostridium difficile* infection due to isolates with reduced susceptibility to metronidazole

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Objectives: Reduced susceptibility to metronidazole (RS-M) among epidemic *C. difficile* (CD) PCR ribotypes has been reported in recent years. We have examined clinical outcomes for CDIs caused by strains with RS-M and compared these with data for matched controls.

Methods: 22 RS-M CD (MICs ⩾4 mg/L) were identified from patients CDI in Leeds, UK, between December 2005 and November 2006. Clinical outcome data were compared for these cases with those for controls (22 patients, CD MIC ⩽0.5–2.0 ml/L, matched for age, sex, hospital location and CD PCR-ribotype (001), by retrospective searching of medical and electronic records. Differences between cases and controls in terms of death by day 30 (from time of positive cytotoxin test), rate of recurrence, and need for vancomycin therapy were assessed by McNemar's test; days from treatment to resolution of symptoms were compared by paired t test.

Results: 3 RS-M cases and 3 controls were excluded due to unavailability of clinical data. No significant differences were observed between cases and controls for demographic variables (median age: 82 vs 81 years; mean days from symptoms to specimen taken: 4.0 vs 3.8; mean number of cytotoxin-positive specimens in previous 12 months: 0.4 vs 0.4; 74% of cases and 68% of controls were male. 16% (n = 3) patients with CDI due to RS-M were dead by day 30 vs 21% (n = 4) controls (p = 1.00). In M-treated patients, there was no difference between RS-M CDI cases and controls for number of days from treatment to symptom resolution (mean days: 8.0 vs 7.3, range 1–16; p = 0.75). 16% (n = 3) patients with RS-M associated CDI had a recurrence compared with 26% (n = 5) control patients (p = 0.69). Equal numbers (n = 3) of cases and controls received supplementary vancomycin (V) treatment (p = 1.00).

Conclusions: Despite evidence of RS-M in epidemic CD isolates, we found no significant differences in clinical outcome for cases (RS-M CD) vs matched controls (M-susceptible CD). Response to metronidazole was generally poor (slow and prone to recurrence). Notably, patients were typically elderly and frail with very poor outcome (21% mortality rate by day 30). Previous M treatment in most patients who then received V precluded conclusions regarding V efficacy. Much larger study groups, ideally with less frail patients, are needed if the true clinical significance of CD isolates with RS-M is to be delineated.

O500 Success of a national service, *Clostridium difficile* Ribotyping Network for England & N. Ireland (CDRN), to help control epidemic strains and incidence of *C. difficile* infection

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Objectives: To describe the key epidemiological findings concerning *C. difficile* infection (CDI) in England in 2009–10 versus the previous two years as determined by The *Clostridium difficile* Ribotyping Network for England and N. Ireland (CDRN).

Methods: CDRN provides ribotyping and enhanced DNA fingerprinting to identify cross-infection, reduce transmission, optimise management of outbreaks and determine the epidemiology of *C. difficile*.

Results: In 2009/10 CDRN processed 5,720 faecal samples from 172 healthcare facilities, which was a 22% increase over 2008/09 when 4,682 samples were received 190 healthcare facilities. Thus, on average 33 and 25 samples were submitted to CDRN by each participating hospital in 2009/10 and 2008/09, respectively. The number of reports of *C. difficile* recorded by the mandatory scheme in England has decreased from 55,498 to 36,095 to 25 604 (in the financial years 07/08, 08/09 & 09/10). Submissions to CDRN have continued to increase year-on-year; in 2009–10 almost a quarter of the *C. difficile* cases in England were submitted for ribotyping by the CDRN. Marked changes in ribotype prevalence have occurred in the 3 years since CDRN was launched in 2007 (Table). There was a striking decrease in the prevalence of *C. difficile* ribotype 027 (from 55% to 36% to 22%), with 'compensatory' increases in the other main types, including ribotype 078. Ribotype 106 has also declined markedly. There was a significant association (in univariate and multivariate analyses) between all cause mortality and CD 027 (OR = 1.9; p < 0.001).

Conclusions: Since CDRN was introduced in 2007 there has been a marked decrease in incidence of CDI and associated deaths in England. The changes coincided with a 60% decrease in the relative prevalence of ribotype 027 cases. The improvements may reflect the success of control measures to reduce cross-infection in hospitals caused by epidemic strains. Access to timely typing data is important for infection control teams to identify and control transmission of *C. difficile*.

Ribotype	2007/08 (n,%)	2008/09 (n,%)	2009/10 (n,%)	Prevalence change [07/08 and 09/10](%)	Prevalence change [08/09 and 09/10](%)
027	1152 (55.3)	1468 (36.1)	1102 (22.1)	-33.2	-14.0
106	270 (13.0)	517 (12.7)	364 (7.3)	-5.7	-5.4
001	181 (8.7)	297 (7.3)	371 (7.4)	-1.3	+0.1
002	57 (2.7)	231 (5.7)	302 (6.0)	+3.3	+0.3
014/020	57 (2.8)	218 (5.4)	128 (2.6)	-0.2	-2.8
015	50 (2.4)	215 (5.3)	330 (6.6)	+4.2	+1.3
078	37 (1.8)	144 (3.5)	285 (5.7)	+3.9	+2.2
005	29 (1.4)	118 (2.9)	213 (4.3)	+2.9	+1.4
023	21 (1.0)	109 (2.7)	149 (3.0)	+2.0	+0.3
026	5 (0.2)	87 (2.1)	41 (0.8)	+0.6	-1.3

O501 Does *C. difficile* infection per se or other factors lead to death in severe disease

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A prospective hospital-based cohort study was performed for analysing the effect of infection with *C. difficile* (CDI) on the risk of death in 185 hospitalized patients with CDI (CDI patient) compared with all the other hospitalized patients (non-CDI patient, n = 38644). The outcome of interest was pre-discharge mortality. The measures of the effect of CDI on risk of pre-discharge death were risk ratio, attributable risk (i.e. excess mortality per 100 hospitalized patients) and mortality fraction (%).

Death risk ratio was calculated by death risk in the CDI-patients (exposed group)/death risk in non-CDI-patients (non-exposed group) and the attributable risk of death (attributable mortality) was calculated death riske – death risku. The attributable risk percent, AR% (mortality fraction) was calculated by AR% = 100×(death riske – death risku)/death riske. The Population attributable risk percent (PAR%) was calculated by PAR% = 100×(death risk total – death risku)/death risk total. The difference in risk of death was calculated by using Chi-squared test or Fisher's exact test. A stratified analysis and a poisson regression model were applied to adjust the relative and attributable risk of death for sex, age and co-morbidity using Charlson Index. In the CDI group mean age was 74.3 yrs (72.3–76.4) versus 51.9 yrs (51.6–52.1) in the non CDI group; and low/high comorbidity was 136 (73.5%)/49 (26.5%) versus 32107 (83.5%)/6352 (16.5%), respectively. 153 (82.7%) were health-care-facilities associated cases, 27 (14.6%) community associated cases and 5 (2.7%) were of unknown origin. Independent risk factors for death were age (<65/>65: adjusted RR 5.15; 95% CI 4.44–5.98), sex (m/f: RR 1.21; 95% CI 1.07–1.36) Co-morbidity (moderate+severe/low: RR 2.24 95% CI 1.97–2.54) and CDI (CDI : Non-CDI: RR 2.74 95% CI 1.82–4.10).

