Oral presentations

Emerging issues in β-lactamase-mediated resistance (Symposium jointly arranged with FEMS)

S1 Class D carbapenemases: origins, activity, expression and epidemiology of their producers

L. Poirel (Le Kremlin Bicetre, FR)

Oxacillinases are class D β-lactamases, grouping very diverse enzymes usually not sensitive to β-lactamase inhibitors. Some oxacillinases hydrolyse only narrow-spectrum β-lactams, some others expanded-spectrum cephalosporins, but more worrying are those oxacillinases hydrolysing carbapenems. Those latter oxacillinases named CHDLs for "Carbapenem-Hydrolysing class D β-Lactamases" have been identified in a variety of Gram-negative bacterial species. They do hydrolyse penicillins and carbapenems at a low level, but their hydrolysis spectrum does not include expanded-spectrum cephalosporins. They are not inhibited by clavulanic acid but are inhibited by NaCl in vitro.

Some CHDLs correspond to naturally-occurring β-lactamases encoded on the chromosome of a variety of species, such as *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Ralstonia pickettii*. The corresponding genes are often poorly expressed, and the impact of those carbapenemases on the carbapenem susceptibility of the corresponding species seems marginal. However, it has been demonstrated that overexpression of the naturally-occurring blaOXA-51-like gene of *A. baumannii* (linked to insertion of an IS element upstream of that gene) can lead to decreased susceptibility to imipenem.

By contrast, other CHDLs are considered as acquired, leading to esistance to carbapenems. Most of these acquired CHDLs have been identified in *A. baumannii*, being of three types (OXA-23, OXA-40, and OXA-58). Those acquired enzymes may be either plasmid- or cchromosome-encoded. Those oxacillinases have been identified worldwide, always identified in carbapenem-resistant isolates, those latter being very often at the origin of nosocomial outbreaks. Interestingly, the source of the OXA-23-encoding gene has been very recently identified, being the chromosome of the carbapenem-susceptible *Acinetobacter* radioresistens species, in which blaOXA-23 is poorly expressed.

Another acquired CHDL is OXA-48 identified firstly in a carbapenem-resistant *Klebsiella pneumoniae* isolate from Turkey, which has now widely disseminated in Istanbul, responsible for large outbreaks. The blaOXA-48 gene is plasmid-encoded and has been recently evidenced in other enterobacterial species in the same country. Interestingly, the source of that acquired CHDL was shown to be the Gram-negative, environmental and waterborne species *Shewanella oneidensis*.

In conclusion, besides the acquisition of metallo-β-lactamases or even class A carbapenemase suck as the KPC-type enzymes conferring carbapenem resistance in Gram negatives, the current emergence and spread of CHDLs represents a worrying threat. The dissemination of such mechanism is difficult to trace and thus to control, considering that no currently available test allows detection of CHDLs.

S2 Metallo-β-lactamase-producing Enterobacteriaceae: phenotypes, genetics, prevalence and clinical significance

V. Miriagou (Athens, GR)

While production of serine β-lactamases of the molecular classes A and C remains the most clinically significant β-lactam resistance mechanism among enterobacteria, there is an increasing concern as to the dissemination of strains with zinc-dependent class B metallo-β-lactamases (MBLs). These acquired enzymes display an extremely wide spectrum of hydrolysis that includes also carbapenems. The MBL-encoding genes commonly occur as cassettes in integrons carried by a variety of transferable plasmids and enterobacterial chromosomes underscoring their spreading potential. Indeed, a physical linkage of MBL integrons with transposable elements has been, in some instances, documented. VIM and IMP β-lactamases – the main MBL types found in enterobacteria – have already achieved a global spread, the southern Europe and the Far East being the most affected regions. There are quite a few epidemiological studies unveiling the mode of spread of MBL-producing enterobacteria. Nevertheless, our understanding of what MBL production entails in terms of clinical impact is still limited. It is not yet clear if MICs of MBL producers must be consider at face value or these isolates must be reported as potentially resistant to carbapenems. Moreover, performance of the routine detection methods based on EDTA-β-lactam synergy is not optimal and not yet standardised. The aim of this presentation is to discuss recent advances on genetics, epidemiology and clinical significance of MBL-producing enterobacteria.

State-of-the-art treatment of imported parasitic diseases

S6 How to deal with chronic infections of *Trichinella*

C.M. Cretu (Bucharest, RO)

Trichinellosis, parasitic disease due to the presence of *Trichinella* spp. larvae in muscle tissue, is an emerging disease and seems to become a re-emerging disease, as in many endemic countries human clinical cases are related and consequent to a breakdown of local legislation.

In Central and Eastern Europe, *Trichinella* infection still represents an important health problem, according to the statistical data registered by ICT.

Many species of *Trichinella* larvae, encapsulated or not, are responsible for human cases, according to data collected in Reference Laboratory in Rome, each one having a typical clinical appearance and geographical distribution: *T. spiralis*, *T. pseudospiralis*, *T. britovi*, *T. nativa*, *T. nelsoni*, *T. murelli*, *T. papuae*.

In endemic countries, medical attention should be paid on both acute and chronic trichinellosis. Acute trichinellosis is familiar for medical doctors: short incubation period, followed by gastrointestinal phase (20–30 days), acute stage (weakness, chills, fever, headache, sweating, tachycardia, eyelid/periocular/generalised oedema, muscle pain) and, sometime, complications (cardiovascular, ocular, neurological, respiratory etc).

Differentiations between convalescent stage (residual myalgia), sequels or chronic trichinellosis still remain not very well defined.

Chronic trichinellosis is either symptomatic (general discomfort, chronic muscle pain, tingling, neuropsychiatric signs, persistent sweating), in patients with history of trichinellosis, or asymptomatic, accidentally discovered, when muscle biopsy is examined for other reasons (i.e. laryngeal or tongue neoplasm), when residual IgG antibodies level is proved (persistence of residual viable larvae), chronic hypereosinophilia or when bioelectric muscle disturbances persist.

The role of chronic trichinellosis larvae as co-factor in the development of cancer is under debate, but further studies are necessary to be conducted in order to confirm the direct relationship between *Trichinella* larvae and neoplasm (chronic inflammation, direct carcinogenetic role, co-factor in carcinogenesis?).

The algorithms for diagnosis of acute or chronic trichinellosis should be taken into account, in order to initiate the appropriate treatment and to prevent severe complications.
Laboratory diagnosis is simple and relevant during the acute stage, while, during the chronic stage or in people who received corticosteroids, ELISA can be negative and only confirmation tests (imunobloting, multiplex PCR) or muscle biopsy can determine the diagnosis.
Epidemiology should evaluate the geographical regions and the different *Trichinella* species, as clinical evolution of the disease is closely related to the parasitic burden, parasitic species and host immune response.

S7 How to cope with the wormy world? Treatment of helminths

A.M.L. Van Gompel (Antwerp, BE)

Helminth infections are among the most common infections in men.
The different helminthic infections that currently may be imported in our European countries by returning travellers, expatriates, immigrants and refugees coming from endemic countries will be succinctly presented in the following way:

- nematodes or roundworms (intestinal nematodes: ancylostomiasis and other hookworm infections, angiostrongyliasis, ascariasis, capillariasis, enterobiasis, strongyloidiasis, trichostrongyliasis, trichuriasis; filarial nematodes and dirofilariasis; tissue nematodes, e.g.: angiostrongyliasis, cutaneous larva migrans, gnathostomiasis, toxocariasis, trichinosis),
- cestodes or tapeworms (cysticercosis, diphyllobothriasis, echinococcosis, taeniasis and other intestinal tapeworms) and
- trematodes or flukes (chlonorchiasis and opistorchiasis, fasciolasis; intestinal flukes; paragonimiasis; schistosomiasis)

... and their treatment modalities will be highlighted.
The number of effective antihelminthic agents is small relative to the vast array of antibacterial agents. The mechanism of action of most antiparasitic drugs is not always well understood. Especially the role of the following very frequently used anthelmintics will be illustrated: the benzimidazoles (albendazole, mebendazole, flubendazole, triclabendazole), ivermectin, praziquantel and diethylcarbamazine; but other products still in use will also briefly be mentioned. Newer "players" as nitazoxanide, the artemisinin-derivatives (possibly new player for the early stages of schistosomiasis? fasciolase) and doxycycline (adjunctive role in the treatment of filariasis) will be highlighted.
An opportunity to obtain an electronic copy in pdf of the powerpoint will be offered at the end of the lecture.

European MIC breakpoints for antimicrobial susceptibility testing are now harmonised by EUCAST (Symposium arranged with EUCAST)

S9 Why European harmonisation?

D. Brown (Cambridge, UK)

At least seven different MIC breakpoint committee guidelines for antimicrobial susceptibility testing have been used in Europe. Consequently Europe has had several different sets of antimicrobial breakpoints and a range of variations in technical methods. It became increasingly evident that harmonisation of breakpoints was necessary both for therapy and resistance surveillance. ESCMID set up EUCAST in 1997 with a representative from each European country and 6 representatives from industry. In 2002 EUCAST was restructured and the major responsibility for the work of EUCAST was taken on by the active national breakpoint committees in Europe. A Steering Committee was formed, currently comprised of a representative from the 6 active national breakpoint committees, 2 from the EUCAST General Committee (which has a representative from each European country), a Chairperson, a Scientific Secretary and a Clinical Data Coordinator. A decision making process was established whereby proposals made by the Steering Committee are distributed to the EUCAST General Committee, relevant expert groups and industry for consultation. The final decision is made by consensus in the Steering Committee, taking account of any comments made during consultations. In this process the expertise of the national breakpoint committees is utilised, there is wide consultation on proposals and the national committees take responsibility for implementation of decisions. Subcommittees have been set up to deal with specific topics including susceptibility testing of fungi and anaerobes, and expert rules in susceptibility testing. A website has been established (http://www.EUCAST.org) that gives details of EUCAST activities, EUCAST breakpoints and publications. Another website has been developed for the collection of MIC data and its presentation as species-specific wild type MIC distributions. EUCAST has been funded by ESCMID, the national breakpoint committees, a grant from the EU and now by ECDC. Industry does not contribute financially but is asked to contribute data required for determining breakpoints and to comment on proposed breakpoints. EUCAST has achieved harmonisation of most existing breakpoints in Europe. It has a formal relationship with EMEA regarding the setting of breakpoints for new agents and the revision of breakpoints for existing agents. The process has been applied to several new drugs. Documents on various aspects of susceptibility testing have also been published.

S12 Implementation of European breakpoints and the future of EUCAST

G. Kahlmeter on behalf of EUCAST

EUCAST will soon have harmonised European breakpoints for existing antimicrobials. Also, as part of the EMEA process for approval of new drugs, EUCAST has determined breakpoints for several antimicrobials. The work of the committee now enters a wider implementation phase.
Existing classes of drugs: At the end of 2008 there will be a complete set of EUCAST clinical breakpoints and epidemiological cut-off values. By early 2009 the clinical breakpoints will be implemented in the AST systems of BSAC (UK), CA-SFM (France), CRG (Netherlands), DIN (Germany), NWGA (Norway) and SRGA (Sweden).
New antimicrobials: EUCAST determines breakpoints as part of the EMEA approval process for new antimicrobials. EUCAST breakpoints are the only breakpoints included in the Summary of Product Characteristics (SPC). Daptomycin and tigecycline are already approved and another 4–6 drugs will be handled during 2007–9.
Antimicrobial susceptibility testing devices: Work is ongoing to implement EUCAST clinical breakpoints in Phoenix (BD) and VITEK 2 (BioMerieux) and it is expected that EUCAST breakpoints will be available for both in early 2009. The fact that EUCAST breakpoints and national breakpoints will be the same will simplify the development of test panels as well as benefiting users.
EUCAST disk diffusion method: Preliminary results from a questionnaire to determine the expectations of clinical microbiologists in Europe indicate that EUCAST should take the lead in developing a disk diffusion test based on Mueller-Hinton agar.
The future of EUCAST: EUCAST has been financed by ESCMID and the national breakpoint committees of France, Germany, Norway, Sweden, The Netherlands and the UK for many years. Over the last 4 years the EU and ECDC have contributed financially. It is hoped that this will be continued by ECDC.
There is a need to sustain a European Committee on Antimicrobial Susceptibility Testing beyond the breakpoint harmonisation process. New antimicrobials will need breakpoints. Companies with approved antimicrobials will seek approval for extensions of clinical or microbiological indications or modified dosages. New resistance mechanisms occasionally necessitate the review of existing breakpoints. The establishment of a European disk diffusion test is a major undertaking and there will be a need continually to develop it to accommodate new antimicrobials and new resistance mechanisms. All these efforts are best served by a common European committee, EUCAST.

New bugs – old drugs: frustrations in antimicrobial treatment in primary care

S17 Urinary tract infections

T. Christiaens (Ghent, BE)

Urinary tract infections (UTI) are the most common bacterial infections in women. The spectrum includes an umpteenth, patient-familiar episode of a recurrent cystitis to a fatal UTI-linked gramnegative sepsis. In primary healthcare (PHC) three major clinical pictures are important: acute cystitis or uncomplicated lower UTI, recurrent cystitis and acute pyelonephritis.

Discussing treatment of cystitis in PHC regards essentially (1) the growing resistance of uropathogens against familiar drugs used in cystitis, (2) the place of newer drugs such as the oral chinolones, (3) and the duration of therapy. Therapeutic dilemmas in community acquired acute pyelonephritis are: (1) which patients can be treated ambulatory and (2) with which drug.

In acute and recurrent cystitis treatment choices are mostly empirical. This implies that bacterial species and resistance have to be anticipated reliably. In recent years alarming resistance data in uropathogens have persuaded physicians to choose broadspectrum antimicrobial agents. But most of these data come from surveillance studies on all uropathogens in regional bacteriological laboratories. Important selection of urine samples occur in this setting: most samples sent to the bacteriological laboratory came from complicated infections, immune-incompetent patients or people with urologic problems such as pyelum stones. In contrast, the majority of cystitis is encountered in healthy women, so extrapolation of these resistance data is speculative. We performed a surveillance in this healthy population with cystitis and found no alarming resistance at all; moreover, a new surveillance 10 years later showed no increase of resistance in uropathogens encountered in these women. This means that nitrofurantoin remains the first choice in the Belgian GP-guideline (the same applies to the Dutch and the French guideline) Trimethoprim can still be useful but in GB and in the Netherlands more resistance has been observed. Chinolones are efficient but not superior. Because of high resistance, amoxicillines cannot be given empirically, which is a major problem in pregnant women for whom it is the safest drug.

The ideal duration of treatment remains controversial, but a recent Cochrane review showed equivalence in success in treatment s of 3 days compared with 5 days or more in all studied drugs. In this case, we are more uncertain about the use of nitrofurans, they are frequently prescribed for five days because of lack of data for 3 days. Physicians have to be aware that with these short treatments most patients will still have symptoms at the moment in which drug intake is stopped.

The field of recurrent cystitis is very unpopular, so study data are scarse. Nitrofurans seem most active; of course renal impairment should be taken into account. By resulting in less drug intake for equal efficacity, self-treatment is more attractive than chronic use.

Acute pylonephritis is not so common as the former infections, but it is of course a potential life-threatening situation. Hospitalisation has been the rule until recently the chinolones has proven to be a safe ambulatory oral treatment in otherwise healthy individuals. Only by reserving chinolones for serious infections we do not induce chinolone-resistance in Gram-negative bacteria and spoil life-saving drugs in uncomplicated infections. UTI are an interesting field to develop and to study rational antbiotherapy.

S18 How to single out the sinusitis patients who can benefit from antibiotic treatment

M. Lindbaek (Oslo, NO)

Background: Respiratory tract infections (RTIs) are common in general practice and comprise approximately 15% of daily practice consultations. They often result in antibiotic prescription and make up more than 60% of all antibiotic prescriptions in general practice. Thus they play an important part in the development of antimicrobial resistance. A 10-fold difference in proportion of penicillin resistant pneumococci has been demonstrated between the highest and lowest prescribing countries in Europe.

The need of antibiotic prescription for the most common RTIs such as acute otitis media, sore throat, acute bronchitis and acute sinusitis has been highly debated for many years. Many studies have demonstrated only limited benefit from antibiotics for most of such patients in general practice, and most studies have failed in demonstrating clinically significant differences between antibiotic treatment and placebo. In the US and UK more than 90% receive antibiotics for acute sinusitis, while in Holland and Scandinavia, the proportion is lower – 68–80%. For this condition the situation is particular since two different guidelines for the treatment of acute sinusitis have been published in Clinical Evidence: One for patients with a confirmed sinusitis, stating that antibiotics have a moderate effect, and one for patients with a clinical sinusitis where no antibiotics is recommended.

Diagnostic problems in acute bacterial sinusitis: In general practice setting it is difficult to distinguish between bacterial and viral sinusitis. A number of studies have, by use of various reference standards, demonstrated some findings that may be of value. However, they have not proven to be of prognostic value as to duration of disease in non-selected populations. Furthermore: Even in the studies with reference standards, more than half of the patients had less or none symptoms by day 10. Also in patients with a high predictive value for bacterial infection, the spontaneous rate of recovery is high.