Compared with all the other hospital patients the absolute risk of pre-discharge death in hospital patients with CDI due to CDI is 10.3/100 individuals, regardless of the co-morbidity. Thus, removing CDI would result in 79.2% reduction of hospital mortality in this patient group. Only 20% of hospital patients with CDI, who die during hospital stay, will die due to other reasons than CDI. The PAR% due to CDI in the total hospital population of the study hospital was found to be insignificant. Therefore, elimination of CDI from the study hospital not impact the hospital mortality in this hospital.

Table Crude RR, excess mortality and mortality fraction and RR, excess mortality and mortality fraction stratified by severity of co-morbidity

	Risk of death in CDI-patients n/N (%)	Risk of death in Non-CDI patients n/N (%)	RR (95%CI)	CDI AR of hospital mortality per 100 individuals	CDI Mortality fraction (95%CI)	CDI PAR%
Total	24/185 (13%)	1021/38459 (2.7%)	4.89 (3.35-7.13)	10.3/100	79.2% (70.1%-86%)	2% (-6.4%-9.9%)
Co-morbidity severity						
Sub-cohort I: Low co-morbidity	15/136 (11%)	616/32107 (1.9%)	5.75 (3.54-9.32)	9.1/100	82.7% (71.8%-89.3%)	2% (-9.9%-12.3%)
Sub-cohort II: moderate / severe co-morbidity	9/49 (18.4%)	405/6352 (6.4%)	2.88 (1.58-5.24)	12/100	65.2% (36.7%-80.9%)	1% (-12.4%-13.8%)

O502 A national enhanced DNA fingerprinting service to investigate potential *C. difficile* infection case clusters sharing the same ribotype

M. Wilcox, W. Fawley on behalf of the Clostridium difficile Ribotyping Network for England and N. Ireland (CDRN)*

Objectives: To describe the use of the centralised enhanced DNA fingerprinting service of the CDRN for the investigation of potential CDI case clusters sharing the same ribotype. To determine the likelihood that clusters of cases sharing the same ribotype are found be highly related/indistinguishable using multi-locus variable number of tandem repeat analysis (MLVA).

Methods: We investigated potential case clusters (defined as patients related in time and/or place yielding isolates with the same ribotype) submitted to CDRN from institutions in England. Enhanced DNA fingerprinting was carried out using MLVA. Case clusters were defined according to the summed total of tandem repeats between isolates (STRD). Cases with isolate STRD $\leqslant 2$ were considered to be related (REL); conversely, if isolates were clearly distinguishable than cases were not related (NOT REL); case clusters containing both highly related and clearly distinguishable isolates were termed mixed (MIX).

Results: Between 2008–210 we investigated 279 *C. difficile* isolates from 54 potential CDI case clusters (2–41 patients) in 27 institutions that shared one of 10 ribotypes (001, 005, 014, 014, 017, 023, 027, 078, 106, 277). The most common ribotype investigated was 027 (192 isolates in 32 case clusters). Of the 54 case clusters, 24 (44%) and 17 (32%) were found to be REL and MIX, respectively. Of the NOT REL potential case clusters, 8/13 were caused by ribotype 027 isolates.

Conclusions: Despite suspicion of case clustering, enhanced DNA fingerprinting by MLVA determined that in 24% of instances isolates sharing the same ribotype, including epidemic 027 strains, were not related. These finding emphasise the value of MLVA to confirm or refute case clusters. Access to timely typing and fingerprinting data is important for infection control teams to identify and control transmission of *C. difficile*.

O503 Time-interval of increased risk for *Clostridium difficile* infection after exposure to antibiotics

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Objectives: *Clostridium difficile* infections (CDI) are common in developed countries and affect more than 25,000 people annually in England and 250,000 in the United States. The most important risk factor for the disease is antibiotic therapy. Development of CDI is associated with certain antibiotic classes (e.g. cephalosporins and clindamycin), the number of antibiotics used, their dosage and the duration of therapy. The exact time-interval of increased risk for CDI after exposure to antibiotics is however unknown.

Methods: A case-control study was performed in nine hospitals in the Netherlands. Each hospital participated for a minimum of six months between March 2006 and May 2009. Hospitalized patients with diarrhoea and a positive test for the toxin of *Clostridium difficile* (CDI patients) were matched on hospital, ward and time of diagnosis to patients without diarrhoea (non-diarrhoeal). Besides antibiotic classes, number and quantity of antibiotic therapy as risk factors for CDI, we evaluated the time-interval of increased risk for CDI after exposure to antibiotics by means of a conditional logistic regression.

Results: In total, 362 CDI patients and 348 non-diarrhoeal patients were included in the analysis. All antibiotic classes, except for first generation cephalosporins and macrolides, were significantly associated with CDI. Cephalosporins and carbapenems were the most potent risk factors for CDI. Patients with CDI used a larger amount of antibiotics and more antibiotic classes, compared to non-diarrhoeal patients. At time of diagnosis, CDI patients more frequently used an antibiotic compared to non-diarrhoeal patients (36% vs. 24%). During antibiotic therapy and the first month after cessation of the therapy, patients had a six to ten fold increased risk for CDI. This risk declined in the period between one and three months after the antibiotic was stopped (OR 2.72; 95% CI: 1.20 to 6.15).

Conclusion: Antibiotic use increases the risk for CDI during therapy and in the period of three months after cessation of antibiotic therapy. A six to tenfold higher risk is found in the first month after cessation of the antibiotic.

O504 Immunosuppression and the risk of death, cure rates and disease recurrence among patients with *Clostridium difficile* infection

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Objectives: CDI is emerging in hospitals and is associated with substantial morbidity and cost of care. It is associated with increasing frequency of treatment failure and mortality. While some microbial factors, such as the BI/NAP1 strain, are linked to more severe CDI, the relation between the host immune status and CDI outcome is less clear. We examine the role of immunosuppression (IS) on CDI outcome.

Methods: We used data from 2 large, randomized phase 3 clinical trials in which adults with CDI symptoms and a positive toxin test received oral fidaxomicin or vancomycin for 10 days. IS was characterized according

to its severity, and type. The net state of IS, encompassing host and iatrogenic factors, was calculated. The independent effect of IS on CDI outcome was assessed using multivariate analysis.
Results: Of the total of 1105 patients in the mITT population, treatment failure, death and CDI recurrence rates were observed in 13%, 7% and 20%, respectively. Mortality was higher among those with a net state of IS (11% vs 3%; $p < 0.01$), those receiving any IS medications (9% vs 6%; 0.04) and specifically systemic steroids (14% vs 5%; <0.01), moderate or high-dose steroids (25% vs 5%, <0.01), and those with neutropenia (16% vs 6%; 0.01). In those with a non-BI/NAP1 strain, 92% of all deaths occurred among IS patients. Mortality was lowest among immunocompetent patients infected with a non-BI/NAP1 strain (0.6%). CDI cure rates were lower among immunosuppressed patient and particularly among those receiving moderate or high-dose steroids (75% vs 88%; <0.01). IS was not associated with CDI recurrence. In a multivariate analysis, IS remained independently associated with higher mortality and a lower cure rate. Cure and mortality rates were similar between fidaxomicin and vancomycin. Rates of CDI recurrence among those treated with fidaxomicin were substantially lower among both immunocompetent (13% vs 26% for vancomycin; <0.01) and IS (16% vs 27%; <0.01).
Conclusion: The net state of IS is predictive of CDI outcome. IS is associated with higher death and lower CDI cure rates. The rate of CDI recurrence remains substantially lower among patients treated with fidaxomicin regardless of their level of immunosuppression.