Treatment challenges: A high proportion of spontaneous recovery has been demonstrated in patients with bacterial sinusitis. A study of risk factors for long duration of symptoms among patients with acute sinusitis, showed significant differences for the most affected and the older patients. However, newer relevant studies have demonstrated few predictors for long/short duration. A Danish study demonstrated that a high CRP-value was predictive of higher cure rate with antibiotics in patients with much pain as compared to placebo.

Still, a small proportion of patients with acute sinusitis that has high fever, much pain and a deteriorated general condition, will benefit from antibiotic treatment. Otherwise a wait-and-see approach is reasonable. In our own studies a delayed prescription strategy has been demonstrated to reduce antibiotic treatment as less than 50% of the prescriptions have been dispensed from the pharmacy. This demonstrates that a considerable proportion of antibiotics prescribed for acute sinusitis in general practice can be avoided. The role of topical steroids is also debated as the results of various studies have given conflicting results.

S19 Acute exacerbations of chronic obstructive pulmonary disease

A. Torres (Barcelona, ES)

Exacerbations of chronic obstructive pulmonary disease (COPD) are a frequent clinical problem both in the primary care and in the hospital. The microorganisms that are associated with exacerbations are well recognised and include viruses, *Chlamydia* and bacteria such as *S. pneumoniae*, *H. influenzae* and *P. aeruginosa*. The severity of the COPD and the exacerbation are associated with different pathogens.

In the primary care setting usually these exacerbations are mild and do not need hospitalisation. The routine care of these patients is to administer antibiotics. According to some investigations a great part of these mild exacerbations would not require antibiotic treatment because they are viral in origin or because they are not infectious. However, in the clinical practice is difficult to distinguish between these two situations.

A recent study from our group in COPD exacerbated patients confirms that the existence of sputum purulence referred by the patient is a sensitive and specific variable associated with positive bacterial cultures in bronchoscopic samples.

We do not know if this information can be extrapolated to ambulatory patients but probably yes.

Of course, there are patients that cannot expectorate and some of them may have a bacterial infection. In these

Cases, perhaps biological markers could help to differentiate between viral and bacterial infections. Procalcitonin (PCT) has been studied in COPD exacerbation and in one randomised trial it has been useful continuing or discontinuing antibiotics without recurrence problems. The limitations of this method are the need of a blood sample and the availability of methods able to detect small amounts of PCT.
In terms of pathogens one of the most controversial issue is the role of *Pseudomonas aeruginosa*. Some studies in some countries confirm that *Pseudomonas aeruginosa* is isolated in around 10 to 15% of exacerbations. However, the pathogenic mechanisms of *Pseudomonas aeruginosa* in COPD exacerbations is unclear but the current recommendations advise to treat these patients with antipseudomonal antibiotics
Risk factors are known from individual studies. The ERS guidelines include among them the following: FEV1 < 30%; prior antibiotic treatment, long-term treatment with steroids and prior colonisation or isolation of *P. aeruginosa*. When two out of these four variables are present antipseudomonal treatment is recommended.

S20 Skin infections by community-acquired MRSA

S. Kaplan (Houston, US)

Meticillin-resistant *S. aureus* isolates causing community-acquired infections (CA-MRSA) are a major problem around the world. In some communities, CA-MRSA account for more than 70% of community *S. aureus* isolates. These isolates typically have a unique staphylococcal chromosomal cassette (SCCmec IV) that carries the antibiotic resistant genes and is lower in molecular weight than the cassette carried by the typical nosocomial MRSA isolates. CA-MRSA isolates generally are susceptible to clindamycin, trimethoprim-sulfamethoxazole and doxycycline. CA-MRSA *S. aureus* isolates also typically carry the genes coding for Panton-Valentine leukocidin (PVL) although the role of PVL in the pathogenesis of infections remains unclear. In the United States one particular clone identified by pulsed field gel electrophoresis (PFGE), USA300, accounts for the vast majority of CA-MRSA isolates. Other clones predominate in different areas of the world. The genomes of two CA-MRSA isolates, USA300 and USA400 (MW-2) have been fully sequenced and may eventually provide clues to why USA300 has been so successful in spreading and causing infection. By far the most common infections associated with CA-MRSA isolates are skin and soft tissue infections including abscesses and cellulitis. CA-MRSA as a common aetiology of abscesses is much better documented because of the ease of culturing purulent drainage. In children the most common sites of infection are the buttock, perineum and extremities. CA-MRSA isolates are also associated with serious invasive infections, especially osteomyelitis and pneumonia. Recurrent soft tissue infections and infections within the family caused by CA-MRSA isolates are common. The most important aspect of management of superficial skin and soft tissue infections is appropriate surgical drainage. Most clinicians also administer an effective oral agent such as TMP-SMX or clindamycin, although the contribution of antibiotic therapy after surgical incision and drainage is under investigation. Empiric vancomycin or clindamycin is typically administered for more serious and invasive infections such as osteomyelitis, septic arthritis or suspected Staphylococcal pneumonia in regions where CA-MRSA is common (>5–10% of community *S. aureus* isolates). Clindamycin is efficacious in treating CA-MRSA infections caused by susceptible organisms. Effective measures to prevent CA-MRSA infections remain unclear.

Epidemiology of community-acquired meticillin-resistant *Staphylococcus aureus*

S21 Origins and evolution of MRSA clonal lineages

A. van Belkum (Rotterdam, NL)

Staphylococcus aureus essentially is a clonal bacterial species. This implies that various lineages exist that appear to be ecologically highly succesfull. For both meticillin susceptible and resistant strains it has been demonstrated that many of these belong to such pandemic clones. Based on these observations as well as on the comparative analysis of the many available staphylococcal genome sequences it has been suggested that genome complexity in *S. aureus* can be pictured in a simple model. The genome of *S. aureus* consists of a core element (approximately 80% of the entire genome), a core variable element and a so called variable or accessory genome. This model, attractive because of its simplicity, would suggest that the core genome would encode the overall *S. aureus*-characteristic traits whereas the core variable and variable moieties encode factors that provide *S. aureus* with more strain or clone specific traits.
The talk will describe the genomic tools available for defining *S. aureus* population structures and how these stucutres can be used to define the emergenc of novel lineages. In addition, those factors as present in the accessory genome and defining traits such as host specificity, invasiveness or epidemicity will be discussed. Finally, recent research has suggested that not all human individuals share genotypes that would permit *S. aureus* to colonise the nasopharynges. This would also suggest that the interaction between S. aurteus and humans requires intricate matching of both bacterial and human features. The relevance of *S. aureus* genome flexibility in establishing host-microbe ointeractions will be discussed.

S22 Emergence and dissemination of community-acquired meticillin-resistant *Staphylococcus aureus* in Europe

M.A. Dominguez (Hospitalet. Barcelona, ES)

During the past decade, meticillin-resistant *Staphylococcus aureus* (MRSA) has emerged as cause of community acquired (CA) infections, among patients without established risk factors for MRSA. The differentiation between CA-MRSA and hospital-associated (HA) MRSA is becoming difficult since CA-MRSA could spread into hospitals. CA-MRSA cause skin and soft-tissue infections, but can also cause necrotising tissue infections and fulminant pneumonia. Most CA-MRSA strains produce the Panton-Valentine leucocidin (PVL), a leukocyte pore-forming toxin strongly associated with invasive disease and virulence. Transmission of CA-MRSA from person to person has been well documented and ascribed to close contact, e.g. during sport practice or prison stays. Also transmission can occur among family members. In the United States, CA-MRSA has been reported as the most frequent cause of soft-tissue infections. The predominant CA-MRSA clone is the USA300, belonging to Clonal Complex (CC) 8, characterised by a particular pulsed field gel electrophoresis pattern, SCCmec type IV, and encoding PVL production. The CA-MRSA prevalence in Europe is diverse. Although rates of HA-MRSA are very high in England and South Mediterranean countries, CA-MRSA has been more frequently reported in Northern and Central European countries. Isolates of CC80-SCCmecIV have been the predominant genotype in many European countries since late 1990s (Denmark, Sweden, Norway, Netherlands, Germany, England, France or Switzerland). However, another dominant genotype, CC8-SCCmecIV, related to clone USA 300, emerged in 2003 and spread through many countries (Netherlands, France, Spain, Switzerland, Greece or Norway). In Spain, with high endemic rates of HA-MRSA, isolation of PVL-positive CA-MRSA has been increasing in the last years. The predominant clone of CA-MRSA in Spain is the CC8-SCCmecIV, affecting family groups and patients with recent travel or family relation to countries in South America. A worrisome feature is that isolates of pandemic genotype CC5-SCCmecIV (paediatric clone), highly prevalent among HA-MRSA in many European countries, have been found to carry PVL toxin and to be CA-MRSA. Prevention and control of CA-MRSA infections outside the healthcare setting represent a real challenge for the health system and may be a serious problem in containing the spread of CA-MRSA within the hospital population.

S24 Animals as a source of MRSA infections in humans

J.S. Weese (Guelph, CA)

As MRSA has emerged as a significant community-associated (CA) pathogen, it is perhaps not surprising that MRSA has been identified in various animal species. MRSA is a prime example of the often-overlooked close relationship between humans and animals, with a potential for transmission of pathogens in both directions. Animals could play a role in human MRSA by direct contact, environmental contamination or food, however direct contact is the only route that has been clearly demonstrated. Animal populations could also be reservoirs of MRSA. The epidemiology, clinical aspects and human risks of MRSA in animals vary greatly between species.

Direct transmission of MRSA between household pets and their human contacts has been demonstrated, and pets may be particularly important in recurrent MRSA in households. MRSA strains found in pets tend to be the predominant strains found in humans in the geographic area and it is likely that humans are the source of MRSA for most household pets.

The epidemiology is different in horses, where an uncommon human epidemic clone has predominated, suggesting it is relatively horse-adapted. Zoonotic infections of horse owners and veterinary personnel have been identified. These groups also have high rates of MRSA colonisation and tend to be colonised with the clone most commonly found in horses, suggesting frequent zoonotic transmission.

In contrast, recent reports involving food animals, particularly pigs, have described predominance of ST398 strains that have not historically been important causes of human disease. This clone is now an important cause of CA-MRSA infection, first in persons with pig contact and now in the general population in some regions. There is significant concern that a reservoir of MRSA in may complicate aggressive MRSA control programmes. Identification of MRSA in food animals logically leads to concerns about the potential for foodborne transmission of MRSA. These risks are currently unclear but are being investigated.

The overall role of animals in human MRSA infections is still unclear but it is likely that animals can play a direct or indirect role in at least some cases. The apparent spread of ST398 from pigs to the broader community in Europe and the potential role of pets as household reservoirs are perhaps the greatest concerns. Failure to properly investigate the role of animals in human MRSA infection and in the changing epidemiology of CA-MRSA could compromise control measures.

Community-acquired bacterial infections of the respiratory tract

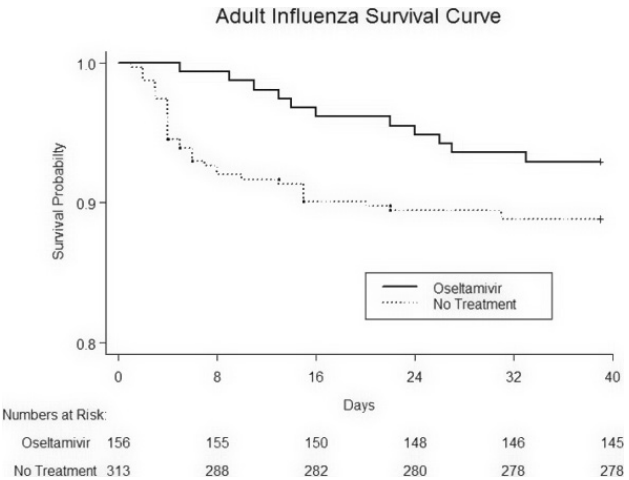
O25 Severe disease due to influenza in adults in Toronto, Canada, 2005–2007: impact of antiviral therapy

A. McGeer, K. Green, J. Raboud, D.E. Low on behalf of the Toronto Invasive Bacterial Diseases Network

Background: Few data describe the clinical features and outcomes of severe influenza (FLU)-associated illness, or the impact of specific therapy.

Methods: Since 1/1/2005, TIBDN has performed population-based surveillance for laboratory confirmed FLU associated with hospital admission in Toronto/Peel (population 3.8M). Consenting patients hospitalised for illness associated with a rapid antigen test, culture, and/or PCR positive for FLU are enrolled.

Results: From 1/1/2005 to 30/4/2007, 485 adult (>15yo) patients with community-acquired disease have been identified. Median age was 76.7yrs (range 15.1–98.8y), 250 (52%) were male, 105 (225) had no chronic underlying illness; 279/426 (65%) for whom data were available had received influenza vaccine; 86 (18%) were residents of nursing homes. There were 415 cases (86%) of FLUA, and 70 (14%) of FLUB. For CA cases, the most common diagnoses were: influenza (with/without other diagnoses): 266 (55%), pneumonia 111 (23%), other respiratory infection: 28 (6%), fever/viral syndrome 26 (5%), other respiratory diagnosis (eg. COPD) 23 (5%). 27 patients (5.6%) had culture-confirmed complicating bacterial infection (15 *S. aureus*, 7 *S. pneumoniae*, 3 *H. influenzae*, 1 group A streptococcus, 1 *E. coli*, 1 *M. catarrhalis*). 87 (18%) required ICU admission. Median LOS was 4 days (range 1–45d) for those <65yrs, and 6 days (range 1–103d) for those 65 years of age and older. 421 (88%) of patients were treated with antibacterials; 160 (33%) received antivirals: 3 amantadine, 157 oseltamivir. 106/154 (69%) treated cases received their 1st dose of oseltamivir >48h after symptom onset. Fifteen day mortality was 8.5% (41/485). In multivariable survival analysis, ICU admission (OR 7.3, 95% CI 4.1, 13, $P < 0.001$), nursing home residence (OR 2.1, 95% CI 1.1, 4.0, $P = 0.02$), charlson co-morbidity index (OR per point 1.2, 95%CI 1.0, 1.3, $P = 0.04$), shorter time from symptom onset to hospital admission (OR per 10 hrs 1.3, 95% CI 1.1, 1.4, $P = 0.002$), and failure to treat with antivirals (OR 2.0, 95% CI 1.1, 5.0, $P = 0.03$) were associated with death.



Conclusions: Seasonal influenza is a significant cause of serious illness. Oseltamivir treatment significantly reduces mortality in severely ill patients even when therapy is started late.

O26 Healthcare-associated bacteraemic pneumonia: aetiology, severity of disease and outcomes

M. Salvado, L. Lozano, E. Calbo, N. Freixes, M. Riera, M. Xercavins, M. Rodriguez-Carballeira, J. Garau (Terrassa, ES)

Introduction: Recent studies have been focused on healthcare-associated pneumonia (HCAP) trying to distinguish them from truly community-acquired (CAP) or hospital acquired pneumonia (HAP). The aim of our study was to describe the aetiology and outcomes of a cohort of patients with bacteraemic pneumonia (BP), regarding their relation to the healthcare-system.

Material and Methods: From Jan 2004 to June 2007, consecutive patients with BP were identified through the records of the Clinical Microbiology Laboratory in a 450-bed acute care teaching hospital. Data obtained included demographics, co morbidities, aetiology, severity of disease (Pitt score), presence of shock, relation with the healthcare-system and in-hospital mortality.

Results: 175 episodes of BP were identified. HAP was diagnosed in 25 patients (14.2%), CAP in 115 (65.3%) and HCAP in 36 (20.5%); mean age was 62.5 (SD 17.3), 57.8 (SD 20), and 75.6 y (SD 12.48), respectively ($p = 0.001$). Men represented 56%, 69.6% and 55.6% of the HAP, CAP and HCAP ($p = 0.2$), respectively. Co morbidity, measured by Charlson score, was 2.57 in HA, 1.55 in CAP and 2.03 in HCAP ($p = 0.05$). *Streptococcus pneumoniae* was isolated in 36%, 93% and 71.4% ($p = 0.001$); *Staphylococcus aureus* was present in 12%, 1.7% and 2.8 ($p = 0.037$) and *Pseudomonas aeruginosa* in 24%, 0.9% and 11% of the HAP, CAP and HCAP ($p = 0.001$). Pitt score was 2.8, 1.03 and 1.14, respectively ($p = 0.01$). Shock was present on admission in 20% of HAP, 10.4% CAP and in 11% of HCAP ($p = 0.4$). The in-hospital mortality

rate was 40% in HAP, 10% in CAP and 43% in HCAP (p = 0.001). In the multivariate analysis, severity of disease (OR=1.2; CI95% 1.06–1.5, p = 0.007), HAP (OR=3.9 CI95% 1.2–13, p = 0.02) and HCAP (OR=5.6; CI95% 1.9–16.6, p = 0.001) were independent predictive factors for increased mortality.
Conclusions: The majority of BP are seen in patients with CAP. Severity of disease and relation with the healthcare-system are important predictive factors of increased mortality. Although the aetiology in HCAP is similar to CAP, its mortality parallels that of HAP, showing the great impact of host factors on outcome.