Young Investigator Award

K509 Emergence of antimicrobial resistance, linked to optimal prescribing

S. Malhotra-Kumar (Antwerp, BE)*

Laboratory testing is often a critical component in the proper diagnosis and optimal management of patients presenting with a broad range of disease states. This is especially true of infectious disease, where presenting signs, symptoms, and history are often insufficient to identify a pathogen, or sometimes even suggest an infectious etiology. Practitioners often rely on laboratory values as an aid in the differential diagnosis of infection. Increasingly, laboratory assays are being paired with imaging modalities to further increase diagnostic accuracy. One recent synergy is the use of an in vitro blood test for liver fibrosis (ELF™*) in conjunction with an ultrasound-based imaging technology (ARFI*) that may enhance analysis of hepatic damage through assessment of liver elasticity along with evidence of fibrosis. In patients with chronic viral hepatitis or other forms of hepatic disease, such an approach could potentially reduce the risk and discomfort associated with a liver biopsy through use of less-invasive methodologies. This presentation will focus on key contributions of the laboratory in the accurate identification of an infectious origin by utilizing two separate patient profiles. The case histories will be discussed to exemplify the value of laboratory and other findings in the respective diagnosis and management of two common conditions associated with infection: bacterial sepsis and viral disease.
*Not available in the US.

Enterococci – where are we now?

S513 Current epidemiology of vancomycin-resistant enterococci in Europe

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Objective: To give an overview about prevalence of VRE in Europe and speculate on possible routes of emergence and spread and reservoirs of vanA/B type resistance.
Main topic: Eight types of acquired vancomycin resistance in enterococci are known; however, only VanA and VanB are widely prevalent. The major reservoir of acquired vanA/B type resistance is in *E. faecium*; mainly among clinical strains. Ampicillin- and vancomycin-resistant *E. faecalis* remain still rare. The supposed non-hospital reservoir of vanA type *E. faecium* in animal husbandry and of the vanB gene clusters in non-enterococcal, intestinal colonizers could be significant but their exact impact on a corresponding resistance gene pool in hospital strains cannot be assessed properly so far.
Population analysis of *E. faecium* has revealed a distinct subpopulation of hospital-associated strain types increasingly prevalent among the nosocomial setting and showing higher rates of acquired antibiotic resistances. Acquired ampicillin resistance and increased number of high-level ciprofloxacin resistance are important phenotypic markers of hospital-acquired *E. faecium* in Europe and experience has shown that these features often precede increasing rates of vancomycin resistance with a delay of several years. Hospital-associated strains can be differentiated by molecular typing methods (MLVA, MLST) from human commensal and animal strains and have additional genomic contents (accessory genome) including several factors known or supposed to be virulence-associated.
Several conditions are known to promote VRE colonisation, transmission and subsequent infection; however, despite having populations with similar predispositions, rates of vancomycin resistance vary all over Europe between <2% and >30% among all clinical *E. faecium* isolates. Nevertheless, VRE rates were constant during recent years and significant trends could not been identified. Few exceptional developments will be discussed.
Outlook: A deeper molecular analysis of the population structure of (hospital-associated) *E. faecium* and *E. faecalis* strain types and their mobile genetic elements encoding vancomycin resistance including detailed transposon, plasmid and genome analyses up to complete genomic sequencing is inevitable for a better understanding of the epidemiology of VRE and will allow establishing models of emergence and spread of vanA/B type resistance among enterococci.

Do you know the concept of anti-anaerobic quinolones?

S518 Clinical significance of anti-anaerobic quinolones in VAT and VAP

H. Mikamo, Y. Yamagishi (Aichi, JP)*

Ventilator-associated pneumonia (VAP), ventilator-associated brachiotronchitis (VAT) and aspiration pneumonitis (AP) represent a spectrum of aspiration syndromes. All diseases are common problems encountered in the ICU, with VAP occurring in approximately 25% of patients undergoing mechanical ventilation, and AP commonly occurring in patients admitted to the ICU with an altered level of consciousness. AP follows macroaspiration of oropharyngeal and/or gastric contents in patients with an altered level of consciousness, dysphagia, or bowel obstruction. VAP is widely believed to result from the microaspiration of oropharyngeal material colonized by pathogenic microorganisms. In fact, anaerobic bacteria have been frequently isolated from the oropharyngeal flora, their pathogenetic role in VAP, VAT and AP has been established. In general, anaerobes have been considered to be common pulmonary pathogens, and they have been believed to play a major role in aspiration and nosocomial pneumonia. These are based on many studies conducted in the 1970s, when transtracheal aspiration was used for the collection of uncontaminated respiratory secretions. Consequently, antimicrobial agents with anaerobic coverage would be recommended in patients with aspiration pneumonia and nosocomial pneumonia.
Older fluoroquinolones, such as ciprofloxacin and ofloxacin, are inactive or only partially active against anaerobic bacteria. Newer quinolones, such as sparfloxacin and levofloxacin, have improved in vitro activities against anaerobic bacteria but still have limited activities against certain Gram-positive and Gram-negative anaerobic bacilli. Some of the newer quinolones, such as moxifloxacin, sitafloxacin, and garenoxacin, and so on, are much more active to both Gram-positive and Gram-negative

anaerobic bacteria. Those antianaerobic quinolones would be useful for the treatment of VAP, VAT and AP, which would be highly associated with anaerobes.

Microbiol pathogenesis, pathophysiology of infections diseases

O519 Relative fitness of tigecycline-susceptible versus resistant isolates of *Acinetobacter baumannii* recovered from a single patient

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Objectives: *Acinetobacter baumannii* is an important nosocomial pathogen that is of increasing concern owing to the many isolates that are resistant to nearly all available antimicrobials. Tigecycline usually remains active against otherwise multidrug-resistant *A. baumannii* (MDRAB) though there are reports of resistance emerging, including during tigecycline therapy. The fitness cost of such resistance was investigated in vitro, under normal and stressed laboratory growth conditions and in vivo, using an insect model of *A. baumannii* infection and a pre- and post-treatment pair of clinical isolates.
Methods: The emergence of efflux-mediated tigecycline resistance during on-label usage in a representative of the *A. baumannii* UK lineage 'OXA-23 clone 1' has been described recently. The in vitro fitness cost of tigecycline resistance was determined using the pre-therapy isolate, AB210 and the post-therapy isolate, AB211 in LB broth and a microtitre plate-based growth kinetics assay. The influence of stress was investigated by growing the isolates in: (i) LB at pH 4.5; (ii) LB supplemented with 200 mM NaCl; (iii) one-third strength LB diluted in saline; (iv) LB supplemented with 200 μM 2,2'-dipyridyl (iron chelator). Relative fitness was calculated as follows: doubling time of parent/doubling time of derivative. The ability of the isolates to form a biofilm was assessed in a microtitre plate using a crystal violet-based assay. Virulence of AB210 & AB211 was assessed using a *Galleria mellonella* (greater wax moth caterpillar) model of infection. An in vivo competition assay was performed with a starting ratio of 1: 1 (AB210: AB211).
Results: The results of the in vitro studies are summarised in Table 1. Pre-therapy isolate AB210 performed better than its tigecycline-resistant counterpart, post-therapy isolate AB211, both under normal (full strength LB broth) and stressed laboratory conditions except iron-limitation (LB supplemented with 200 μM 2,2'-dipyridyl). AB211 was better able to form biofilms than AB210. AB211 outcompeted AB210 in vivo by a median ratio of 6: 1.
Conclusions: In comparison with the pre-therapy, tigecycline-susceptible isolate (AB210), the post-therapy, tigecycline-resistant isolate (AB211) was: (i) less fit in vitro under all conditions except iron-limitation; (ii) a better biofilm producer and hence may persist longer; (iii) able to outcompete AB210 in vivo and thus may be better adapted for survival within the host.

Table 1. Characteristics of *A. baumannii* isolates used

Isolate	Origin	TGC MIC (mg/L)	Relative fitness under different growth conditions (± SD)*				
			LB	LB (pH 4.5)	LB (1/3)	LB (200 mM NaCl)	LB (200 μM DIP)
AB210	pre-therapy clinical isolate	0.5	1	1	1	1	1
AB211	post-therapy clinical isolate	16	0.7 (0.07)	0.87 (0.1)	0.57 (0.08)	0.79 (0.02)	1.92 (0.09)

*relative to AB210

O520 Hospital- and community-acquired methicillin-resistant *Staphylococcus aureus* differ greatly in their ability to invade bone cells, persist intracellularly and induce cell damage

J.P. Rasigade, S. Trouillet, Y. Lhoste, T. Ferry, S. Tigaud, J. Etienne, F. Vandenesch, F. Laurent (Lyon, FR)*

Objectives: *Staphylococcus aureus* is the leading cause of bone and joint infections (BJIs). Most methicillin-resistant *S. aureus* (MRSA) causing BJIs are hospital-acquired (HA-MRSA), but community-acquired (CA)-MRSA are an emerging cause of BJIs in outpatients. HA- and CA-MRSA belong to distinct genetic backgrounds and are associated with varying clinical presentations, CA-MRSA causing often more severe and acute BJIs than HA-MRSA, which are frequently associated with relapse. Underlying mechanisms for these differences are not fully elucidated. We compared the intracellular persistence of, and cytotoxicity induced by *S. aureus* clinical isolates belonging to major HA-MRSA and CA-MRSA genetic backgrounds, in a model of intracellular bacterial challenge of cultured osteoblasts.
Methods: In a gentamicin protection assay, osteoblastic MG-63 cells were infected for 2h using clinical isolates (1 isolate per clone) representative of HA-MRSA (ST8-Lyon and ST5-Geraldine clones) and CA-MRSA (ST80-European clone). An ST80 MSSA variant was also included to assess whether methicillin resistance impacts virulence. Viable intracellular bacteria after 3, 24 and 48h incubation were enumerated by plate counting after host cell lysis. Cytotoxicity was assessed by lactate deshydrogenase (LDH) release assay after 24 and 48h incubation. Differences were tested for statistical significance using Mann Whitney U-test.
Results: The number of viable intracellular bacteria per well after 3, 24 and 48h incubation was 5.8-, 9.5- and 34.0-fold higher, respectively, for HA-MRSA isolates than for CA-MRSA/ST80 isolates ($p < 0.0001$ for all differences). Conversely, excess LDH release after 24 and 48h incubation was 2.9- and 2.0-fold higher in CA-MRSA/ST80- than HA-MRSA-infected cells ($p < 0.0001$ for both differences). ST80 MRSA and MSSA yielded identical phenotypes, ruling out a role for methicillin resistance in this model.
Conclusion: *S. aureus* clinical isolates differed greatly in their ability to invade and persist within bone cells. HA-MRSA isolates were able to survive intracellularly while inducing moderate cell damage. Conversely, CA-MRSA/ST80 isolates were highly cytotoxic and exhibited impaired intracellular survival, a finding consistent with the acuteness of CA-MRSA BJIs.

O521 Immunological and molecular mechanisms induced in an in vitro model of co-culture of *Helicobacter pylori* and dendritic cells

A. Hocès De La Guardia, C. Staedel, I. Kaafarany, A. Clément, P. Blanco, F. Mégraud, P. Lehours (Bordeaux, FR)*

Objectives: In gastric MALT lymphoma (GML) the B lymphocyte proliferation is not directly induced by the contact with *H. pylori* but is dependent on the presence of tumour infiltrated T lymphocytes (TL). These TL recognize *H. pylori* probably via the dendritic cells (DCs). GML occurs in a Th0/Th2 inflammatory context in contrary to the Th1 duodenal ulcer. Our aim is to understand this phenomenon and among the developed research axes, the in vitro interaction between human DCs and *H. pylori* is studied.
Methods: DCs were generated from monocytes isolated from human blood by immunomagnetic sorting on CD14 and differentiated in the presence of IL-4 and GM-CSF. These DCs were co-cultivated for 48h with GML *H. pylori* strains. The impact of Multiplicity Of Infection (MOI) and of released bacterial factors (Transwell model) was also tested. The activation of DCs was evaluated by measuring the expression of activation markers (CD40, CD80, CD83, CD86, CD197, HLA) by flow cytometry and by analyzing the production of cytokines by ELISA and flow cytometry. The expression of two microRNAs, miR146 and

miR155 was quantified by RT-PCR. *H. pylori* activated DCs were co-cultivated during 5 days with autologous TL. Their ability to induce TL proliferation was measured by BrdU incorporation.
Results: All 9 tested strains were able to induce a significant DC activation, a strong production of INF-g, IL-10, IL-6, TNF-a, IL-8 and IL-23, and an overexpression of miR146 and miR155. Activation markers and miR155 decreased when MOI increased, and miR146 increased with MOI. The cytokines adopt a Th1 profile at low MOI but a Th2 profile at high MOI. Pulsated DCs in the Transwell model expressed HLA and miR146 only. The other activation markers, cytokines and miR155 were thus contact-dependent. The pulsated DCs were able to induce autologous TL proliferation, in a MOI-dependent manner. TL then created a Th2 type environment. TL proliferation was not maximal in presence of pulsated DCs in a Transwell model.
Conclusion: *H. pylori* strains associated with GML are thus able to activate human DCs, and to promote an autologous TL response, in a Th2 type environment favourable to the GML development. They also induce the expression of microRNAs that probably regulate the inflammatory response. The The NFkB pathway seems to be here the major regulator of this signalisation, in particular via the TLRs recognition.

O522 MicroRNA expression profile in human macrophages infected with *Mycobacterium tuberculosis*

L. Furci, E. Schena, P. Miotto, P. Mantegani, D. Cirillo (Milan, IT)*

Objectives: *M. tuberculosis* (Mtb) can persist alive and replicate into the host due to its ability to interfere with the cellular mechanisms that allow inactivation and clearance of bacteria inside macrophages. MicroRNAs have been recently reported to be critical regulators of the mammalian immune system through the dynamic fine-tuning of gene expression. Bacterial pathogens have been described to overcome host defense by encoding proteins that suppress pathways regulated by these miRNAs. Aim of this study is to search for miRNA expression profiles in human macrophages infected with *M. tuberculosis* complex members.
Methods: Monocyte derived macrophages (MDM) were isolated from healthy, Mantoux negative, donors PBMCS by plastic-adhesion and differentiated in vitro for 7–10 days. Differentiated MDM were infected with a virulent Mtb strain (H37Rv), either live or formaldehyde-inactivated, and an attenuated Mtb strain (BCG). At selected time-points cells were collected and solubilized for total mRNA extraction and retro transcription. MicroRNA expression was measured and quantified by qRT-PCR using TaqMan Array Human MicroRNA Card, a 384-well micro fluidic card containing dried TaqMan primers and probes for the miRNAs best characterized up to now. Analysis of miRNA targets was performed using the public database, Targetscan 4.2.
Results: We have examined changes in miRNA expression level in four pools of 10 donors before and after infection: 2hr, to evaluate which miRNAs are influenced by the attachment-entry of Mtb; 24hr and 72hr, to study miRNAs involved in the processes of Mtb persistence/elimination from the host cells. Formaldehyde inactivated Mtb was selected in order to discriminate the active effect of live Mtb from that mediated by antigenic components of the Mtb wall. We found that Mtb infection significantly modulated (cut off 1.7 fold) 107 miRNA over 377 miRNA analyzed. Among these we could discriminate a set of miRNAs differentially expressed during the early phase of the infection process and others specifically associated with the intracellular persistence of mycobacteria into the target cells.
Conclusion: This work describes for the first time the miRNAs expression profile of human macrophages infected with *M. tuberculosis*. The identification of miRNAs crucially involved in Mtb persistence in target cells will be a fundamental step towards the comprehension of the pathogenesis of tuberculosis and the design of new drugs and vaccine strategies.