O27 Factors associated with prolonged length of hospital stay in community-acquired pneumonia

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Objectives: Considerable variability exists in length of hospital stay (LOS) for patients with community-acquired pneumonia, in spite of being a key point in the quality and cost of care. The objective of the study was to identify factors associated with prolonged LOS (>8 days).
Methods: Observational analysis of a prospective cohort of nonseverely immunosuppresed adults with community-acquired pneumonia requiring hospitalisation from February 1995 through December 2006.
Results: We documented a total of 2,739 consecutive episodes of community-acquired pneumonia. Patients who required intensive care unit admission from the emergency department (n= 107), those who died during hospitalisation (n= 367), or patients with LOS >30 days (n= 60) were excluded from the analysis. The median duration of hospital stay was 8.0 days (IQR 6.0–11.0). No differences were found regarding time to institution of initial antibiotic therapy (door-to-needle time) when comparing patients with prolonged LOS and the remaining patients (5.8 vs 5.9 hours). Factors independently associated with prolonged LOS by step-wise multiple logistic regression analysis were female sex (OR 1.26; 95% CI 1.03–1.53), alcohol abuse (OR 1.48; 95% CI 1.16–1.86), high-risk Pneumonia Severity Index class (OR 2.02; 95% CI 1.65–2.48), bacteraemia (OR 1.75; 95% CI 1.30–2.37), aspiration pneumonia (OR 1.84; 95% CI 1.18–2.87), pleural empyema (OR 2.73; 95% CI 1.85–4.02), phlebitis (OR 1.42; 95% CI 1.05–1.91) and prior outpatient antibiotic therapy for current episode of pneumonia (OR 0.72; 95% CI 0.58–0.89). Time to switch from intravenous to oral antibiotics was longer among patients with prolonged LOS as compared with the remaining patients (6.1 vs 3.4 days; p < 0.001).
Conclusion: According to our data, several independent factors increased LOS in adult patients with community-acquired pneumonia. These factors should be considered when evaluating the adequacy of the duration of hospitalisation in a determined institution as well in future studies investigating new strategies aimed to reduce LOS.

O28 Repetitive C-reactive protein measurements in follow-up can predict adequacy of empiric antibiotic therapy in patients with severe community-acquired pneumonia

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Objectives: Despite the introduction of new inflammatory markers, C-reactive protein (CRP) remains commonly used in community-acquired pneumonia (CAP), however evidence for its diagnostic and prognostic value is still unclear. We therefore studied the discriminative value of CRP in aetiology and the value of repetitive CRP measurements in follow-up.
Methods: In a prospective multicentre trial, CRP levels were measured on admission, day 3 and day 7 in patients hospitalised with severe CAP. Patients were clinically followed for 28 days. Aetiology was determined by standard microbiological cultures, urinary antigen tests for *Streptococcus pneumoniae* and *Legionella pneumophila* and serological examinations for *L. pneumophila*, *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*.
Results: 289 patients with severe CAP were included (mean PSI score 113±25.7; age 69.7±13.8 year). In 137 patients (47.4%) aetiology could be determined. Median admission CRP levels were significantly higher in *S. pneumoniae* infection (278.0mg/l; p < 0.001), lower in *M. pneumoniae* infection (49.0mg/l; p = 0.05) and lower in patients with unknown aetiology (140mg/l; p = 0.002) as compared to patients with other aetiologic diagnoses. However, the diagnostic value of CRP for any aetiologic diagnosis was low (Area Under receiving operator Curve <0.70). In follow-up, normalisation patterns of CRP were different among aetiologic pathogens and slowest in *L. pneumophila* infection (ANOVA p = 0.05). In multivariate linear regression analysis, *S. pneumoniae* infection (beta coefficient (b) = 0.23) and chronic obstructive pulmonary disease (COPD) as comorbidity (b = 0.30) were independently associated with a rapid decline in CRP levels within the first three days of hospitalisation, whereas antibiotic treatment prior to hospital admission (b = –0.17) was associated with a slow decline in CRP levels (p < 0.008). A <60% decline in CRP levels within the first three days of hospitalisation was independently associated with inappropriate empiric antibiotic treatment (odds ratio 5.1; 95%CI 1.2–22.8; p = 0.03).
Conclusions: CRP is useful in follow-up rather than in establishing a bacterial aetiologic diagnosis. Repetitive CRP measurements in follow-up of severe CAP provide important information about adequacy of empiric therapy. Nevertheless the causative micro-organism, comorbidity as COPD and previous antibiotic use need to be considered when interpreting CRP measurements in follow-up correctly.

O29 Severe community-acquired pneumonia: validation of the ATS/IDSA guidelines to predict admission to the ICU

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Background: In the recent ATS/IDSA guidelines for the management of adults with CAP the decision for ICU admission is based on a predictive rule that identifies patients with severe CAP (CID 2007;44:S27–72). The purpose of this study was to validate this rule in the clinical practice.
Methods: We studied 2199 episodes of CAP (1574 men (62%), age 65±19 yrs) admitted to a university hospital from 2000 to 2006. The predictive rule consists of at least 1 of 2 major (shock or need of mechanical ventilation) or 3 of 9 minor (tachypnea, hypoxaemia, multilobar, confusion, uraemia, leucopenia, thrombocytopenia, hypothermia and hypotension) criteria. We assessed the agreement between the predictive rule and the clinical decision for ICU admission and the operative indices.
Results: 241 (11%) episodes were admitted to the ICU, while the predictive rule identified 456 (21%) episodes of severe CAP. The agreement between the predictive rule and the clinical decision for ICU admission was 1852 (84%) episodes (kappa coefficient 0.4, RR for admission to the ICU of severe CAP 12.3, 95% CI 9.1–16.6, p < 0.001). The predictive rule was highly accurate for ICU admission; ROC curves identified 1 major or 3 minor criteria as optimal predictor (AUC 0.868, sensitivity 76%, specificity 85%, positive predictive value 34%, negative predictive value of 97%, p < 0.001). The presence of minor criteria only was also accurate to predict ICU admission; ROC curves showed 2 criteria as the optimal predictor (AUC 0.793, sensitivity 78%, specificity 67%, p < 0.001). Mortality was 4% in the ward and 18% in the ICU.
Conclusion: The predictive rule of the ATS/IDSA guidelines to identify severe CAP for ICU admission is accurate in the daily clinical practice.
Funded By: CibeRes (CB06/06/0028), 2005 SGR 00822, ERS Fellowship, IDIBAPS

O30 Community-acquired pneumonia occurring in immunocompromised older patients: incidence, causative organisms, and outcome

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Objectives: We sought to determine the incidence, causative organisms and outcome of community-acquired pneumonia (CAP) occurring in immunocompromised older patients.
Methods: Prospective observational multicentre study of a cohort of patients aged 65 years or older hospitalised with CAP from May 2005 to January 2007 in 5 teaching hospitals in Spain. A comparison between cases of CAP occurring in immunocompromised patients and the remaining cases was performed.
Results: Over the study period, we documented a total of 320 cases of CAP; 115 (36%) of which occurred in immunocompromised patients. Main underlying conditions among these patients included one or more of the following: solid cancer or haematological malignancy (97 patients), treatment with corticosteroids or other immunosuppressive drugs (44), solid organ or stem cell transplant (5), and other conditions (8). An aetiologic diagnosis of CAP was more frequently established in immunocompromised patients than in the remaining cases (44% vs 32%; p = 0.03). The most common causative organism was *Streptococcus pneumoniae* in both groups (29% vs 21%; p = 0.08). No significant differences were observed between groups regarding the incidence of *Haemophilus influenzae* (2% vs 1%), *Legionella pneumophila* (3% vs 6%) and atypical agents (1% vs 2%). Gram-negative bacilli were more frequently encountered among immunocompromised patients (5% vs 0.5%; p = 0.009), particularly *Pseudomonas aeruginosa* (3% vs 0%; p = 0.04). Nocardiosis was only observed among immunocompromised patients (2 cases). The occurrence of bacteraemia was similar in both groups of patients (12% vs 9%). Most patients were given initial empirical antibiotic monotherapy (61% vs 62%). No significant differences were found concerning the percentage of patients requiring ICU admission (8%, in both groups), and length of hospital stay (12.5 vs 10. 4 days). The early (<48 hours) (3.5 vs 0.5%; p = 0.05) and overall case-fatality rates (12% vs 3%; p = 0.003) were higher in immunocompromised patients.
Conclusions: A substantial number of older patients currently hospitalised for CAP are immunocompromised. Although relatively uncommon, CAP due to Gram-negative bacilli, including *P. aeruginosa*, is more frequent among these patients. CAP occurring in immunocomprised patients causes significant case-fatality rates.

O31 *Pseudomonas aeruginosa* in sputum at admission in hospitalised patients for acute COPD exacerbation: prognostic implications

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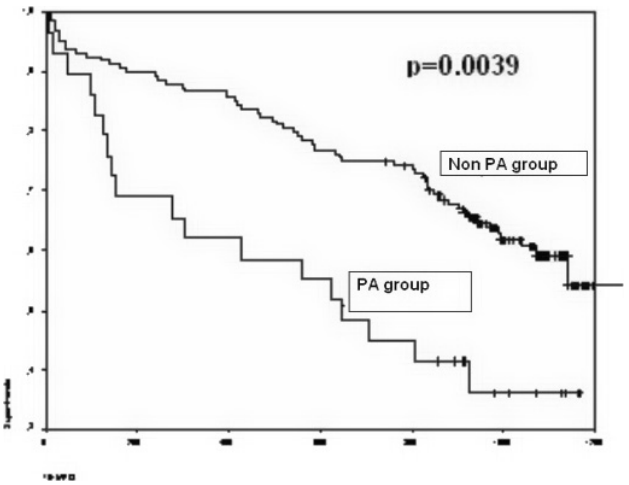
Background: *Pseudomonas aeruginosa* (PA) isolation in sputum in hospitalised patients for acute COPD exacerbation has been associated with an advanced stage of the pulmonary disease. However, information regarding the relation between PA isolation and mortality is lacking. The aim of this study was to analyse the relation between PA isolation in patients hospitalised for COPD exacerbation and long-term mortality.
Methods: We prospectively recorded clinical information and sputum cultures of all COPD patients with an acute exacerbation that were admitted to our hospital between June 2003 and September 2004. All readmissions during the following year were recorded. Mortality was assessed on January 2007 and Kaplan-Meyer analysis was made.
Results: 188 patients with a mean of age 72 years (SD 11) were included. Ninety five (50.5%) patients were classified as severe disease and 26 patients (13.8%) as very severe disease following Gold criteria. Among this cohort, a total of 469 episodes of hospital admission due to COPD exacerbation were recorded. Valid sputum was collected in 220 episodes, 49% of these had mouth flora. The prevalence of PA isolation was 23.2% of all episodes, becoming the most frequently isolated species. *H. influenzae* (11%) and *S. pneumoniae* (10%) remained a common aetiology. Overall mortality at 3 years was 61.3% in patients with PA isolation versus 37.2% in patients without PA isolation (OR 2.18; CI 95% 1.28–3.71; p = 0.004). After adjustment for age and FEV1, the relation between PA isolation and mortality remained significant.



Conclusion: PA isolation in sputum in hospitalised patients for acute COPD exacerbation is a marker of high 3-year mortality, independently of respiratory function and age.

O32 Fluoroquinolones versus β-lactam antibiotics for the treatment of acute bacterial sinusitis: a meta-analysis of randomised controlled trials

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Background: The presumed, based on laboratory data, superiority of newer fluoroquinolones for the treatment of acute bacterial sinusitis is not established on clinical grounds.
Methods: We performed a meta-analysis of randomised controlled trials (RCTs) comparing the effectiveness and safety of fluoroquinolones to β-lactams in acute bacterial sinusitis.
Results: Eight RCTs involving moxifloxacin, levofloxacin, and gatifloxacin as the newer fluoroquinolone agents, were identified. In the primary effectiveness analysis, involving the intention-to-treat population, clinical cure or improvement was not different, between fluoroquinolones and β-lactams [fixed effect model (FEM), odds ratio (OR)=1.09, 95% confidence interval (CI)=0.85–1.39, 5 RCTs, 2133 patients] at the test-of-cure assessment (the time of determination of the primary clinical effectiveness outcome, which varied between 10–31 days after the beginning of treatment). Fluoroquinolones were associated with a higher chance of clinical success in the clinically evaluable population (including patients who satisfied the eligibility criteria for clinical evaluation), (FEM, OR=1.29, 95% CI=1.03–1.63, 8 RCTs, 2797 patients), and in the analysis limited to 4 blinded RCTs. Findings were not statistically significant in the comparison of fluoroquinolones to amoxicillin/clavulanate. Bacteriologic success (eradication or presumed eradication of pre-treatment isolated pathogens) was more likely with fluoroquinolone treatment (FEM, OR=2.11, 95% CI=1.09–4.08, 3 RCTs, 506 patients). In the primary safety analysis, adverse events did not differ between compared treatments (random effects model, OR=1.17, 95% CI=0.86–1.59, 6 RCTs, 2732 patients). More adverse events were related to fluoroquinolone use in 2 blinded RCTs. The above associations were generally consistent when 3 more studies involving ciprofloxacin and sparfloxacin were included in the analysis.

Conclusion: Fluoroquinolone treatment for acute bacterial sinusitis did not differ compared to β-lactams, with regard to the primary effectiveness and safety outcomes of this meta-analysis.

O33 Should *Legionella* urinary antigen test be applied in any case of community-acquired pneumonia?

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Objectives: Despite the availability of a simple and rapid test such as *Legionella* urinary antigen (LUA), that allows the diagnosis of up 80% of infections caused by *L. pneumophila* sg 1, its systematic use in community-acquired pneumonia (CAP) is controversial. In this study we evaluated the incidence of *Legionella* infection observed during periods of routine and non routine use of the LUA test in our hospital.
Methods: Four different periods were evaluated (Table 1). During period I the LUA test was never applied. In period II a prospective study on the aetiology of CAP was carried out and the LUA test was used in almost all cases of CAP. At the end of this study the use of the LUA test decreased and was limited to patients with epidemiologically or clinically suspected LD (period III). From February 1998 to October 2006, the use of the LUA test was included in the hospital CAP guidelines and routinely performed (period IV).
Results: Table 1 shows the number of diagnoses of CAP by *Legionella* in each period related to the cases of CAP admitted to the hospital during the same period.

Table 1. Periods studied and cases of Legionnaires disease (LD)

Period	Number of CAP	Number CAP/year	LD cases	Number LD/1 000
Period I (30 months) (January 1992–June 1994)	540	216	1	1.85/1000
Period II (20 months) (July 1994–February 1996)	582	350	42	72.16/1000
Period III (23 months) (March 1996–January 1998)	832	435	6	6/1000
Period IV (105 months) (February 1998–October 2006)	4201	480	148	35.22/1000

Conclusions: As observed in this study, the incidence of CAP by *Legionella* rose notably when doctors considered this entity because of an ongoing investigation or a hospital policy that considered the use of LUA when pneumonia is diagnosed. To the contrary, when *Legionella* infection was considered a rare entity (period I) or it was only investigated when clinical data was suggestive of LD the incidence declined significantly (period III). When extrapolating these data to all the cases of CAP, this figure would increase considerably and probably thousands of patients would not be diagnosed with LD worldwide.

O34 Viral aetiology of hospitalised patients with community-acquired pneumonia in Hong Kong

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Objectives: Community-acquired pneumonia (CAP) is a leading infectious cause of death throughout the world. Pandemic avian influenza is an impending concern and prevention remains one of highest priority in Hong Kong where previous H5N1 influenza cases occurred. Diagnostic exclusion remains one of importance in the exclusion of new pathogen. A multiplex PCR that examined 17 viruses, and including influenza H typing was applied to nasopharyngeal aspirates from 857 adults hospitalised with community acquired pneumonia during 2004–5.
Methods: A prospective observational study of consecutive inpatients with CAP was performed in a university hospital in the New Territories of Hong Kong. Adult patients with CAP were recruited prospectively from Jan, 2004 to June, 2005. Nasopharyngeal aspirates (NPA) were collected and assessed by polymerase chain reaction (PCR) and viral isolation. Five groups of nested multiplex PCR assays targeting 17 respiratory viruses, namely influenza virus type A subtypes H1N1, H3N2, H5N1, influenza virus type B, Parainfluenza viruses group 1 to 4, human respiratory syncytial viruses A and B, Coronaviruses incl. SARS, OC43 and 229E, human adenovirus, Metapneumonvirus, human rhinovirus, human enterovirus, were included.
Results: 857 episodes of CAP cases were recruited. The F:M ratio of this cohort was 1:1.4. The mean age was 70.1 yrs (median 75 yrs, IR25% 62 yrs, IR75% 83 yrs, range 17–103 yrs old). Elderly subjects of $\geqslant$65 yrs of age constituted 73% of all cases. One hundred seventy six episodes (41.5%) yielded positive viral aetiology by PCR/and or by virus isolation. The viruses identified were influenza virus types A/B (25.9%), Metapneumovirus (4.5%), RSV A/B (3.1%), rhinovirus (3.1%), parainfluenza viruses group 1–4 (2.6%), Coronaviruses OC43/229 (2.1%) and Adenovirus (0.2%). Avian influenza H5N1, SARS coronavirus and human enteroviruses were not detected. The overall mortality rate was 5.0% in this cohort and there was no statistical difference to those who were PCR positive to a viral pathogen to those who were negative.
Conclusion: Influenza virus types A/B, Metapneumovirus, and RSV viruses were the common viral aetiological agents in patients hospitalised with CAP in Hong Kong.

Infections in the compromised host

O35 Transmission of viruses through the kidney graft: a study in paediatric transplant recipients

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Objectives: Viral infections, which are serious complications in kidney transplant recipients, can be transmitted from the donor through the kidney graft, by means of infected resident cells or blood cells. Aim of this study is to investigate the presence of viral genome sequences in the kidney graft from young donors and the risk of viral transmission to paediatric recipients.
Methods: In order to set-up a sensitive and non-invasive test for diagnosis of viral infections of the graft before implantation, the presence of EBV, HCMV, BKV, and parvovirus B19 DNA was investigated by TaqMan real-time PCR in graft preservation (PS) and washing solutions (WS), besides in donor kidney biopsies (B). Results were correlated with serological data, viral DNAemia, and clinical data on infections during the first 12 months post-transplantation. A total of 75 consecutive grafts from deceased donors (median age, 12 yr, range 1–58) and their 75 recipients (median age13 yr, range 2–27) were studied.
Results: Overall, considering viral DNA detection in at least one type of sample (B, PS, WS), 68% donor kidney units were positive for at least one virus; B19 was detected in 35 (47%) graft units, EBV in 21 (28%), HCMV in 9 (12%), and BKV in 3 (4%). The prevalence of EBV, HCMV, and BKV DNA was higher in PS and WS than in B, whereas B19 was consistently detected in B, PS, and WS. All EBV-seronegative recipients of an EBV-positive graft showed primary EBV infection with DNAemia within the first months post-transplantation, earlier than in recipients of an EBV-negative graft. Likewise, the only two HCMV-seronegative recipients of positive grafts showed HCMV DNAemia immediately after transplantation and a recipient of a BKV-positive graft developed acute rejection followed by severe BKV-associated nephropathy. Follow-up of B19-positive grafts gave discordant results, with 6 cases of acute prolonged infection, but also several cases of persistent B19 DNA detection in the allograft but lack of seroconversion and DNAemia.
Conclusions: Viral genomes are frequently detectable in donor renal graft units, especially in PS and WS, suggesting that they are mainly carried by circulating blood cells, but, in the case of B19, also by resident kidney cells. The presence of viral DNA in renal graft units

is a significant risk factor for symptomatic infections in seronegative recipients in the early post-transplant period.

O36 Clinical utility of Septifast PCR in neutropenic cancer patients with persistent fever despite antibacterial therapy

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Background: Blood cultures are the standard technique for the microbiological documentation of fever during neutropenia. However, no pathogen is identified in the majority of patients with persistent fever despite broad-spectrum antibacterial therapy. This results in multiple investigations and empirical modifications of antimicrobial therapy. Septifast (Roche) is a new PCR test for the detection of bacterial and fungal DNA in blood.
Objective: To assess the clinical utility of Septifast in neutropenic cancer patients with persistent fever despite antibacterial therapy.
Method: 48 consecutive adult neutropenic patients with persistent fever during $\geqslant$3 days after myeloablative chemotherapy for hematogical malignancies were studied. Febrile episodes were classified as microbiologically (MDI) or clinically documented infection (CDI) and fever of unknown origin (FUO) (ICHS, JID, 1990). Blood cultures were performed on D0 (onset of fever) and D3 (persistent fever). Blood samples for Septifast were drawn on D3. Septifast results were compared with microbiological and clinical documentation of infection.
Results: 53 episodes of persistent fever were analysed (20 MDI, 22 CDI, 11 FUO). Blood cultures detected pathogens in 11/53 (21%) episodes on D0. On D3, blood cultures and Septifast were positive in 4/53 (8%) episodes and 20/53 (38%; $p < 0.001$), respectively. Septifast detected 4 pathogens identified by blood cultures (D0 or D3). In addition, 21 bacterial pathogens (7 G+, 14 G–) were detected by SF only (86% with documented site of infection): these results are summarised in the Table. 6 fungi were detected by Septifast, none by blood cultures. 5 of these positive results were observed in possible or probable invasive mycoses (EORTC-MSG criteria, CID, 2001).

Bacteria detected by Septifast only (D3)

	Site of infection			
	Upper/lower GI tract (n = 16)	Cath/skin (n = 1)	Airways (n = 1)	No site of infection
Gram-positives (n = 7)				
S. aureus	2	1		
E. faecium	3			
S. pneumoniae	1			
Gram-negatives (n = 14)				
P. aeruginosa	6		1	2
Enterobacteria	4			
A. baumannii				1

Conclusions: Septifast PCR is clinically useful in neutropenic cancer patients with persistent fever despite antibacterial therapy.

O37 Post-operative risk factors as a cause of infection after heart transplantation in a cardiovascular clinic in Medellin, Colombia

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Objectives: We tried to identify possible risk factors to acquire infections after heart transplantation. The procedures were performed in a Cardiovascular Clinic in Medellin, Colombia, between January of 1997 and July of 2007. Our Clinic is the leader Institution in heart and lung transplantation in Colombia (South America) and this is the first time we tried to find out the scope of our transplant-related infections.
Methods: A cross sectional study with case and control analysis was made. A case was defined as an episode of postoperative infection in a heart transplant receptor, while a control was a patient who did not develop infection after a similar surgery. A review of the medical charts of all patients infected and not infected was done. Presumed preoperative (sex, age, diabetes, COPD, renal failure, previous hospitalisations), surgical (duration of ischemy and perfusion time) and postoperative (cardiac tamponade, bleeding, diabetes, ventricular failure, graft rejection, etc.) risk factors were assessed.
Results: 141 patients were included in the study. 62 of them appeared with at least one episode of infection as defined by CDC criteria. An association was found ($p < 0.05$) with the following post transplant events: acute renal failure, diabetes, delay to start of cyclosporin therapy, hours of mechanical ventilation, prolonged stay at ICU. Among surgical events only the duration of ischemy during the procedure had statistic relevance.
Conclusion: In our study the main risk factors to acquire a heart transplant related infection were postoperative. It is important to attempt to control the factors that depend on medical interventions like stay at an ICU, days of mechanical ventilation and onset of cyclosporine therapy as a measure to prevent the presence of infections.

O38 Investigation of viral infections in kidney transplant recipients unveils the pathogenic role of Parvovirus B19 in chronic allograft injury

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Objectives: The relevance of viral infection of the kidney allograft in the development of allograft lesions is still unclear, although some viruses have been implicated. Aim of this study is the investigation of both systemic and intrarenal viral infections in kidney transplant recipients and their association with the risk of acute rejection and chronic allograft injuries predictive of long-term dysfunction.
Methods: Screening for genome sequences of all human herpesviruses, polyomaviruses, and parvovirus B19 in baseline and 6, 12, 24 months follow-up allograft biopsies performed in 69 transplanted children. Correlation of virological findings with clinical data, viral DNAemia, renal function tests, and allograft histology.
Results: Overall, viral DNA was detectable in 46% baseline biopsies and in about 70% follow-up biopsies. The most frequently detected viruses were parvovirus B19 and HHV-6, already present in donor kidneys, and BKV and EBV, usually acquired during follow-up. In most cases, viral DNA persisted in the kidney allograft during follow-up. Univariate and multivariate cox-proportional hazard regression analysis demonstrated that, among viruses, only the intrarenal persistence of B19 DNA was significantly associated with the development of chronic allograft injury, whereas HCMV DNAemia, but not allograft infection, was a risk factor for acute rejection. Analysis of matched data on intrarenal viral DNA detection and DNAemia indicated that HCMV did not involve the kidney allograft in the course of systemic infection. Instead, B19 targeted the kidney, where it established prolonged infection. Both the early transcript NS1 and the late lytic transcripts VP1, VP-2, and 11kDa were expressed in biopsies from patients with primary acute infection and DNAemia, whereas only NS1 was expressed in biopsies from patients without DNAemia. These results indicate that, after acute infection, characterised by expression of all viral genes, B19 establishes persistent infection in the kidney, characterised by expression of the early gene NS1 only. Kidney injury might be linked to persistent B19 replication in the allograft, but also to expression of the pro-apoptotic NS1 protein.
Conclusions: Unprecedented so far, this study demonstrates that persistent intrarenal parvovirus B19 infection is associated with chronic allograft injury in kidney transplant recipients. Moreover, this study underlines the role of HCMV as risk factor for acute rejection.

O39 A clinical model for predicting severe medical complications in cancer patients with bloodstream infections

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Objective: Cancer patients with bloodstream infections (BSI) and concurrent medical conditions are at high risk of mortality. This study aimed to identify patients at low risk of developing severe medical complications and death.
Patients and Methods: A prediction score was derived from a prospective cohort study involving 773 consecutive episodes of BSI (2003–2005) in cancer patients hospitalised at National Cancer Institute in Brazil. Main outcome was the development of severe medical complications in the first 72 h of hospitalisation, and defined as the presence of acute respiratory failure, septic shock, persistent infection and severe bleeding. All variables reaching statistical significance ($P < 0.05$) in the univariate analysis were included in a stepwise, multivariate logistic regression analysis. A simple model score was developed, assigning one point to the presence of each statistical significant independent variable. The model was validated with 2000–2002 data from the same hospital, on 859 episodes of BSI. Discriminative capability of the score was assessed by area under receiver operating characteristic (AUROC) curves.
Results: On the derivation set, 315 patients (40.7%) had severe medical complications and 81 died (25.7%). There were no deaths in the group of 458 patients without severe medical complications. Multivariate analysis identified poor PS (<70), more than 2 coexisting illnesses, polymicrobial infection, isolation of *Pseudomonas aeruginosa* and the presence of other sites of infection as independently associated with severe medical complications. The score points varied from 0 to 5 and all necessary information was available in the first 72 h. In this group, the AUROC curve was 0.751 and 0.847 for the prediction of severe medical complications and death, respectively. The validation set comprised a group of patients with an overall mortality rate of 25.1% (216 deaths) and with an AUROC curve of 0.752 for the prediction of death. In this group, a cut-off point of 1.5 was able to obtain 86.1% of sensitivity, 51.8% of specificity, 90.6% of negative predictive value and 37.7% of positive predictive value.
Conclusion: The prognostic score is able to identify in the first 72h, cancer patients with BSI at low risk of severe medical complications. These results encourage a less aggressive management strategy for a selected subgroup of patients, who might benefit of early hospital discharge with oral therapy.

O40 Prospective study on chemotherapy-induced febrile neutropenia in patients with solid neoplasm in the era of risk stratification

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Aims: (1) Describe chemotherapy-induced febrile neutropenia (FN) in patients with solid neoplasms stratified according to the scale of the Multinational Association of Support Treatment in Cancer (MASCC). (2) Validate the MASCC scale. (3) Describe the patients with FN who may be discharged early (<3 days of hospitalisation) and (4) Evaluate whether the use of the MASCC scale is useful for reducing hospital costs and improving the quality of life of these patients.
Material and Method: We performed a prospective study on the incidence of chemotherapy-induced FN in patients with solid neoplasms from December 2005 to November 2006 and calculated the sensitivity and specificity of the MASCC scale for complications and/or death. We compared the rate of G-CSF administration, mean hospital stay, percentage of FN as the reason for admission in the Oncology Department and mean economic cost in euros per episode of FN derived from antibiotic treatment before and after (periods 1 and 2) the inclusion of the MASCC scale for patient stratification.
Results: We included 80 episodes of FN in 73 patients (7.6 episodes/1,000 chemotherapy cycles), 56.3% being males (45/80) with a mean age of 57.8 years (31–85). Lung and breast cancer were the most prevalent and the cancer was disseminated in 61.3% (49/89). 48.8% (39/80) were of low risk on the MASCC scale. The mean number of granulocytes was of 217.3 (0–500) with 30% having <100. 58.8% (47/80) had no clinical or microbiologic foci with microbiologic foci in 21 (26.3%); the urinary and respiratory tracts and abdomen being the most frequent origin of the latter. Gram-negative bacilli predominated with *Pseudomonas* and enterobacteria being of note. 16.2% (13/80) of the episodes were bacteremic. The related mortality was of 3.7%. The sensitivity and the specificity of the MASCC scale were 86.3% (19/22) and 62% (26/58), respectively. 16 (20%) patients were discharged in <3 days. The use of G-CSF, the percentage of FN among the total number of admissions to oncology and the mean cost per episode and day in euros derived from antibiotic treatment were significantly lower during period 2 than period 1.
Conclusions: The MASCC scale is a safe tool for stratifying the risk of cancer patients and chemotherapy-induced FN. However, greater specificity would allow a greater reduction in hospital costs for this oncologic emergency and improve the quality of life of these patients.

O41 A prospective comparative study of hospitalised adult and paediatric cancer patients with candidaemia: clinical characteristics, comorbid conditions and influences on mortality

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Objective: *Candida* species are among the most common causes of invasive fungal infections in cancer patients and are associated with high morbidity and mortality rates. The study aimed to compare the prognostic impact of the epidemiological characteristics, concurrent illnesses and clinical microbiological data of adults and children with candidaemia.
Patients and Methods: Between January 2001 and June 2005 a prospective cohort study of hospitalised adults (n = 99) and children (n = 130) cancer patients with candidaemia was conducted at a tertiary cancer centre in Brazil.
Results: The crude mortality was higher among adults than in children (37.4% vs. 7.7%). Univariate analysis indicated that in the adult population lymphoma, neutropenia, presence of comorbidities, a poor performance status, an absence of CVC, use of steroid, previous surgery, hypotension, hepatic, severe respiratory dysfunctions and the species *Candida glabrata*, *krusei* and *tropicalis* were risk factors significantly associated with death. Among children the predictors of mortality were acute leukaemia, neutropenia, presence of comorbidities, an absence of CVC, a poor performance status, hypotension, concomitant infected sites besides bloodstream infection, pulmonary infiltrates, severe respiratory dysfunction and the species *Candida glabrata*, *krusei* and *tropicalis*. The survival rates following fungaemia with *C. albicans* and all *Candida* non-*albicans* species were similar in both groups. In multivariate analysis, the presence of comorbidities (odds ratio [OR] 2.61; 95% confidence interval [CI] 1.46–4.67) and neutropenia (OR 10.27; 95% CI 1.80–58.49) affected independently the adults outcome. In children, only comorbidities were associated with mortality (OR 2.22; 95% CI 1.22–4.03).
Conclusion: The study showed important differences of epidemiological, clinical characteristics and risk factors for death between both groups.

O42 Increasing use of immunosuppression and biological therapies: the establishment of a designated clinic to prevent infectious complications

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Introduction: Many patients with Inflammatory bowel disease (IBD) are young and require long term treatment with immunosuppressants to control their disease. Corticosteroids, immunomodulators and more recently biological therapies increase the risk of infections. Current Irish guidelines recommend vaccination against influenza, pneumococcus,

tetanus and varicella zoster virus in patients immunosuppressed by disease or treatment (1).
Aims: To assess the vaccination history and exposure to vaccine preventable diseases in IBD patients on immunosuppression prior to the establishment of a chronic inflammatory diseases assessment clinic.
Method: We conducted a prospective survey of consecutive IBD patients on immunosuppressant medications. A thorough vaccination history was obtained and blood samples were taken to assess past exposure and vaccination status.
Results: A total of 70 IBD patients were assessed (53% Crohn's disease, 33% Ulcerative Colitis, 4% Indeterminate). History of immunosuppressant use included; Azathioprine (51%), Prednisolone (44%) and Methotrexate (2%), Anti-TNF (6%).
91% of patients did not receive their annual influenza vaccination and 80% had never been vaccinated. 97% of patients were not adequately vaccinated against pneumococcus and 86% had never been vaccinated. Varicella Zoster Virus IgG was detected in 100% of patients, 43% had a previous history of chickenpox and 11% had a previous history of shingles. 94% of patients were not vaccinated against hepatitis B, 3% had chronic hepatitis B infection and only 3% were vaccinated.
Conclusion: This study indicates that vaccination uptake amongst IBD patients on immunosuppressant regimens is extremely poor. Current Irish guidelines recommend vaccination in these patients. Ignoring these guidelines puts patients at risk of preventable diseases.
The establishment of a designated infectious disease assessment clinic will commence to address vaccination requirements and provide health-care advice pertaining to infectious complications of immunosuppressive therapies in particular biological agents. We intend to expand this service to other medical specialties utilising these immunosuppressive regimens.

O43 Virological and immunological monitoring of human cytomegalovirus infection in heart and small bowel/multivisceral transplantation

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Objectives: This study was carried out to assess the diagnostic and prognostic value of cytomegalovirus (CMV) determination in whole blood (heart and intestinal recipients) and in biopsy tissue samples (intestinal recipients) from transplant patients during virological surveillance and analyse the CMV T cell response after transplantation.
Methods: We monitored 30 heart transplant recipients (HTR) comprising three R–/D+ and 34 intestinal/multivisceral transplant (SBMTR) comprising three R–/D+. CMV pp65 antigenaemia and Real Time PCR were monitored once or twice weekly during the first month, every week for the next two months, every two weeks until the sixth month and monthly from then onwards. Additional blood and biopsy samples were taken if clinically indicated.
Immunological surveillance was done in 12 HTR and 9 SBMTR. The study of the CMV-specific T-cell response consisted of two stages: i) the collection of a blood sample per patient at the time of transplantation and ii) the collection of a sample per patient each month during the post-transplant follow-up. T lymphocyte suspensions obtained using a Ficoll gradient were processed by ELISPOT, an immunoenzymeassay based on the search for T cells with viral specific antigens of one of the protein markers of cell activation (IFN-gamma).
Around 2000 blood samples and 1200 intestinal biopsy specimens were processed for virological tests and 56 were analysed by ELISPOT.
Results: 70% (21/30) and 44% (15/34) of HTR and SBMTR developed active CMV infection, respectively. Of the 21 HTR infected four had a mild-moderate symptomatic infection (leucopenia and fever). Of the 15 SBMTR infected patients two had a severe symptomatic CMV infection (pneumonia, enteritis and rejection) which led to the patient's death and three had a mild infection (fever).
Conclusion: Quantitative determination of CMV in blood and organ biopsy by molecular tests is the elective assay for monitoring viral load, since it directly correlates with viral replication and clinical symptoms. The preliminary outcome of immunological monitoring shows i) a CMV T-cell immune response in the first month after transplantation was associated with a reduction in mean and peak CMV viral load and; ii) a good and early reconstitution or development of the CMV-specific T-cell response can shorten the duration of CMV infection and control the risk to incur repeated episodes linked to a recurrent infection.