O523 Toll-like receptor 4 polymorphism Asp299Gly and its association with nasopharyngeal colonisation of *Moraxella catarrhalis* in Finnish infants

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Objectives: Nasopharynx is a cpmplex ecosystem that contains various bacteria species among which *Moraxella catarrhalis* is a common one. *M. catarrhalis* is an exclusively human pathogen and is responsible for 15%-20% of acute otitis media episodes in children. Toll-like receptors (TLRs) are key components of human innate immunity and act as a first line of defence against invading microorganisms. TLR4 is involved in recognition of bacterial lipids. Single nucleotide polymorphisms (SNPs) in gene encoding TLR4 have been reported. TLR4 Asp299Gly is associated with decreased response to lipopolysaccharide of Gram negative bacteria. The aim of this study is to investigate whether there is an association between nasopharyngeal bacterial colonization of *M. catarrhalis* and TLR4 polymorphism Asp299Gly in Finnish infants.
Methods: From August 2008 to August 2010, 489 nasopharyngeal swabs (NPs) and 412 blood samples were taken from 3-month-old asymptomatic Finnish infants in a prospective cohort study carried out in Turku, Finland. The semi-quantative culture was used for identification of different bacterial species and the pyrosequencing-based method was used for detection of TLR4 polymorphism Asp299Gly.
Results: Of the 489 NP swabs, 290 (59%) were positive for at least one of the four bacterial species:
122 (25%) for *Staphylococcus aureus*, 114 (23%) for *M. catarrhalis*, 55 (11%) for *Streptococcus pneumoniae* and 3 for *Haemophilus influenzae*. Only 24 (5%) swabs were bacterial culture negative. The prevalence of other bacterial species was 27% for *Streptococcus* sp, 21% for *Staphylococcus* sp, 48% for *Corynebacterium* sp, 1% for *Neisseria* sp and 0.5% for *Haemophilus parainfluenzae*. Of 412 infants with blood samples, 72 (17%) were found to have polymorphism Asp299Gly of TLR4. Colonization rate of *M. catarrhalis* was significantly higher in infants with TLR4 polymorphism Asp299Gly than those infants without (39% vs 21%, $P = 0.002$). However, no such associations were found for other bacterial species studied.
Conclusion: This study clearly shows that *M. catarrhalis* colonizes more often the nasopharynx of infants with TLR4 polymorphism Asp299Gly.

Acinetobacter baumannii: current situation

O524 Trends in antimicrobial resistance in *Acinetobacter* spp. from the European TEST programme

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Background: *Acinetobacter* spp. are a frequent cause of nosocomial as well as community-acquired infections and represent increasingly difficult therapeutic challenges. The Tigecycline European Surveillance Trial (T.E.S.T.) has been monitoring global resistance patterns of significant pathogens, including *Acinetobacter* spp., since 2004. This report summarizes the susceptibility trends seen in Europe from 2004–2010.
Methods: Clinically significant *Acinetobacter* spp. were obtained from 27 European countries during 2004–2010. MICs for piperacillin-tazobactam (PT), levofloxacin (LVX), ceftriaxone (CAX), cefepime (CPE), amikacin (AK), minocycline (MIN), ceftazidime (CAZ), tigecycline (TIG), imipenem (IMI), and meropenem (MER) were determined using supplied broth microdilution panels and interpreted according to EUCAST guidelines.
Results: The % susceptible and MIC_{90} (μg/ml) for 5,455 *Acinetobacter* spp. isolated in Europe over seven years are shown in the following table:
Conclusions: While the activity of all compounds tested (except meropenem) decreased over time, only ceftriaxone, minocycline, and piperacillin-tazobactam decreased significantly ($p < 0.001$). Tigecycline

exhibited the lowest MIC_{90} values for all years (1–2 μg/ml), but has no EUCAST breakpoint for *Acinetobacter*. Among drugs with breakpoints, minocycline showed the highest %S each year tested.

Year (N)	2004 (454)		2005 (260)		2006 (592)		2007 (823)		2008 (1360)		2009 (1575)		2010 (391)	
	MIC_{90}	%S	MIC_{90}	%S	MIC_{90}	%S	MIC_{90}	%S	MIC_{90}	%S	MIC_{90}	%S	MIC_{90}	%S
AK	> 64	68.9	> 64	73.1	> 64	71.4	> 64	70.7	> 64	59.3	> 64	55.4	> 64	67.3
CPE	32	58.6	> 32	57.3	> 32	58.3	> 32	62.1	> 32	52.6	> 32	52.1	> 32	55.5
CAZ	> 32	55.7	> 32	54.2	> 32	55.9	> 32	53.1	> 32	46.8	> 32	47.3	> 32	49.9
CAX	> 64	40.5	> 64	40.0	> 64	36.8	> 64	35.4	> 64	28.8	> 64	25.1	> 64	26.9
IMI	> 16	81.2	16	83.7	8	78.4	nt	nt	nt	nt	nt	nt	nt	nt
LVX	> 8	52.9	> 8	52.3	> 8	56.6	> 8	55.7	> 8	46.7	> 8	46.2	> 8	46.6
MER	nt	nt	nt	nt	16	57.7	> 16	71.4	> 16	61.3	> 16	57.8	> 16	62.2
MIN	8	89.9	2	95.8	2	97	4	95.5	8	87.8	8	83.4	8	80.1
PT	> 128	61.0	> 128	60.4	> 128	63.2	> 128	57.4	> 128	44.9	> 128	43.1	> 128	46.6
TIG	1	na	1	na	1	na	1	na	2	na	2	na	2	na