O44 *Listeria monocytogenes* outbreak in a comprehensive cancer and transplantation centre

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Objective: *Listeria monocytogenes* infection is rare in man, but known to occur in the immunocompromised host with high fatality rates. *L. monocytogenes* is widely distributed in the environment, has the ability to multiply even at low temperatures and is a feared food-borne pathogen. We describe an outbreak in a university hospital in Oslo, Norway. The hospital is primarily a cancer and transplantation centre.
Methods: Identification of *L. monocytogenes* was based on Gram stain, beta-haemolysis, catalase, VITEK 2 identification and CAMP-test. Resistance pattern, serotyping and MLVA were performed to confirm an outbreak.
Results: From the 1st until the 30th of October, *L. monocytogenes* was isolated from blood cultures (N = 13), pus (N = 1) or faeces (N = 1) from 10 women and 5 men with a median age of 65 years (range 37–84).
Fourteen of the patients had predisposing underlying disease, 13 had received immuno-modulating therapy and 7 had received antacids. Thirteen patients had fever, 6 patients had diarrhoea, none had classical symptoms of meningitis, but 6 were somnolent and/or had a headache. Three patients (20%) died before the diagnosis was confirmed. The remaining patients received appropriate treatment after *L. monocytogenes* was isolated, and have recovered. Eight patients received empirical monotherapy with cephalosporins after onset of fever – 2 of these patients died.
All blood cultures were positive within 2 days. Further identification confirmed that the isolates from our 15 patients were of serotype 1 and had identical MLVA profiles (7–7–9–10–6) and patterns of resistance. A pasteurised camembert cheese was the source of the outbreak.
Conclusions: We report a *L. monocytogenes* outbreak with a mortality of 20% in a setting of predominately immunocompromised patients. The use of empirical cephalosporins as monotherapy in a setting with immunocompromised patients may be hazardous as *L. monocytogenes* is resistant to these antibiotics. Food-regulations in hospitals seem warranted.

Typing: from species to clones (Symposium arranged with ESGEM)

S57 MLST – 10 years of experience

M. Maiden (Oxford, UK)

MLST was proposed in 1998 as a portable approach to the identification of bacterial clones. It was based on three developments: (i) the increasing availability and decreasing cost of high-throughput sequencing; (ii) the improving understanding of bacterial population biology and its implications for epidemiology; and (iii) the increasing availability and power of the Internet as a means of data sharing. Since that time MLST has become a gold standard method for the characterisation of many bacterial pathogens and a number of non-pathogens. MLST has made it possible to compare the bacterial isolates obtained in different parts of the world and at different times simply and accurately using generic techniques but without the necessity of sharing reagents or isolates. In addition to solving many of the problems inherent in the characterisation of isolates from diverse recombinogenic bacteria, MLST has also provided data that have enabled extensive studies of bacterial population

genetics, speciation, and evolution, providing substantial added value to epidemiological studies. The MLST approach also provides indications as to the most efficient means of exploiting the next generation of DNA sequencing machines.

S58 Use of DNA microarrays in molecular typing

J. Lindsay (London, UK)

Microarrays are solid supports spotted with thousands of tiny DNA probes that can be used to quickly determine which genes are present in a bacterium or sample. They have caused a revolution in our understanding of bacterial population structures and how they vary and evolve, and have also helped to identify markers of virulence or epidemiology. Microarrays are also extremely valueable for developing and validating improved routine typing methods. In the typing laboratory where methods need to be cheap, rapid, accurate and reproduceable, microarrays technology is expensive and experimental. But this is changing. When printed with appropriate probes and validated, their uses are endless, and are only limited by our ability to interpret the data generated.

S59 MLVA and CRISPR web services for bacterial genotyping

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Multiple loci variable number of tandem repeats (VNTR) analysis (MLVA) is a typing technique based on the polymorphism of certain tandemly repeated DNA sequences. Although their biological function and evolution mechanism are not fully understood, they have diverse practical applications including strain identification in bacterial epidemiology. In a typical MLVA assay, a few to more than twenty VNTRs, distributed over the entire bacterial genome, are analysed, and a code corresponding to the number of repeats at each locus is determined. This code is easily stored into databases and can be used for strain clustering and epidemiological studies. We will show a list of the currently proposed MLVA assays and briefly describe representative examples, including open questions. We will compare MLVA to other approaches, and discuss issues related to standardisation and possibilities offered by the internet in terms of shared databases for MLVA (MLVA Web Services).
Clustered Regularly Interspaced Short Palindromic Repeats (CRISPRs) loci are part of a recently discovered mechanism used by some bacteria and most archaea as a defence against genetic elements in particular phages. These structures consist in repeated sequences (23–47bp long) called direct repeats (DR) separated by unique sequences of similar size called spacers. Their polymorphism can be used to differentiate strains inside a species. It is due to either acquisition of new motifs (a DR and a spacer) in a polarised fashion or to the interstitial deletion of one or several consecutive motifs. The spoligotyping method which has been widely applied to the genotyping of *Mycobacterium tuberculosis*, is based on the analysis of a CRISPR locus. To assess the interest of CRISPR analysis as a genotyping method it is important to first identify these structures in the genomes of interest, then extract and classify the constituting elements.
When a CRISPR has been identified in a sequenced strain (in silico analysis, Phase 1) then its polymorphism is investigated in the species by PCR amplification and sequencing (Phase 2). The last phase consists in the classification and numbering of the spacers in order to compare the strains and produce a tentative phylogenetic tree. We will present the Web services tools we have developed for these purposes, and will discuss the efficiency and informativity of the CRISPR analysis as a genotyping tool, depending on the species diversity.

Infection control guidelines

S62 Copy and paste – is the search and destroy policy suitable for Europe?

C.M.J.E. Vandenbroucke-Grauls (Amsterdam, NL)

Since the early 1980s the Netherlands has adopted a search and destroy policy with respect to meticillin-resistant *Staphylococcus aureus* (MRSA). This policy implies a national guideline for control, with search for carriers among defined groups of patients and healthcare workers, strict isolation of carriers, and treatment of carriers. The guideline is enforced by the Health Inspectorate. In addition, a national surveillance system has been implemented. From this surveillance we know that, as of 2006, MRSA was detected in approximately 2000 persons (both patients and healthcare workers; the number comprises carriers and infected persons). This very low number is confirmed by the EARSS data: the Netherlands is one of the countries in Europe with the lowest burden of MRSA.
The stringent Dutch policy has been applicable for so many years, because the number of carriers is so low. We realize that screening and strict isolation "the Dutch way" are impossible when the number of MRSA carriers becomes too high. From the Dutch experience, however, lessons can be learned and might provide a basis for "copy and paste" of part of the Dutch approach in countries with a higher burden of MRSA. Among these are the national approach and the national surveillance, which provide support to all healthcare workers involved and motivation to keep up the efforts.

S63 epic2: National evidence-based guidelines for preventing healthcare-associated infections in National Health Service facilities in England

R.J. Pratt (Brentford, UK)

All successful strategies for preventing healthcare-associated infections require a multifaceted evidence-based approach that includes providing practitioners with best evidence for clinically effective practice and then supporting them to understand and use this evidence to minimise infection risks and increase patient safety. This presentation will describe how national evidence-based guidelines from the Department of Health and the National Institute for Health and Clinical Excellence in England form the foundations for ensuring the availability of best evidence to practitioners in the National Health Service (NHS) and, how the development of an associated e-learning/blended learning programme is now supporting all practitioners to effectively use this evidence to protect patients from the risk of preventable infections during care. By incorporating these national guidelines into local policies and protocols and then into routine daily clinical practice, patient safety can be enhanced and the risk of patients acquiring an infection during episodes of healthcare in NHS facilities in England minimised.

Emerging infections in Europe

S66 Bartonellosis and other emerging infection in homeless people in Europe

P. Brouqui (Marseille, FR)

Homeless people are particularly exposed to ectoparasite. The living conditions and the crowded shelters provide ideal conditions for the spread of lice, flea, ticks and mites. Body lice have long been recognised as human parasites and although typically prevalent in rural communities in upland areas of countries close to the equator, it is now increasingly encountered in developed countries especially in homeless people or inner city economically deprived population. Fleas are widespread but are not adapted to a specific host and may occasionally bite humans. Most common fleas that parasite humans are the cat, the rat and the human

fleas, *Ctenocephalides felis*, *Xenopsylla cheopis* and *Pulex irritans*, respectively. Ticks belonging to the family Ixodidae, in particular the genera *Dermacentor*, *Rhipicephalus* and *Ixodes*, are frequent parasites in humans. *Sarcoptes scabiei* var. *hominis* is a mite (Arachnida class) responsible for scabies. It is an obligate parasite of human skin. The hematophagic biting mite, *Liponyssoides sanguineus*, is a mite of the rat, mouse and other domestic rodents but can also bite humans. The threat posed by the ectoparasite in homeless is not the ectoparasite themselves but the associated infectious diseases that they may transmit to humans. Except for scabies all these ectoparasites are potential vector for infectious agents.

Three louse borne diseases are known at this time. Trench fever caused by *Bartonella quintana*, epidemic typhus caused by *Rickettsia prowazekii* and relapsing fever caused by the spirochete *Borrelia recurrentis*. Fleas transmit plague (*Xenopsylla cheopis*, *Pulex irritans*), murine typhus (*Xenopsylla cheopis*), flea-borne spotted rickettsiosis due to the recently described species *Rickettsia felis* (*Ctenocephalides felis*), and occasionally cat-scratch disease due to *Bartonella henselae* (*Ctenocephalides felis*). The role of fleas as potential vector of *B. quintana* has recently been suggested. Among the hematophagic biting mites, *Liponyssoides sanguineus*, is responsible for the transmission of *Rickettsia akari*, the aetiologic agent of rickettsialpox. Virtually, no data are available on tick-borne disease in this population.

This review will deal with epidemiology, diagnosis, prevention and treatment of these ectoparasite and the infectious diseases they transmit to the homeless people.

S67 Hantavirus infections

T. Avsic-Zupanc (Ljubljana, SI)

Hantaviruses belong to the emerging pathogens having gained more and more attention in the last decades. These viruses are members of the family Bunyaviridae and are grouped into a separate genus known as Hantavirus. Unlike most other Bunyaviridae, hantaviruses are not arthropod-borne (arboviruses), but are ROdent-BOrne, roboviruses. Each hantavirus is primarily carried by a distinct rodent/insectivore species although a few host switches seem to have occurred during the tens of millions of years of their co-evolution with their carrier animals. Hantaviruses are maintained by cyclical transmission between persistently infected rodents, with incidental infection of humans. Transmission occurs primarily by inhalation of aerosols from infected rodents' urine, faeces or saliva. Hantaviruses are found worldwide and are known to cause two serious and often fatal human diseases: haemorrhagic fever with renal syndrome (HFRS) and hantavirus cardiopulmonary syndrome (HCPS). More than 20 hantaviruses have been identified, approximately half of which are known to cause HFRS or HPS. The serotypes Hantaan (HTN), Seoul (SEO), Puumala (PUU), and Dobrava (DOB) virus predominantly cause haemorrhagic fever with renal syndrome (HFRS), a disease characterised by renal failure, haemorrhages, and shock. Whereas hantavirus cardiopulmonary syndrome (HCPS) is a severe cardiopulmonary illness most often caused by the Sin Nombre virus. The illness begins as a nonspecific febrile prodrome, and then patients quickly develop noncardiogenic pulmonary edema, respiratory failure, and shock. The overall case fatality rate of HCPS is approximately 40 percent. The pathogenesis of HFRS is poorly understood. However, it is known that β3 integrins can mediate the entry of pathogenic hantaviruses and that hantaviruses can regulate apoptosis. Also there is evidence and that increased capillary permeability is an essential component in the pathogenesis of both HFRS and HCPS, although different target tissues, kidneys and lungs are affected in the two diseases. HFRS patients show locally increased levels of TNF-α in the plasma and kidneys and high levels of urinary secretion of the proinflammatory cytokine IL-6. The diagnosis of acute hantavirus infection is primarily based on serology, since viral RNA cannot be regularly detected in the blood or urine of patients. Both immunofluorescence tests and enzyme immunoassays are widely used for detection of specific IgM or low-avidity IgG antibodies, characteristic of acute infection. Vaccines against hantavirus infections have been used for years in China and Korea, but not in Europe or the Americas. No specific therapy is used in Europe, although both Ribavirin and interferon-α have been successfully used in trials in China. The high rate of mortality could be reduced if effective therapeutics could be discovered for treatment of this illness.

Sepsis 2008 (Symposium arranged with the International Sepsis Forum)

S69 TREM-1 improves the inflammatory response and outcome to *Streptococcus pneumoniae* infection by reducing levels of negative regulators in the lung

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Background: Triggering receptor expressed on myeloid cells (TREM-1) is a cell surface receptor present on both monocytes/macrophages and neutrophils that has been shown to amplify cytokine and chemokine production in response to bacterial Toll like receptor (TLR) ligands. Blocking studies revealed TREM-1 as a valuable target to prevent overwhelming inflammation during sepsis. At the same time, TREM-1 is highly expressed within the pulmonary compartment with soluble TREM-1 being a valuable marker indicating the presence of pneumonia in humans. The biological role of TREM-1 during community acquired pneumonia, such as pneumococcal pneumonia is not known. The aim of the present study was to determine the function of TREM-1 in the inflammatory response to *Streptococcus pneumoniae* infection in vivo, the mechanism where by this occurred, and the outcome of this on infection.

Methods: C57BL/6 mice were intranasally infected with *S. pneumoniae* followed by i.p. treatment with PBS, isotype Ab or agonistic TREM-1 mAb. Bacterial counts, lung histology, neutrophil influx, chemokine/cytokine responses and signaling mediators as well as survival were evaluated in vivo in a time dependent manner. In vitro, immortalised lung alveolar macrophages (MHS) and respiratory epithelial cells were utilised to study the response to *S. pneumoniae* treatment.

Results: Mice pre-treated with agonistic TREM-1 displayed a significantly amplified cytokine and chemokine responses (TNF, MIP-2 and IL-6) as well as neutrophil influx to the lungs 6h after induction of pneumonia. This enhanced early inflammation was associated with lower numbers of bacteria in the lungs and reduced pulmonary inflammation at 48hr, resulting in improved survival. In vitro studies corroborated these results at the early time point. A reduction of negative regulators of TLR signaling in lungs was observed in mice pre-treated with TREM-1 in vivo.

Conclusion: TREM-1 boosts the early inflammatory response during *S. pneumoniae* infection in vivo through lowering levels of negative regulators in the lung, which results in accelerated bacterial clearance and ultimately improved survival.

S72 Tropical sepsis

T. van der Poll (Amsterdam, NL)

Sepsis is as a clinical syndrome that results from a systemic response of the host to an infection. The outcome of sepsis is poor, and even in the developed world mortality rates remain as high as 30–40%. In the western world, sepsis most frequently is caused by bacteria. The most commonly isolated pathogens in Gram-positive sepsis are *Staphylococcus aureus* and *Streptococcus pneumoniae*, the most common Gram-negative causative bacteria are *Escherichia coli*, *Klebsiella* sp. and *Pseudomonas aeruginosa*. Although these bacteria also are isolated from patients with sepsis in tropical areas, several other causes must be considered. Potentially lethal causes of sepsis in the tropics include malaria, viral haemorrhagic fevers, leptospirosis, typhoid fever and melioidosis. Many aspects of the host response to these tropical

infections resemble the host response to sepsis as seen in the western world, including activation of inflammatory and procoagulant pathways. Studies comparing the host response to "western sepsis" with that to "tropical sepsis" will be discussed.