O525 Molecular epidemiology of *Acinetobacter baumannii* in Germany, 2005–2009

X. Schleicher, P.G. Higgins, H. Wisplinghoff, D. Stefanik, M. Kresken, H. Seifert (Cologne, Rheinbach, DE)*

Objectives: *Acinetobacter baumannii* is one of the most prevalent nosocomial pathogens contributing significantly to morbidity and mortality, mainly among patients in the ICU setting. Recent data suggest a clonal population structure with several highly prevalent world-wide clones. This study was conducted to evaluate the molecular epidemiology of *A. baumannii* in Germany.
Methods: A total of 377 *Acinetobacter* isolates from three nationwide surveillance studies prospectively collected from 17 centers between 2005 and 2009 (as part of the German Tigecycline Evaluation Surveillance Trial, G-T.E.S.T.) were identified to species level using reference methods. Among these, 140 *A. baumannii* were analyzed using rep-PCR (DiversiLab®). A clonal cluster was defined as >2 isolates from different centers showing >95% similarity. Antimicrobial susceptibility was determined by broth microdilution. Presence of carbapenem-hydrolyzing oxacillinases (OXA) was investigated by PCR.
Results: *Acinetobacter* genomic species 3 (GS3) was the most prevalent species (n = 190; 50%), followed by *A. baumannii* (n = 140; 37%) and *Acinetobacter* genomic species 13TU (GS13TU) (n = 8; 2%). 25 *A. baumannii* had an imipenem MIC ⩾8 mg/L and all had either an OXA-58-like or OXA-23-like. The imipenem susceptibility rates decreased from 96% in 2005 to 71% in 2007 and 76% in 2009. Among imipenem-resistant *A. baumannii* the prevalence of OXA-58-like decreased from 100% in 2005 to 0% in 2009 while the prevalence of OXA-23-like increased to 31% in 2007 and to 100% in 2009. All GS3 and GS13TU isolates were susceptible to the carbapenems. However, OXA-58-like (n = 5) and OXA-23-like (n = 2) genes were detected in GS3 but not in GS13TU isolates.
Among *A. baumannii* isolates, world-wide clone 2 (WW2) was the most frequently detected clonal lineage (n = 35) followed by WW1 (n = 6) and WW4 (n = 4). However, the majority of isolates (n = 94) did not cluster with any previously identified WW-clone and were found as either single isolates or as small clusters of up to 4 isolates from a single center. Carbapenem-resistance was significantly more frequent among isolates of WW2 (64% vs. 8%; $p < 0.001$).
Conclusion: The population structure of *A. baumannii* in Germany is highly diverse. Among the few clonal clusters detected, WW2 was the most prevalent, geographically widespread and carbapenem-resistant. These data underscore the high clonality of carbapenem-resistant *A. baumannii* isolates.

O526 Aminoglycoside resistance and distribution of resistance genes in sporadic and outbreak strains of *Acinetobacter baumannii* isolated in Abu Dhabi

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Objectives: The aim was to investigate the differences in the level of resistance and the distribution of aminoglycoside resistance genes between sporadic and outbreak strains of *Acinetobacter baumannii*.
Methods: Sixty three non-repeat *A. baumannii* strains clustered into seven distinct outbreak clones by macro-restriction and genotyping were compared to 50 sporadic strains isolated in Abu Dhabi in 2008. Aminoglycoside resistance genes (aadA, aac(3)-Ia, aac(3)-Iia, aac(6′)-Ih, aph(3’)-VI, ant(2’)-Ia, aph(3’)-Ia, aac(6′)-Ib, armA, strAB, rmtA, rmtB, rmtC, rmtD, npmA) were detected by PCR. Antibiotic susceptibility was tested against a wide range of drugs by disc diffusion and to ceftazidime, imipenem, meropenem, ciprofloxacin, tigecycline, colistin, gentamicin, amikacin, streptomycin, kanamycin, netilmicin, spectinomycin and tobramycin by E-test.
Results: All outbreak strains and 48% of the sporadic isolates were MDR (arbitrarily defined as resistance to carbapenems and at least to further two non-β lactam classes of drugs). The MIC values of different aminoglycosides to different groups of strains are shown in Table 1. The most frequent aminoglycoside resistance gene was armA among both outbreak and sporadic isolates (61.9% and 52.0%, respectively) followed by strAB (57.1% among outbreak strains) and by aadA and aph(3′)-VI (38.0% each among sporadic isolates). The most common combination of aminoglycoside resistance genes carried by members of the two largest outbreak clones were: armA, strAB (with or without aac(3)-Iia) (Clone D, N = 14) with high level of resistance to all aminoglycosides and aph(3’)-VI, ant(2’)-Ia (with or without armA) (Clone F, N = 19) showing high level of resistance to amikacin, gentamicin and kanamycin with varying levels to other drugs.
Conclusion: Aminoglycoside resistance is wide-spread among local outbreak isolates but also common among sporadic strains. In this latter group it is also strongly linked to resistance to other antibiotic classes, i.e. to multi-drug resistance. Particularly alarming is the wide-spread distribution of armA, often localized on plasmids and spreading rapidly in other parts of the world. Close monitoring of resistance clones and resistance genes are needed to reveal the dynamics of rapidly increasing aminoglycoside resistance in *A. baumannii*.

ANTIBIOTICS	MIC	ALL STRAINS	OUTBREAK	SPORADIC		
				ALL	NON-MDR	MDR
		N=113	*N=63*	*N=50*	*N=26*	*N=24*
Amikacin	50	256	>256	4	3	256
	90	>256	>256	>256	48	>256
Gentamicin	50	192	>1024	6	0.5	192
	90	>1024	>1024	>1024	32	>1024
Streptomycin	50	>1024	>1024	96	12	>1024
	90	>1024	>1024	>1024	>1024	>1024
Kanamycin	50	>256	>256	3	3	>256
	90	>256	>256	>256	>256	>256
Netilmicin	50	8	12	4	1.5	8
	90	>256	>256	>256	4	>256
Spectinomycin	50	256	1024	96	24	>1024
	90	>1024	>1024	>1024	1024	>1024
Tobramycin	50	32	192	1.5	0.75	2
	90	>1024	>1024	>1024	32	>1024

O527 Ceftazidime-resistance in *Acinetobacter baumannii* of clonal complex 92 in Korea

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Objectives: The aim of this study was to define the epidemiological traits of oxymino-cephalosporin-resistant *Acinetobacter baumannii* clinical isolates from Korea.
Methods: A total of 378 non-duplicate clinical isolates of *A. baumannii* were collected from 19 different hospitals in Korea in 2008. Antimicrobial susceptibilities were tested by the disk diffusion assay. PCR experiments were performed to detect genes encoding ESBLs, MBL genes, and OXA carbapenemases. PCR products were subjected to direct sequencing. Surrounding regions of the blaPER-1 gene was investigated by sequencing overlapping PCR fragments. Southern blot experiments were performed to identify location of the blaPER-1 gene. Strain typing was performed by multilocus sequence typing (MLST) experiments.

Results: 291/378 *A. baumannii* isolates were identified as clonal complex (CC) 92, including sequence type (ST) 69, ST75, ST92, ST137, and ST138, by MLST. Most (288/291) *A. baumannii* isolates of CC92 showed resistance to ceftazidime, in contrast to only 7/87 isolates of non-CC92 did. The ISAba1 element upstream of the blaADC gene was detected in 282/291 isolates of CC92 and 7/87 isolates of non-CC92. The blaPER-1 gene was detected in 116/291 isolates of CC92 but not in 87 isolates of non-CC92. The blaPER-1 gene was located in the transposon Tn1213 (ISPa12-blaPER-1-Deltagst-ISPa13) in 93 isolates and in a complex class 1 integron (orf513-blaPER-1-ATP binding protein gene) in 6 isolates. The probe specific for blaPER-1 hybridized with I-CeuI-digested fragments, which also hybridized with a probe specific for 16S rRNA, suggesting the chromosomal location of the gene.
Conclusion: The present data show that *A. baumannii* isolates of CC92 are widely disseminated in Korea and they acquire ceftazidime-resistance by production of PER-1 and/or overproduction of ADC enzymes.