S73 Innate immunomodulating therapies

T. Calandra (Lausanne, CH)

The innate immune system assumes an essential role in the natural host defences against microbes. Sensing of microbial pathogens, either in tissue in contact with the host's environment or in the systemic circulation after invasion of the bloodstream, is carried out by macrophages, dendritic cells, natural killer cells, granulocytes and monocytes acting as sentinels of the innate immune system. Recognition of invasive pathogens by immune cells relies on their capacity to detect microbial molecular motifs, such as endotoxin, peptidoglycan subcomponents, lipopeptides, glucans, mannans, flagellin and nucleic acids via microbial-recognition receptors. Microbe-associated molecular motifs bind to a family of microbial recognition molecules expressed by immune and non-immune cells, including CD14, Toll-like receptors, peptidoglycan-recognition proteins, nucleotide-binding oligomerisation domain-like receptors, helicase-domain-containing like receptors, such as retinoic acid-inducible gene I (RIG-I) or melanoma-differentiation-associated gene 5 (Mda5), C-type lectin receptors (such as dectin-1) and the triggering receptors expressed on myeloid cells (TREM) receptor family. Ligand-activated receptors turn on signal transduction pathways and the transcription of immune genes resulting in the expression of co-stimulatory molecules at the cell surface and in the release of immunoregulatory effector molecules in the extracellular compartment. Until recently increased knowledge of the pathophysiological basis of sepsis did not translate into clinical benefit. Lately, however, several treatment approaches have for the first time yielded encouraging, albeit still arguable results (i.e. early-goal directed therapy, activated protein C, hydrocortisone therapy, and intensive insulin therapy). New immunomodulating therapies targeting components of the innate immune system, such as TLR4, are currently underway.

Clinical trials of antibiotics

O74 Efficacy and safety of novel amoxicillin/clavulanic acid formulation versus originator film-coated tablets in adult patients with lower respiratory tract infections

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Objectives: To compare tolerability and clinical efficacy of novel formulation of amoxicillin/clavulanic acid (ACA) – dispersible tablets – with originator film-coated tablets in hospitalised adults with bacterial lower respiratory tract infections (LRTIs).
Methods: In this comparative, two-centre, open-label study adult patients hospitalised for non-severe community-acquired pneumonia (CAP) or bacterial exacerbation of chronic obstructive pulmonary disease (COPD) were randomly assigned (1:1) to receive 5–10-days course of treatment with either ACA dispersible tablets, 500/125 mg, t.i.d. (group A) or ACA film-coated tablets, 500/125 mg, t.i.d. (group B). Clinical outcomes were assessed at test of cure and follow-up visits (5–10 days and 25–30 days after the end of treatment, respectively), tolerability at day 3 of treatment, end of treatment (5–10 days), test of cure and follow-up visits on the basis of reported adverse events (AE) and laboratory abnormalities. Because all treatment regimens were routine and no other than standard procedures implemented, there was no necessity for approval of the study by the Ethics Committee.
Results: Per-protocol (PP) population comprised 200 patients. CAP was diagnosed in 81 and 70 of patients in group A and B, respectively. There were no significant differences in demographic data and clinical presentation at baseline between groups. Clinical success rate at follow-up visit was 95/100 (95%) and 94/100 (94%) for group A and group B. No difference in symptoms resolution of LRTIs was observed. AE were recorded in 15% and 31% (P = 0.01) of patients in group A and group B respectively and comprised predominantly gastrointestinal complaints (diarrhoea, nausea, meteorism, abdominal pain). Tendency towards less percentage of diarrhoea in group A patients on comparison with those in group B was observed (6% vs. 17%, P = 0.027). There were no clinically significant laboratory abnormalities revealed. AEs resulting to withdrawal were registered in 2% and 4% in group A and B, respectively (P > 0.05).
Conclusion: A novel formulation of ACA dispersible tablets given at the dosage of 500/125 mg t.i.d. for 5–10 days in hospitalised adults with bacterial LRTIs was as effective as originator film-coated tablets. Tendency towards lesser gastrointestinal AE (mainly diarrhoea) was observed in group A, however this required to be proven in large-scale clinical trials.

O75 Telavancin for hospital-acquired pneumonia, including ventilator-associated pneumonia: the ATTAIN studies

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Objectives: Telavancin (TLV), a rapidly bactericidal, investigational, lipoglycopeptide with a multifunctional mechanism of action, is active against a broad range of clinically relevant Gram-positive bacteria, including meticillin-resistant *Staphylococcus aureus* (MRSA). MRSA is an important pathogen causing hospital-acquired pneumonia (HAP) worldwide. The ATTAIN studies were designed to compare the efficacy and safety of TLV with vancomycin (VAN) in patients with HAP, including patients with ventilator-associated pneumonia (VAP).
Methods: ATTAIN 1 & 2 were identical, multinational, multicentre, randomised, double-blind, Phase 3 studies. Patients ⩾18 years with HAP caused by suspected or confirmed Gram-positive pathogens were randomised (1:1) to TLV 10 mg/kg IV every 24h or VAN 1 g IV every 12h (dosages adjusted per site-specific guidelines) for 7–21 days. The primary efficacy analysis in each study was non-inferiority of TLV compared with VAN in clinical cure rates in clinically evaluable (CE) patients. In addition, pooled analyses of the two studies were specified prospectively.
Results: A total of 1503 patients were randomised and treated (TLV=749, VAN=754); 658 of these patients were CE. In each study, the demographic and baseline characteristics were similar in the two treatment groups. Non-inferiority was achieved for both the All-treated (AT) and CE populations in each study as well as in the pooled analysis. The pooled clinical cure rates are displayed in the table below. Further, the clinical cure rate in CE patients with VAP was numerically higher in the TLV group (Table).

	Clinical Cure Rate		Difference, TLV – VAN
	TLV	VAN	(95% CI)
Hospital-acquired Pneumonia			
AT (n = 1503)	58.9%	59.5%	–0.7% (–5.6%, 4.3%)
CE (n = 658)	82.7%	80.9%	1.8% (–4.1%, 7.7%)
Ventilator-associated Pneumonia			
AT (n = 427)	49.1%	53.1%	–4.0% (–13.5%, 5.5%)
CE (n = 139)	80.3%	67.6%	13.2% (–1.8%, 26.8%)

AT = All-treated; CE = Clinically-evaluable; TLV = telavancin; VAN = vancomycin; CI = confidence interval.

The overall incidence of adverse events (AEs) in both groups was comparable (82% for TLV, 81% for VAN). The most common AEs, which occurred at similar rates in both groups, were diarrhoea, constipation, and anaemia.
Conclusion: To our knowledge, ATTAIN was the largest programme conducted in patients with HAP due to Gram-positive bacteria. Data

from the ATTAIN studies support the once-daily use of TLV for the treatment of Gram-positive HAP, including patients with VAP.

O76 Genotyping of Gram-negative uropathogens isolated pre and post-treatment from subjects in a doripenem clinical trial for complicated urinary tract infections

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Objectives: Doripenem (DOR) a parenteral carbapenem was approved in the US for treatment of complicated intraabdominal and complicated urinary tract infections including pyelonephritis (cUTI). In a cUTI trial comparing DOR and IV levofloxacin (LVX), the microbiologic cure rate at test of cure (TOC, day 6–11 post therapy) for the microbiologically evaluable population was 82.1% (230 / 280) in the DOR group and 83.4% (221 / 265) in the LVX group. Gram-negative pathogens isolated from the same subject pre- and post-treatment (microbiological failures) were genotyped to determine if the pre-treatment isolate persisted or if a new infection occurred.
Methods: Baseline *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Pseudomonas aeruginosa* isolates were compared to TOC or late follow-up (LFU, day 28–35 post therapy) isolates by pulsed-field gel electrophoresis (PFGE). TOC and LFU isolates with $\leqslant$6 DNA band differences to the matched baseline isolate were classified as persistent infections; isolates with >6 differences were considered to be a different strain (new infection). Isolates producing smears by PFGE were non-typeable (NT).
Results: For *P. mirabilis*, *P. aeruginosa*, and *K. pneumoniae* microbiologic failures in the DOR and LVX treatment groups were usually due to persistence of the baseline pathogen. For *E. coli*, microbiologic failures due to a new infection were higher in the DOR treatment group (44%) then the LVX group (29%) for TOC isolates. Prevalence of new *E. coli* infections increased at LFU to about two-thirds for both treatment groups. When a LVX-resistant *E. coli* was present at baseline, microbiologic failures due to persistence was 78% and 57% in the LVX and DOR treatment groups, respectively.

		No. Microbiologic Failures (%)					
		DOR Treatment Group			LVX Treatment Group		
Baseline Pathogen	Isolate	N	Persist	New Infect	N	Persist	New Infect
E. coli	TOC	23*	9 (39)	10 (44)	14*	6 (43)	4 (29)
	LFU	31*	8 (26)	19 (61)	26*	6 (23)	18 (69)
P. mirabilis	TOC	6	6 (100)	–	2	2 (100)	–
	LFU	4	4 (100)	–	2	1 (50)	1 (50)
P. aeruginosa	TOC	5	4 (80)	1 (20)	2	2 (100)	–
	LFU	2	1 (50)	1 (50)	1	1 (100)	–
K. pneumoniae	TOC	1	1 (100)	–	2	2 (100)	–
	LFU	0	–	–	2*	1 (50)	–

1 *Some isolates were NT and not analysed.

Conclusion: In a cUTI trial, microbiologic failures in the DOR and LVX treatment groups for *P. mirabilis*, *P. aeruginosa*, and *K. pneumoniae* were usually (>80%) due to persistence of the baseline isolate. *E. coli* failures were due to replacement of the baseline isolate (new infection) in >50% of the cases.

O77 Justification for non-inferiority margin in community-acquired pneumonia

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Objective: To determine the NI margin for clinical trials in mild-moderate community-acquired pneumonia (CAP) when placebo-controlled trials of adequate design are unavailable.

Methods: ICH guidelines identify 5 types of control groups: placebo, no treatment, different dose, different active treatment, external or historical. Efficacy is established when new drug demonstrates clinically meaningful superiority over a control regimen. An active control trial can demonstrate efficacy when a new treatment is similar (non-inferior) to a known effective treatment when the new treatment is not less effective than control by a predetermined margin (M2), identifying the lower boundary of a 95% CI. M2 can only be determined after the benefit of the active control over placebo (M1) has been demonstrated in studies showing superiority over placebo or a less effective active control. No placebo-controlled trials have been conducted in CAP. Historical evidence of antibiotic efficacy in CAP has been derived from estimates of mortality. Thus, an estimate of clinical response for M1 can only be derived indirectly. Some assumptions are made:1) efficacy can only be estimated for an antibiotic with in vitro activity; 2) efficacy in CAP is derived from patients with bacterial infection; 3) clinical response results in improvement within 72 hrs; 4) M2 should preserve 50% of M1. The natural history of spontaneously resolving (non-fatal) pneumococcal pneumonia in the pre-antibiotic era was estimated. Publications and Summary Basis of Approvals for antibiotics from 1996–2006 were reviewed for microbial aetiology and treatment response.
Results: For contemporary CAP, 30% are caused by typical bacteria, 20% atypical pathogens and 50% viral or other causes. Clinical success is 87% in mild-moderate CAP. Clinical improvement does not occur before 72 hrs in untreated non-fatal pneumococcal pneumonia. Thus, for an antibiotic with activity against only typical bacteria, M1 equals 0.30(% CAP typical pathogens) $\times$ 0.87 (clinical response). Using these data and assumptions a M2 margin of 13% would provide evidence of treatment efficacy in mild-moderate CAP when an appropriate control drug is used.
Conclusion: The M2 margin for CAP can be derived from knowledge of spontaneous recovery in the pre-antibiotic era combined with a careful analysis of contemporary aetiologies and treatment response of CAP.

O78 Comparison of antibiotics with placebo for the treatment of presumed acute bacterial sinusitis: a meta-analysis of randomised controlled trials

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Objectives: There is controversy regarding the benefit of antibiotics for the treatment of patients with presumed acute bacterial sinusitis.
Methods: We performed a meta-analysis of randomised, placebo-controlled trials (RCTs) evaluating the effectiveness and safety of antibiotics for this indication.
Results: Sixteen RCTs were included, (among which 3 performed exclusively in children), involving 21 antibiotic arms (8 amoxicillin, 4 penicillin V, 3 amoxicillin/clavulanate, and 6 other antibiotics). Compared to placebo, use of antibiotics is associated with higher proportion of cure or improvement [2785 patients, random effects model (REM) odds ratio (OR)=1.60, 95% confidence interval (CI)=1.31–1.96, data from 16 RCTs], speedier resolution of symptoms, and a trend towards fewer complications (1840 patients, REM, OR=0.36, 95% CI=0.10–1.30, 9 RCTs). However, it is also associated with higher proportion of adverse events (2228 patients, REM, OR=1.94, 95% CI=1.29–2.92, 13 RCTs) although it is not associated with significantly more withdrawals due to adverse events (2705 patients, REM, OR=1.42, 95% CI=0.74–2.72, 15 RCTs).
Conclusion: Antibiotic treatment of presumed acute bacterial sinusitis compared to placebo is associated with a rounded 10% added benefit in clinical outcomes, at a cost of an approximately equal increase in the rate of adverse events. Considering that observed adverse events are generally not serious, and that not using antibiotics may carry an appreciable risk of disease complications, we believe that clinical benefits obtained from antibiotic use, justify the risks associated with such treatment.

O79 Ertapenem for complicated intra-abdominal infections: a meta-analysis of randomised controlled trials

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Objective: Ertapenem has been FDA approved, among other indications, for the treatment of complicated intra-abdominal infections (cIAIs). Additional studies have been performed on its clinical use for this important type of infections. We evaluated the effectiveness and safety of ertapenem treatment for cIAIs.
Methods: We performed a meta-analysis of randomised controlled trials (RCTs) that compared treatment with ertapenem versus other antimicrobial regimens, in patients of all ages, with cIAIs. The primary outcomes evaluated were clinical success (cure or improvement) in the modified intention-to-treat population, and clinical adverse events. We searched in PubMed, the Cochrane Central Register of Controlled Trials, and Scopus database for relevant studies.
Results: Six RCTs (3 with a double-blind design; one performed in children) that compared ertapenem treatment against piperacillin/tazobactam, ceftriaxone plus metronidazole, and ticarcillin/clavulanic acid (in 3, 2 and 1 RCTs, respectively) were included. No difference was found between patients treated with ertapenem versus comparators, regarding clinical success (6 RCTs, 2067 patients, fixed effect model (FEM), odds ratio =1.13, 95% confidence interval=0.90–1.41); clinical adverse events (5 RCTs, 1635 patients, FEM, OR=0.81, 95% CI 0.59–1.13); microbiological success; mortality; and withdrawals due to adverse events. Ertapenem was associated with more laboratory adverse events (5 RCTs, 1633 patients, FEM, OR=1.63, 95% CI 1.09–2.44), but none was reported as serious.
Conclusion: This meta-analysis provides additional evidence that ertapenem can be used as effectively and safely, as other recommended antimicrobial regimens, for the treatment of complicated intra-abdominal infections.

O80 Efficacy and safety of linezolid versus vancomycin for the treatment of complicated skin and soft-tissue infections proven to be due to meticillin-resistant *Staphylococcus aureus*

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Objectives: Linezolid (LZD) is an oxazolidinone antibiotic used to treat infections caused by Gram-positive pathogens, including MRSA. A randomised, open-label, controlled, multicentre phase 4 study was conducted comparing LZD to vancomycin (VAN) in the treatment of complicated skin and soft-tissue infections (cSSTI) proven due to MRSA.
Methods: Subjects ⩾18 years with defined, proven MRSA cSSTI were randomised to receive either LZD (600 mg IV/PO q12h) or VAN (15 mg/kg IV q12h, adjusted for CLCR) for 7–14 d. Aztreonam and metronidazole coverage were permitted. The primary efficacy endpoint was clinical outcome at end of study (EOS; 7–10 d after the last dose) in subjects with MRSA who met inclusion/exclusion criteria (per protocol [PP] population). Secondary endpoints included clinical outcome at end of therapy (EOT) and microbiological outcome at EOS and EOT as well as analyses based on the modified intent-to-treat (mITT) population. Noninferiority was assessed by a 2-sided 95% confidence interval (CI) for the difference in the success rate at EOS (delta = 10%).
Results: Subjects included 537 randomised to LZD and 515 to VAN, for a total of 1052. There were 481 with abscess (243 LZD, 238 VAN), 234 with surgical wound infection (111 LZD, 123 VAN), 106 with diabetic ulcer (61 LZD, 45 VAN), and 231 with other cSSTI (122 LZD, 109 VAN). 452 subjects were in the PP population (235 LZD, 217 VAN) and 654 in the mITT group (329 LZD, 325 VAN). Success at EOS was comparable between the 2 groups (193 LZD [83.2%] 171 VAN [79.5%]; P = 0.321; 95% CI [–0.036, 0.109]) demonstrating that LZD was noninferior to VAN (Table). For microbiological success, treatment groups were comparable at EOS. At EOT, there was a statistically significant difference between LZD and VAN (211 LZD [85.8%]; 158 VAN [69.3%]; P = 0.000; 95% CI [0.091, 0.239]). Overall, in the ITT population, the mean treatment durations were comparable for the groups, with 8.8 d for LZD subjects and 7.6 d for VAN subjects. PP mean IV treatment lasted 5.4 d (LZD) and 10.3 d (VAN). The 3 most common drug-related adverse events were gastrointestinal (65 LZD [12.1%], 23 VAN [4.5%]), infection/infestation (26 LZD [4.8%], 10 VAN [1.9%]), and skin/subcutaneous tissue disorder (10 LZD [1.9], 35 VAN [6.8%]). There were 18 deaths: 11 LZD (2.0%), 7 VAN (1.4%), and 1 VAN considered study drug-related.

Table. Clinical and microbiological response in the per protocol (PP) population

	Linezolid N (%)	Vancomycin N (%)	*P*-value	95% CI
Clinical outcome EOT				
Subjects in analysis	246 (100)	227 (100)		
Success	224 (91.1)	199 (87.7)	0.231	(–0.022, 0.090)
Failure	22 (8.4)	28 (12.3)		
Clinical outcome EOS				
Subjects in analysis	232 (100)	215 (100)		
Success	193 (83.2)	171 (79.5)	0.321	(–0.036, 0.109)
Failure	39 (16.8)	44 (20.5)		
Microbiology outcome EOT				
Subjects in analysis	246 (100)	228 (100)		
Success	211 (85.8)	158 (69.3)	0.000	(0.091, 0.239)
Failure	35 (14.2)	70 (30.7)		
Microbiology outcome EOS				
Subjects in analysis	234 (100)	215 (100)		
Success	174 (74.4)	147 (68.4)	0.160	(–0.024, 0.143)
Failure	60 (25.6)	68 (31.6)		

Conclusion: LZD was at least as effective as VAN for the treatment of proven MRSA cSSTI, and was well-tolerated.

O81 Fluoroquinolones versus β-lactam-based regimens for the treatment of osteomyelitis: a meta-analysis of randomised controlled trials

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Objective: To compare fluoroquinolones to β-lactams for the treatment of osteomyelitis. Treatment of osteomyelitis remains a real challenge in medicine necessitating the use of broad-spectrum antibiotics, because of the variety of the pathogens causing the infection and the fact that the infected bone may become necrotic and avascular preventing systemic antibiotics from adequately penetrating to the infection site.
Methods: A literature search was performed by two reviewers independently (PubMed database and the Cochrane Central Register of Controlled Trials).
Results: We identified 7 studies eligible for inclusion in our meta-analysis; ciprofloxacin, ofloxacin and pefloxacin were used in 3, 3, and 1 study, respectively, while various β-lactams (mainly in the intravenous form) were used as comparators. There was no difference in treatment success for osteomyelitis between fluoroquinolones and β-lactams [194 patients, fixed effect model (FEM), odds ratio (OR)=0.99, 95% confidence interval (CI) 0.51–1.91], bacteriological success (201 isolates, FEM, OR=0.88, 95% CI 0.45–1.70), superinfections (173 patients, FEM, OR=1.75, 95% CI 0.63–4.90), relapses (153 patients, FEM, OR=1.23, 95% CI 0.46–3.31), or adverse events (170 patients, FEM, OR=0.47, 95% CI 0.21–1.06).
Conclusion: Fluoroquinolones are as effective as β-lactams for the treatment of osteomyelitis and can be considered as a useful alternative in the physician's armamentarium. The value of fluoroquinolones for the treatment of osteomyelitis lies in the fact that they can be administered in an outpatient setting. However, they should be used with caution, so as to preserve their activity against increasingly resistant bacteria.

O82 Effectiveness and safety of short versus long duration of antibiotic therapy for acute bacterial sinusitis: a meta-analysis of randomised trials

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Objectives: Traditionally, 10 to 14-day duration antibiotic therapy is recommended for acute bacterial sinusitis (ABS). Shorter duration regimens, if proven equally effective, may be associated with better patient compliance and less toxicity, antimicrobial resistance development, and economic burden. To evaluate the effectiveness and safety of short-course antibiotic treatment for ABS compared to treatment of longer duration, by performing a meta-analysis of randomised controlled trials (RCTs).
Methods: Relevant studies were retrieved by searching PubMed and the Cochrane Central Register of Controlled Trials. We included RCTs that compared short-course (up to 7 days) versus long-course therapy (at least 2 days longer than short-course), with the same antimicrobial agent, in the same daily dosage, for patients with ABS.
Results: Twelve RCTs (10 with double-blind design) involving adult patients with radiologically confirmed ABS, were included. There was no difference in the comparison of short-course treatment (3–7 days) to long-course treatment (6–10 days) regarding clinical success (cure or improvement) [12 RCTs, 4430 patients, fixed effect model (FEM), odds ratio (OR)=0.95, 95% confidence interval (CI)=0.81–1.12]; bacteriologic efficacy (eradication or presumed eradication); relapses; adverse events (10 RCTs, 4172 patients, random effects model, OR=0.88, 95% CI=0.71–1.09); or withdrawals due to adverse events. Findings were not significant in the subset analysis limited to β-lactam agents (6 RCTs). In the sensitivity analysis comparing 5 to 10-day regimens (7 RCTs), clinical success was not different but adverse events were fewer with short-course treatment (5 RCTs, 2151 patients, FEM, OR=0.79, 95% CI=0.63–0.98).
Conclusion: In patients with ABS, short-course antibiotic treatment proved equally effective to a longer course with the same antibiotic. An initial strategy of a shorter than traditionally recommended duration of treatment may be favoured, particularly for patients without complicating factors, considering the advantages of fewer adverse events and potentially lower rates of resistance development.

O83 Efficacy and tolerability of moxifloxacin versus clindamycin in the treatment of odontogenic abscesses and inflammatory infiltrates (MOCLI Study)

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Objectives: Odontogenic infections of bacterial origin penetrate primarily into the soft and bony oromaxillofacial tissues to produce submucosal infiltrates and abscesses there. These infections are typically mixed infections, and anaerobic bacteria are thought to have a central aetiologic role. Often taking a mild course, these infections may, depending on a patient's immunocompetence and the site of the inflammatory process, also produce life-threatening complications. The availability of effective and efficient antibiotics for the treatment of odontogenic infections is therefore essential. Preclinical studies have shown good oromaxillary tissue penetration for moxifloxacin (MXF), and early clinical reports suggest that MXF may be effective in the treatment of odontogenic infections. The objective of our study was to systematically evaluate the efficacy of MXF in a larger comparative clinical trial.
Methods: A prospective, randomised, double-blind, multicentre, phase II trial compared the efficacy and tolerability of MXF with that of clindamycin (CLI) in the treatment of inflammatory infiltrates and odontogenic abscesses. Patients received either MXF 400 mg once daily or CLI 300 mg four times daily for 5 days. The primary efficacy endpoint was the percent reduction in patients' perceived pain on a visual analog scale (VAS) on day 2–3 from day 1.
Results: The primary endpoint analysis included 21 MXF-treated patients and 19 CLI-treated patients with inflammatory infiltrates and 15 MXF and 16 CLI patients with odontogenic abscesses (intention-to-treat population). Mean pain reduction among patients with inflammatory infiltrates was 61.0% (SD, 46.9%) with MXF versus 23.4% (SD, 32.1%) with CLI ($p = 0.006$). Mean pain reduction among patients with odontogenic abscesses was 55.8% (SD, 24.8%) with MXF versus 42.7% (SD, 48.5%) with CLI ($p = 0.358$).
Conclusion: Moxifloxacin was significantly more effective at reducing pain in patients with inflammatory infiltrates on day 2–3, compared with clindamycin. No significant differences between the treatment groups were found among patients with odontogenic abscesses.

Molecular and clinical epidemiology of emerging MDR Gram-negatives

O84 Association of plasmid-mediated quinolone resistance genes, DHA-1 β-lactamase, and extended-spectrum β-lactamase SHV-12 in *Enterobacter cloacae* isolated in Moroccco

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Objective: To determine the genetic environment of qnr genes among Enterobacteriaceae isolated at the university Hospital Ibn Rochd Casablanca, Morocco.
Methods: Third generation cephalosporin resistant unrelated *E. cloacae* strains ($n = 8$) have been isolated at the Ibn Rochd Hospital (Casablanca) during a survey conducted between October 2006 and March 2007. Antibiotic susceptibility (disc diffusion method and MIC) and screening for Extended Spectrum Beta-Lactamase (ESBL) were performed according to the French Society for Microbiology guidelines. Characterisation of quinolone resistance genes (qnrA, qnrB, qnrS, aac(6′)-Ib-cr) was investigated by PCR. Identification of ESBL (TEM, SHV, CTX-M1, CTX-M2, CTX-M8 and CTX-M9 groups) was performed by PCR. Identification of plasmid AmpC β-lactamases was performed by multiplex PCR. All PCR products were sequenced on both strands. Conjugation experiments were performed using azide-resistant *Escherichia coli* K12J5 as a recipient strain.
Results: Among the 8 ESBL-producing strains, 4 were resistant to all third generation cephalosporins tested except cefepime (MIC 1–4 mg/l). qnrB4, DHA-1, TEM-1, SHV-12, aac(6′)-Ib-cr (except 1 strain with aac(6′)-Ib) were identified in all strains. Transfer of plasmid DNA was successfully obtained for 3 strains. Transconjugants were confirmed to be *E. coli* using biochemical tests. All transconjugants expressed resistance to third generation cephalosporin except cefepime. With specific primers, qnrB4, DHA-1, aac(6′)-Ib-cr were proved to be co-transferred for the 3 transconjugants. SHV-12 was co-tranferred in two transconjugants.
Conclusion: This study demonstrated the association between qnrB4, aac(6′)-Ib-cr and DHA-1 determinants in *E. cloacae* strains isolated in Casablanca. Moreover, this is the first report of *E. cloacae* isolates containing transmissible plasmid-mediated DHA-1.

O85 The high prevalence of plasmid-mediated quinolone resistance gene (qnr) and aac(6′)-Ib-cr in clinical isolates of Enterobacteriaceae from nine teaching hospitals in China

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Objective: To investigate the prevalence of plasmid-mediated quinolone resistance qnr and aac(6′)-Ib-cr genes in Enterobacteriaceae in China.
Methods: A total of 197 of *Escherichia coli*, *Klebsiella pneumoniae*, *Citrobacter freundii*, and *Enterobacter cloacae*, with ciprofloxacin $\geqslant$ 0.25 ug/ml, cefotaxime $\geqslant$2.0 ug/ml) were screened from the 974 non-repetitive clinical isolates collected from the nine teaching hospitals in China. qnrA, qnrB, qnrS and aac(6′)-Ib genes were detected by PCR. aac(6′)-Ib-cr gene was further identified by the digestion with BtsCI

and direct sequencing. Conjugation experiment was performed for 20 isolates. Plasmid AmpC and ESBL genes were detected by PCR for the transconjugants. The MIC of ciprofloxacin and other antibacterial agents were determined by agar dilution.
Results: Qnr was present in 65.7% of *E. cloacae*, 65.5% of *K. pneumoniae*, 63.3% of *C. freundii*, and 6.5% of *E. coli*, respectively. aac(6′)-Ib-cr was found in 8.6% of *E. cloacae*, 21.8% of *K. pneumoniae*, 26.7% of *C. freundii*, and 16.9% of *E. coli*, respectively. Seventeen isolates carried qnrA, 46 isolates with qnrB, 24 with qnrS, 2 with qnrA and qnrB, and 2 with qnrB and qnrS. Eighteen isolates carried both qnr and aac(6′)-Ib-cr. The 13 transconjugants showed 16- to 250-fold increases in the MICs of ciprofloxacin compared to that of the recipient. Six transconjugants carried plasmid AmpC enzyme (including DHA, ACT and CMY-like) and 7 produced CTX-M ESBL.
Conclusions: Qnr and aac(6′)-Ib-cr widely existed in the enterobacteriaceae and perhaps contribute to the rapid increase of bacterial resistance to quinolones in China.

O86 Independent emergence of quinolone resistance in CTX-M-5 β-lactamase-producing isolates of *Salmonella typhimurium* from Russia, Belarus and Kazakhstan

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Objectives: Extended-spectrum cephalosporins and quinolones are used as primary treatment for severe salmonellosis. Therefore, the emergence of simultaneous resistance to these drugs in clinical isolates of *Salmonella* is of great therapeutic concern. This study was performed to investigate the molecular epidemiology of resistance to cefotaxime (CTX) and nalidixic acid (NA) among S. Typhimurium (Sty) isolates from Russia, Belarus and Kazakhstan.
Methods: A total of 44 clinical Sty isolates collected in 2002–07 were studied including 11 from Gomel region (Belarus), 24 from Irkutsk, Smolensk and Voronezh regions (Russia) and 9 from Karaganda (Kazakhstan). Four additional CTX-M-5-producing strains isolated in 1996–99 in various regions of Russia and Belarus and reported earlier to belong to a single clonal group [Edelstein et al. 2004] were included in this study for comparison. Susceptibility testing was performed by agar dilution method according to CLSI guidelines. Molecular typing was done using fluorescent multiple-locus variable-number tandem-repeats analysis (MLVA). PCR and sequencing were used to identify the CTX-M β-lactamase genes and to characterise their genetic context as well as to determine the gyrA QRDR sequences. The CTX-M-coding plasmids were compared using RFLP analysis with PstI and PvuII endonucleases.
Results: All the isolates studied shared a common phenotype of CTX resistance reversible by clavulanic acid. The resistance was due to production of CTX-M-5 ESBL whose gene was associated with ISEcp1 element and located on small (~7.4 kb) plasmid exhibiting identical RFLP pattern in all the isolates. Eighteen isolates were resistant to NA, none of them were resistant to ciprofloxacin. MLVA grouped the isolates into 8 types linked to each other with only 1 or 2 VNTR loci distinguishing each type. The quinolone-resistant isolates were distributed among 6 MLVA types, 3 of which also included NA-susceptible isolates. Resistance to NA strongly correlated with the presence of known mutations: Ser83-Phe, Asp87-Asn, -Tyr, or -Gly in the GyrA QRDR sequences which were otherwise identical in all isolates. Notably, the isolates of different MLVA types had different GyrA mutations.
Conclusions: This study supports the clonal origin of CTX-M-5-producing Sty isolates which continue to spread in Russia, Belarus and Kazakhstan and provides the evidence of frequent and independent acquisition of quinolone resistance among these isolates.

O87 A nationwide survey of extended-spectrum β-lactamases and metallo-β-lactamases produced in *Pseudomonas aeruginosa* in France, 2007

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Objectives: The aim of this study was (i) to evaluate the incidence of ESBL- and MBL-producing *Pseudomonas aeruginosa* (PA) in a representative panel of French hospitals in 2007 and (ii) to identify the β-lactamases responsible for the resistance to cefazidime (CAZ).
Methods: Isolates of PA resistant to CAZ, according to the French criteria (MIC > 32 mg/l), were collected from diagnostic samples in 85 participating medical centres during June 2007. Cystic fibrosis samples were excluded. Resistance to CAZ was confirmed by agar diffusion in the reference laboratory. Chromosomally-encoded cephalosporinase overproduction was assessed by isoelectrofocusing. ESBLs and MBLs were identified by isoelectrofocusing and gene sequencing.
Results: One-hundred and forty-three of the 2,200 isolates collected were resistant to CAZ (6.5%). Incidence of CAZ-resistant PA ranged from 0 to 50% according to the centres. Average incidence of patient infected/colonised with a CAZ-resistant PA was 0.1 per 1000 days of hospitalisation (from 0 to 0.72). Overproduction of AmpC cephalosporinase was observed in 142 strains (99.3%). Sixteen isolates were found to harbor an additional ESBL: OXA-19-like (n = 11), OXA-28 (n = 1), PER-1 (n = 2), SHV2a (n = 1) or unidentified enzyme (n = 1). Two isolates expressed an additional MBL (VIM-2 and an unidentified enzyme). Incidence of ESBL-producing isolates among CAZ-resistant PA was much higher (11.2%; 0.7% of the whole collection) than that of MBL-producing isolates 0.01%.
Conclusion: These results show the variability in term of frequency and incidence in PA resistance to CAZ among French hospitals. Resistance to CAZ and to imipenem in France is mostly related to the overproduction of cephalosporinase AmpC and to the underexpression of porin OprD, respectively. Interestingly, eleven out of 16 BLSE-producing isolates expressed an OXA-19-like enzyme. Routine tests (double-disk synergy and inhibition by cloxacillin) failed to detect this OXA-type ESBL, which is poorly inhibited by clavulanic acid while inhibited by cloxacillin, like AmpC. In conclusion, ESBL- and MBL-producing PA are still rather unfrequent in France, with the predominance of an underrecognised OXA-type ESBL.

O88 Study of the molecular epidemiology of metallo-β-lactamase-producing *Klebsiella pneumoniae* in Greece

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Objectives: Greece suffers since 2002 from an extensive epidemic of infections in hospitalised patients, due to metallo-β-lactamase (MBL)-producing *Klebsiella pneumoniae*. Our study aimed to contribute to the understanding of the molecular epidemiology of MBL-producing *K. pneumoniae* isolated in Greek hospitals.
Methods: Forty-five MBL-producing *K. pneumoniae* clinical isolates from ten hospitals, isolated during the first semester of 2006, were studied. Susceptibility levels to β-lactams were defined by standard methods. MBL-production was assessed based on the Imp-EDTA disk synergy test and confirmed by PCR. Integron structure was preliminary assessed by PCR. Typing was performed by PFGE. Representative strains were used as donors in mating experiments.
Results: All isolates were resistant to 3rd generation cephalosporins, but MICs to imipenem varied significantly. The Imp-EDTA disk synergy test was positive for all isolates. PCR revealed the presence of blaVIM-1 genes, related to class 1 integrons. Typing of strains showed five genetic types. All but three hospitals had more than one type. Typing revealed also the occurrence of clonal epidemics in two hospitals. Mating experiments revealed the presence of self transferable VIM-harboring plasmids in only two of the five types.