O528 Endemic multidrug-resistant *Acinetobacter baumannii* from animal specimens in German veterinary clinics

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Objectives: Members of the genus *Acinetobacter* comprise well-known nosocomial pathogens responsible for infections and epidemic spread among critically ill human patients including multitrauma patients. The main three clinically relevant species, *A. baumannii*, A. genomic species (gen. sp.) 3 and A. gen. sp. 13TU, and one environmental species, *A. calcoaceticus* form the so-called *A. calcoaceticus-A. baumannii* (Acb) complex whose representatives cannot be distinguished well by phenotypic attributes. Over recent years, an increase in the number of multidrug-resistant *Acinetobacter* isolates from animal specimens was recognized by the microbiology department of the Giessen Veterinary Faculty. The aim of this study was to investigate the species and strain diversity of these organisms and to compare them with a large set of human *Acinetobacter* strains of the Leiden University Medical Center collection.
Methods: From 2000 through 2008, 137 *Acinetobacter* isolates were phenotypically identified as belonging to the Acb complex. Of these, 56 were selected for further investigation. The organisms were characterized by three genotypic methods including amplified ribosomal restriction analysis (ARDRA), macrorestriction analysis (PFGE) and AFLPTM genomic fingerprinting.
Results: Using ARDRA, 52 isolates were identified to *A. baumannii*, three to A. gen. sp. 3 while one strain with a unique profile remained unclassified. With PFGE, three main clusters of strains with highly similar profiles and six unique types were distinguished. These findings were confirmed by AFLP analysis. Moreover, by comparison to reference strains included in the Leiden AFLP database, 19 isolates were identified as belonging to the European clones that are notorious for their association with outbreaks worldwide.
Conclusion: The study indicates persistence and spread of genotypically related strains within and among the German veterinary clinics. The occurrence of the European clones I-III might indicate that, like in human medicine, *A. baumannii* is an upcoming opportunistic pathogen in veterinary medicine. It also raises the question, whether the organisms can spread from animals to humans or whether the animals have acquired the organisms from humans.

Controlling MRSA

S535 Measures to be taken: hand hygiene, isolation, hospital cleaning

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Background: MRSA is major problem worldwide both in healthcare settings and in the community, causing an increasing burden of morbidity, mortality and costs. Control of MRSA spread within healthcare facilities is crucial to avoid complications threatening patient safety during healthcare delivery. Relevant changes in some MRSA national epidemiological trends have been detected over the last years.
Methods: The presentation will be based on systematic reviews of the literature on the effectiveness of measures implemented for MRSA control. In particular, aspects related to the relative impact on the transmission risk, to cost-effectiveness and to implementation feasibility in a range of healthcare settings and according to the available resources and facilities, will be considered. The evaluation will cover both the facility and the country levels.
Results: The best approach to MRSA control is based on multiple actions including early identification and decolonization of carriers, careful application of contact precautions, in particular optimal hand hygiene practices and patient location in single room or cohorting according to the local epidemiology and available facilities, environmental cleaning, and locally tailored approaches to appropriate antibiotic use. Strategies implemented by countries that were able to keep MRSA incidence at low level and, more importantly, by countries that succeeded to reduce high MRSA incidence, in particular bacteraemia, will be presented along with the related results and trends. Controversies in the field of MRSA control will be highlighted; among these: universal versus targeted screening; the advantages and limits of rapid screening methods; the issue of antibiotic resistance following carriers' decolonization; challenges in the application of isolation precautions; the actual role of environmental cleaning, given the relatively low potential role of environmental reservoirs in MRSA transmission; the actual correlation of MRSA incidence reduction and the consumption of alcohol-based handrub.

Milestones in antimicrobial chemotherapy during the past 50 years!

S539 Aminoglycosides

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The first clinically useful aminoglycoside, streptomycin, was isolated from a strain of *Streptomyces griseus* by Waksman and colleagues in 1943. This drug had remarkable activity against many species of Gram-positive and Gram-negative bacteria as well as the tubercle bacillus. However, when used as a single agent for treating tuberculosis, there was rapid emergence of resistance. That was subsequently overcome by using the drug in combination with other antituberculous agents. Over the next quarter century, a number of other aminoglycosidic aminocyclitol antibiotics were discovered, including neomycin (1949), kanamycin (1957), gentamicin (1963), and tobramycin (1967). During this time, extensive studies of the mechanism of action of the aminoglycosides carried out, and based on detailed information concerning mechanisms of enzymatic resistance, it became possible to provide a number of chemical modifications of the basic aminoglycoside structure to overcome resistance. Thus drugs such as amikacin (1972), dibekacin, and others were developed specifically to overcome mechanisms of enzymatic inactivation. Work in this arena has continued to the present, with several aminoglycosides, including SPX-1212 and ACHN-490, in preclinical or early clinical development for the treatment of infections due to organisms resistant to standard aminoglycosides. The issue of nephro- and ototoxicity has plagued all of the aminoglycosides, and although the currently available agents vary in their toxicity, it has been impossible thus far to design a compound in this class of antimicrobial agents completely devoid of oto- and/or nephrotoxicity.

S540 From sulphonamides to fluoroquinolones

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In the early 1930's it was noted that azo dyes were active in vitro against E coli and in vivo against β-hemolytic streptococci and that a sulfonamide moiety para to the azo group mediated in vivo activity;

the first antibacterially active sulfonamide "prontosil" was synthesized in November 1932. Shortly thereafter it was noted that an alkyl side chain – indispensable for antiparasitic action of e.g. plasmoquine or chloroquine – was not required for in vivo activity of sulfonamides. The story of quinolones begins with chloroquine; nalidixic acid was identified as a byproduct of chloroquine synthesis in 1962.

Both, sulfonamides and quinolones, presented opportunities for landmark studies on protein binding (pb) and PK/PD of antibacterials. In 1942/3 Davis worked on the impact of pb on PK of sulfonamides and described that "it is probable that only the unbound drug is bacteriostatically active". Analysis of the impact of pathology on and thermodynamics of pb resulted in the formulation of an "efficacy index" defined as free-drug AUC/MIC, being correlated to in vivo efficacy. Using values for the sulfonamide dissociation constant, pH, blood flow at the focus of infection, pb, metabolism and excretion an "area under the tissue concentration vs time curve" was described as an indicator of probable clinical success. A review on the "pharmacodynamics of sulfonamides" was published in 1949.

In contrast to most antibacterials then available, nalidixic acid showed a broad spectrum of activity against Gram negatives, but resistance developed rapidly. The first FQ, flumequine bearing the F-atom at C-6, was developed in 1976; it was active against nalidixic acid resistant isolates. But till 1980 FQ development did not advance significantly; it was demonstrated then that the presence of a fluorine at C-6 and additional modifications at C-7, C-8 and N-1 improved antibacterial activity and PK. Systematic population PK/PD analysis relating speed of antibacterial action and FQ exposure to infectious outcome demonstrated that pharmacodynamic endpoints "provide a new paradigm for clinical trials".

Both, sulfonamides and sulfones like sulfadiazine and dapsone, as well as FQs like ciprofloxacin, levofloxacin, moxifloxacin are used therapeutically; FQs like delafloxacin, J&J Q2, WCK 771, finafloxacin and ciprofloxacin or levofloxacin for inhalation are being developed demonstrating that both drug classes are still indispensable for treatment of infectious diseases.

Imaging infection

S542 Imaging microbes in vivo

A. Richter-Dahlfors (Stockholm, SE)*

A cornerstone of infection biology is the study of the complex interplay between infecting organism and their host. Combining the power of multiphoton-based live animal imaging with the precision of micro-puncture, we have developed a system that allows us to visually track a live infection from the first interactions between host and pathogen, within a living animal. Using GFP expressing clinical uropathogenic *E. coli* (UPEC) we follow the progressing pathophysiology of pyelonephritis, revealing previously un-described vasal, immune and nephritic events. Isogenic GFP expressing UPEC strains, carrying mutations in virulence factor genes such as Type 1 and P fimbriae, alfa-haemolysin and LPS, have been used to address their role for the kinetics of bacterial colonization in vivo as well as the host's immune and physiological responses. Furthermore, we applied a hypothesis-free approach, based on transcriptional profiling and comparative tissue transcriptomics, to identify components of the multi-cellular tissue responses directing the host response. A core of genes constituting a "General tissue response to early local bacterial infections" was demonstrated, and revealed a marked role of IFN-γ in prompt inter-organ communication in early local tissue response to infection.

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Resistant superbugs: a comparative analysis of Asia versus Europe

S547 ESBLs and carbapenemases

D. Livermore (London, UK)*

Europe and Asia are vast continents with great diversity, including countries with very different infrastructures, public health systems, patterns of antibiotic use and, so far as can be judged, resistance rates. A few surveillance systems – MYSTIC, SMART and SENTRY examine Gram-negative bacteria from both continents. Whilst their coverage is tiny relative to total population, all point to higher rates of cephalosporin resistance and ESBL production in much of Asia compared with Europe. Several show India to have exceptionally high ESBL rates, at around 50–80% in both *Klebsiella* and *E. coli*, whereas ESBL rates of 20–50% are widely seen across East Asia vs. 5–15% for most Western Europe. CTX-M-15 is now the predominant ESBL in Europe except Iberia and in Asia as far east as India, but is replaced by CTX-M-14 in the Far East and, strangely, in Spain. One lineage of uropathogenic *E. coli*, Sequence Type (ST)131, is particularly associated with cephalosporin resistance, usually but not always mediated by CTX-M-15 enzyme; it occurs widely across both continents, also in Africa, N. American and Australia. The dissemination of carbapenemases across the two continents is at an earlier stage than that of ESBLs and these enzymes remain extremely rare in many countries. Nevertheless there is extensive dissemination of NDM-1 metallo-carbapenemase in India and Pakistan, of VIM in Greece, OXA-48 in Turkey and KPC (an enzyme that originated in the US) in Greece. These are being introduced to further countries in both continents by human migration, international lifestyles and medical tourism, with cluster outbreaks of producers in, for example, China, Poland, Italy and the UK. One of the most potentially disturbing, but least clear, aspects is the extent to which resistant Enterobacteriaceae with ESBLs and carbapenemases have entered the wider ecosystem in those countries, largely in South Asia, where there is inadequate separation of drinking and waste water and the extent to which this may drive the problem by allowing gut colonisation of large numbers of residents.

Risk factors other than neutropenia for developing infection in immunosuppressed patients

S549 Toll-like receptors polymorphisms and fungal infections

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Pro-inflammatory cytokines are an essential component of host defence to fungi. Murine models and clinical evidence have revealed significant cytokine derangement in severe fungal diseases raising the suggestion of a causative role for maladaptive cytokine release in the disease course. A number of study is adding to the growing evidence that genetic variation in pro-inflammatory cytokines has a role in susceptibility and survival in many infectious disease. Several polymorphisms have been identified in genes encoding for pro-inflammatory/anti-inflammatory cytokines, such as TNF-α, IL-6, IL-10, INFγ, IL-1Rα, IL-23R, IL-7Rα and IL-10RB, and recently studies have shown association with susceptibility to fungal infections in polymorphism at pattern recognition receptor

(PRR) genes. Largely known for their role in inducing protective immune responses, PRR engagement may paradoxically favour fungal infections, by inducing inflammatory pathology and impairing antifungal immunity. Although the dissection of complex genetic traits modulating susceptibility to fungal infections is complex, the contribution of host immune genetics may hold the key to elucidating new risk factors for these severe, often fatal diseases.
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S550 Complement and fungal infections

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Fungal infections are a serious complication in immunocompromised patients such as human immunodeficiency virus-infected individuals, patients with organ transplantations or with haematological neoplasia. The lethality of opportunistic fungal infection is high despite a growing arsenal of antimycotic drugs, implying the urgent need for supportive immunological therapies to strengthen the current inefficient antimicrobial defences of the immunocompromised host. Therefore, increasing effort has been directed to investigating the interplay between fungi and the host immunity and thus to find starting points for additional therapeutic approaches. Important aspects include the activation of the complement system by the fungal pathogen, the efficiency of the complement-associated antimicrobial functions and the arsenal of immune evasion strategies applied by the fungi. The broad spectrum of complement-associated immune reactions includes the deposition of complement products on the fungal surface to opsonise them for phagocytosis, the activation and chemoattraction of immune cells, and the stimulation of virtually the whole immunodefence network (humoral immunity, T-cell response, and induction of cytokine and chemokine expression). This central role of complement as the first line of antifungal defence could be demonstrated in many cases, e.g. by the fact that complement-deficient mice are extremely susceptible against several fungal infections. The twin functions of complement as an interactive player of the innate immunity and at the same time as a modulator of the adaptive immunity make this defence weapon a particularly interesting therapeutic candidate to mobilise a more effective immune response and to strengthen in one fell swoop a broad spectrum of different immune reactions. The 'Yin-Yang' nature of the complement system in fungal infections, as growing evidence assigns to complement a contributory part in the pathogenesis of fungus-induced allergic manifestations.

S551 Mannose-binding lectin and bacterial infections in patients with haematological cancer

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Genetic defects that impair the recognition of pathogens by the innate immune system, may increase the risk of infectious diseases. Mannose-binding lectin (MBL) an ancestral molecule that survived throughout the evolution, is a C-type lectin receptor. It has a carbohydrate recognition domain that binds to microbial polysaccharides of bacteria, fungi, viruses and protozoa. It activates the complement system and facilitates phagocytosis of microorganisms.
In humans, MBL is encoded by the gene MBL2 located on chromosome 10q25, Exon-1 structural mutations (O variant) and polymorphism in promotor region (LX haplotype) result in low serum MBL concentrations. MBL deficiency has been associated with an increased risk of autoimmune diseases, cardiovascular diseases, cystic fibrosis, cancer and an increased risk of infection with more severe course. Low serum levels of MBL are found in 30–40% of white people and very low levels in about 8–10%. Because many components of the innate immune system are redundant, MBL deficiency appears to be an important risk of infection when it is combined with other immunodeficiencies. These include early childhood with immune immaturity, primary and secondary immunodeficiencies and oncology patients receiving chemotherapy. Patients with haematological malignancies are at high risk of developing chemotherapy-induced neutropenia and a variety of bacterial and fungal infections with life-threatening complications. During this period of immune paralysis of phagocytes and lymphocytes, the non-cellular components of the innate immune systems, not affected by the immunosuppressive drugs, may become an important alternative. Several studies in children and adults tried to assess the role of serum MBL deficiency in this setting. Heterogeneity of patients, small numbers, different cut-off values for defining MBL deficiency have led to conflicting results. We recently reported in a large prospective study that MBL-deficiency patients with haematological malignancy have more severe infections including pneumonia, bacteremia, invasive fungal infections or sepsis, after receiving chemotherapy.
These results have been confirmed in another prospective trial in myeloablative sibling allogeneic hematopoietic stem cell transplantation. These reports suggest that MBL replacement therapy may be useful in patients at high-risk of severe infections in post-chemotherapy period.