Conclusions: Increase of carbapenem resistant *K. pneumoniae* in Greek hospitals seems to be accompanied by the emergence and intra- and inter-hospital spread of multiple VIM-producing clones. Clonal spread identified in some occasions underlines the need of thorough infection control, as a key issue in the strategy to contain the epidemic

O89 Emergence of metallo-β-lactamase producing *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* in Scandinavia

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Objectives: Scandinavian countries have a history of low levels of antibiotic resistance due to effective infection control measures and restricted use of antibiotics. New resistance mechanisms are therefore less likely to emerge from endogenous sources. This study assesses the epidemiology and characterisation of MBL producing clinical isolates detected in Denmark, Norway, and Sweden between 2003–2007.
Methods: MBL genes and their genetic support were evaluated by PCR and DNA sequencing, while epidemiological typing of host isolates was performed by pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST) and serotyping. Susceptibility testing was performed with Etest.
Results: Since the first MBL positive isolate was identified in Sweden in 2003, a total of 17 MBL isolates have been identified in Norway, Sweden and Denmark by 2007, including eleven *Pseudomonas aeruginosa* strains and six *Klebsiella pneumoniae* strains. Many of the infected patients have a history of recent hospitalisation outside Scandinavia including Southern-Europe, Asia and Africa. However, MBL isolates are now being identified in patients precluding travel or hospitalisation outside Scandinavia in the last twelve months. VIM-enzymes constitute the predominant MBL genotype, found in all *K. pneumoniae* isolates and ten *P. aeruginosa* isolates. IMP was detected in one *P. aeruginosa* isolate. Genetic characterisation of the isolates revealed that the genes encoding MBL are located in class 1 integrons including TniC-class 1 integrons of variable size and with a plethora of other resistance gene cassettes. Accordingly, the susceptibility profiles of the isolates show a multi-drug resistant phenotype and some isolates are only susceptible to colistin. Interestingly, the qnrS gene, conferring low-level quinolone resistance, was identified in the two Norwegian *K. pneumoniae* isolates. Serotyping of *P. aeruginosa* indicate that O11 and O12 isolates predominate, and some isolates within the serotypes were found to be related by PFGE and MLST.
Conclusion: The spread of MBL-producing clinical isolates to Scandinavia is likely to initially have occurred through importation of the MBL positive isolates. However, such isolates have now appeared in patients with no history of travel or hospitalisation outside Scandinavia. VIM was the dominant MBL and always present in a multi-drug resistant background. PFGE and MLST revealed that some isolates of the same serotype were related.

O90 Complex emergence of VIM-1 producing *Pseudomonas aeruginosa* in Spain during epidemics involving VIM-1 producing Enterobacteriaceae

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Objectives: Acquired metallo-betalactamases (MBL)-producing isolates are uncommonly described in Spain and mostly confined to VIM-2 and *P. aeruginosa*. We describe the emergence of VIM-1 producing *P. aeruginosa* isolates in our hospital during epidemics involving different VIM-1 producing enterobacterial species (Tato et al. CID 2007; 24:472–3) and identified the genetic context of blaVIM-1.
Methods: Two MBL producing *P. aeruginosa* isolates (PA1 and PA2) were recovered in April-2006 and January-2007, respectively. MBL production was detected by the double-disc synergy test (EDTA-10 mcl 0.5 M versus imipenem and ceftazidime). Clonal analysis was determined by SpeI-PFGE. PCR for blaVIM, class 1 integron, and Tn402 sequences was performed and further sequenced. Transfer of antibiotic resistance was determined by filter-mating using *P. aeruginosa* PAO1 strain.
Results: PFGE revealed different restriction patterns for these *P. aeruginosa* isolates. blaVIM-1 gene was located within class 1 integrons in both cases. In PA1, the MBL gene was carried by a Tn402 derivative lacking the 3′CS but containing tniCTn402 (blaVIM-1-aadB-tniC). A different class 1 integron was detected in PA2 (blaVIM-1-aadA1). These integrons were also different to those found in contemporary MBL-enterobacterial isolates in our hospital. blaVIM-1 gene was transferred (frequency range 10^{-5}–10^{-7}).
Conclusions: We firstly report VIM-1 producing *P. aeruginosa* in our country. The integron platforms containing blaVIM-1 gene in these isolates were different to those found in enterobacterial species contemporarily recovered during a VIM-1-Enterobacteriaceae epidemics in our hospital. Dissemination of blaVIM-1 gene was associated with different genetic structures and bacterial hosts, depicting complex scenario of this emerging threat in our hospital.

O91 Predisposing factors for CTX-M or SHV extended-spectrum β-lactamase-producing *Escherichia coli* in community-acquired infections

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Objectives: Extended-spectrum β-lactamase-producing *Escherichia coli* (ESBLEC) is an increasingly significant cause of community-acquired (CA) infections worldwide. While much attention has been paid to CTX-M-producing isolates, there is scarce data about the epidemiology of SHV in the community. We compared the epidemiological features of CTX-M and SHV-producing ESBLEC causing community-acquired infections.
Methods: A multicentre cohort study including all CA infections caused by ESBLEC from 4 geographical areas in Spain was performed (Feb-2002 to May-2003). ESBL production was inferred following CLSI recommendations. ESBL was characterised by IEF, PCR and sequencing. The following data were collected: demographics, previous healthcare relation, co-morbidities, use of antimicrobials, invasive procedures and type of infection. Patients with CTX-M and SHV-producing isolates were compared using logistic regression.
Results: 122 cases (95% urinary tract infections) were included. ESBL was characterised in 112 isolates; 77 isolates (69%) produced CTX-M enzymes (40 produced CTX-M-9, 25 CTX-M-14), 36 (32%) SHV (31 SHV-12), and 7 (6%) TEM (4 TEM-116); 8 isolates produced >1 ESBL. Also, TEM-1 was produced by 52 isolates. CTX-M were the most frequent ESBL in the 4 areas (range, 56%-82%). Patients with isolates producing only CTX-M enzymes (CTX-M group, n = 70) and only SHV enzymes (SHV group, N=31) were compared; 70% of patients in the CTX-M group and 42% in the SHV group were >60 years old (p = 0.08); Charlson index >2 was found in 14% of patients in the CTX-M group and in 32% in the SHV group (p = 0.03). No differences in underlying diseases, previous healthcare relation, invasive procedures, antibiotic use or type of infections were found. Multivariate analysis including the geographical area showed that Charlson index >2 (OR=4.0, IC 95%: 1.2–12.6) was associated with SHV isolates, while age >60 (4.7; 1.7–12.5) was associated with CTX-M isolates.
Conclusions: SHV-producing ESBLEC are also a significant cause of community-acquired infections in Spain; the clinical epidemiology of such isolates seems much similar to CTX-M-producing ESBLEC. These data suggest that SHV-producing *E. coli* are also spreading in the community.

O92 Emergence of ciprofloxacin-gentamicin resistant *Escherichia coli*: implications for the empirical management of urinary tract infections

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Objectives: An increased incidence of urinary tract infections (UTI) caused by ciprofloxacin-gentamicin resistant *E. coli* (CiGREC) has been observed in our hospital, a tertiary care centre in Canada. Thus, we aimed to determine the risk factors associated with CiGREC UTI and its outcomes, in order to improve the empirical management of patients with UTI pending results of in vitro susceptibility.
Methods: A case control study was conducted between 2000 and 2007. Eighty-four cases and 168 randomly selected controls were identified using laboratory records of patients with $>10^8$ CFU of *E. coli* in a urinary specimen. Cases had *E. coli* with MIC to ciprofloxacin >2ug/L and MIC to gentamicin >8ug/L (CiGREC), and controls had *E. coli* with any other susceptibility pattern to ciprofloxacin and gentamicin. Clinical and laboratory data were collected. Univariate and multivariate analyses were used for case-control comparisons.

Table 1. Risk factors associated with urinary infection with a ciprofloxacin–gentamicin resistant strain of *Escherichia coli*

	Cases (n = 84)	Controls (n = 168)	p-value
Age			
<18	4 (5%)	40 (23%)	<0.001
18–64	29 (35%)	78 (46%)	
⩾65	51 (60%)	50 (31%	
Sex			
Male	36 (57%)	126 (25%)	0.005
Female	48 (43%)	42 (75%)	
Charlson score			
0	24 (29%)	86 (51%)	0.001
1–3	35 (42%)	56 (33%)	
⩾4	25 (30%)	26 (16%)	
Underlying urologic abnormality			
Yes	60 (71%)	74 (44%)	<0.001
No	24 (29%)	94 (56%)	
Acquisition			
Community acquired	21 (25%)	78 (46%)	<0.001
Healthcare associated	26 (31%)	40 (24%)	
Nosocomial	37 (44%)	50 (30%)	
Received quinolones in the last month			
Yes	36 (44%)	9 (5%)	<0.001
No	45 (56%)	158 (95%)	
Received quinolones in the last year			
Yes	32 (40%)	18 (11%)	<0.001
No	47 (60%)	150 (89%)	

Results: Prevalence of CiGREC increased four-fold during the study period, from 0.5% to 2.1%. Strains resistant to both ciprofloxacin and gentamicin were more likely than susceptible strains to harbour concomitant resistance to TMP-SMX (62% vs 11%), ampicillin (90% vs 26%) and tobramycin (47% vs 0.4%) (all with $p < 0.001$), but conserved susceptibility to carbapenems. Risk factors associated with urinary tract infection with the resistant strain were: advanced age, male sex, underlying urologic abnormality, presence of comorbidities with a Charlson score >4, previous use of quinolones in the last month or year and hospital or healthcare acquisition (see table 1). Cases were also more likely to receive inactive antibiotics during the first 48h of treatment (70% vs 28%, $p < 0.001$) and even after the documentation of the resistance (37% vs 2%, $p < 0.001$). They also experienced more frequent relapses (28% vs 15%, $p = 0.03$) and a higher mortality than controls (6% vs 0%, $p = 0.04$).
Conclusion: The incidence of CiGREC as a cause of urinary tract infection is increasing in our hospital and impacts on the outcomes of our patients. Ciprofloxacin and gentamicin should no longer be used as empirical treatment of UTI among patients who have received quinolones in the previous year.

O93 Risk factors associated with the isolation of colistin-resistant Gram-negative bacteria: a matched case-control study

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Objective: The emergence of multidrug resistant Gram-negative bacteria, has led to the re-use of colistin, but resistance to this agent has already been reported. We aimed to investigate the potential risk factors for the isolation of colistin-resistant *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* from hospitalised patients.
Methods: We performed a matched case-control study in a tertiary care hospital in Athens, Greece. Case patients were those that had provided a clinical specimen from whom a colistin-resistant *K. pneumoniae*, *A. baumannii*, and *P. aeruginosa* was isolated. Controls were selected from a pool of patients with susceptible to colistin isolates and were matched (1:1) to cases for species of microorganism and site of isolation. Susceptibility to colistin was determined with the E-test. Data regarding patient demographics, comorbidities, admission to the intensive care unit (ICU), prior antibiotic use, and invasive procedures performed were analysed as risk factors in a matched bivariable model. Variables significantly associated with colistin-resistant isolates ($p < 0.05$) were entered in a backward multivariable logistic regression model.
Results: Forty-one colistin-resistant unique patient isolates were identified from 01/01/2006 until 31/03/2007. These isolates represented infection in 35/41 patients. Risk factors significantly associated with the isolation of colistin-resistant isolates were age, duration of ICU stay, duration of mechanical ventilation, surgical procedures, use of colistin, use of monobactams, and duration of use of 3rd generation cephalosporins. In the multivariable model, use of colistin was identified as the only independent risk factor (adjusted odds ratio=7.78, $p = 0.002$).
Conclusion: Colistin-resistant *K. pneumoniae*, *A. baumannii*, and *P. aeruginosa* pathogens may be encountered in clinical practice, in association with inappropriate colistin use. To prevent this phenomenon, colistin should be used judiciously, given that treatment options for colistin-resistant Gram-negative bacteria are currently limited.

Interesting cases of susceptibility and resistance to antibiotics

O94 Reversal of meticillin resistance in *Staphylococcus aureus* by thioridazine

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Objectives: Thioridazine, a phenothiazine agent, has been shown to reverse oxacillin (meticillin) resistance in meticillin-resistant *Staphylococcus aureus* (MRSA) in vitro. The mechanism behind this observed effect has not yet been clarified. The aim of this study was to determine if thioridazine, alone or in combination with oxacillin affects the transcription of the meticillin resistance gene, mecA and the protein level of the coding protein, Penicillin Binding Protein 2a (PBP2a).
Methods: Growth of MRSA (ATCC 33591) was examined in liquid media in the presence of the meticillin analogue oxacillin and the non-antibiotic thioridazine alone and in combination. Furthermore, the transcription of mecA was analysed by Northern blotting and Primer extension and the protein level of PBP2a was analysed by Western blotting under the same conditions.

Results: We observed an increased susceptibility of MRSA towards oxacillin in the presence of oxacillin and thioridazine compared to bacteria grown with oxacillin or thioridazine alone. Transcription of mecA was reduced with increasing concentrations of thioridazine in the presence of oxacillin compared to bacteria grown with oxacillin or thioridazine alone. Additionally, the protein level of PBP2a was reduced when bacteria were treated with the combination of oxacillin and thioridazine. Thioridazine itself did not affect the growth of MRSA or mecA and PBP2a.
Conclusion: Results of the present study indicate that reversal of meticillin resistance by thioridazine in MRSA may be explained by a reduced transcription of the meticillin resistance gene, mecA, and protein level of the coding protein, PBP2a.

O95 Determination of the minimum inhibitory concentration and mutant prevention concentration of tigecycline against clinical isolates of meticillin-resistant *Staphylococcus aureus*: impact of blood on susceptibility results

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Objectives: Tigecycline, a member of a new class of antimicrobials (glycylcyclines) has been shown to have in vitro activity against clinical isolates of meticillin-resistant (MR) *Staphylococcus aureus* (SA) by minimum inhibitory concentration (MIC) measurements. We were interested in determining if the low MIC values would translate into low MPC values and to investigate if the presence of blood in the test media influences the mutant prevention concentration (MPC) results.
Methods: MIC was in accordance with the recommended CLSI procedure by microbroth dilution using 10^5 cfu/ml tested against doubling drug dilutions in appropriate media. For MPC testing 10^{10} CFUs were added to drug containing agar plates: 1) Mueller-Hinton (MH) agar without blood, 2) MH with 5% sheep red blood cells (MH+SRBC). The lowest drug concentration preventing growth was recorded as the MIC or MPC depending on method.
Results: For the MRSA strains (n = 29) MIC50, MIC90 and MIC range values (μg/ml) were 0.125, 1 and 0.063–1. By MPC testing on MH, the MPC50, MPC90 and MPC range values (ug/ml) were 1, 4 and 0.5–4; on MH+SRBC, the values respectively were 0.5, 4, 0.5–4. In all instances, MPC values were the same or within 1 doubling dilution on MH or MH+SRBC.
Conclusion: By MIC testing, tigecycline was highly active in vitro against MRSA strains with MICs $\leqslant$1 ug/ml. Tigecycline MPC values were $\leqslant$2 ug/ml for 80% of MRSA strains and not influenced by blood in the test media. Tigecycline appears to be a promising agent for treating *Staphylococcus aureus* infections and appears to have a reduced likelihood for selecting for resistance based on MPC measurements.

O96 Comparative antibacterial activity of retapamulin, cephalothin, gentamicin and erythromycin against *Staphylococcus aureus*, including molecularly characterised isolates of MRSA recovered from uncomplicated skin infections

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Objectives: In order to better recognise differences in susceptibility profiles, the in vitro activity of retapamulin, a new topical antibacterial agent, and comparators was determined against *Staphylococcus aureus*, including molecularly characterised isolates of MRSA from uncomplicated skin infections.
Methods: Bacterial isolates were obtained from the skin specimens of patients enrolled in five global phase III clinical trials conducted to evaluate the safety and efficacy of retapamulin. Susceptibility testing was conducted according to current CLSI standards. Susceptibility to retapamulin, cephalothin, gentamicin and erythromycin was determined on all *S. aureus* isolates using broth microdilution panels and susceptibility to meticillin was determined by disk diffusion using cefoxitin and/or oxacillin disks. All meticillin-resistant *Staphylococcus aureus* (MRSA) isolates were further analysed by pulsed-field gel electrophoresis (PFGE), multilocus sequence typing, SCCmec typing, and testing for the Panton-Valentine leukocidin genes.
Results: The overall rate of meticillin resistance among *S. aureus* isolates was 7% (105/1,442). Of the 105 MRSA isolates, 50% (53) were determined to be USA300. MIC90s (μg/mL) against *S. aureus* isolates obtained at the baseline visit are shown in the table.
Retapamulin demonstrated excellent in vitro activity against all *S. aureus* with a MIC range of 0.008–0.5 μg/mL and MIC90s of 0.12 μg/mL. Erythromycin demonstrated poor in vitro activity against all *S. aureus* with MIC90s of $\geqslant$32 μg/mL. Based on MIC90 values, gentamicin was >128-fold more active and cephalothin was 32-fold more active against USA300 MRSA isolates than against non-USA300 MRSA isolates.
Conclusions: Retapamulin was highly active in vitro against all *S. aureus* tested, irrespective of meticillin resistance or PFGE type. However, the MIC results for cephalothin and gentamicin suggest a possible difference between in vitro activity against USA300 and non-USA300 MRSA types. It should be noted that in vitro activity does not always correlate with clinical efficacy.

Baseline Pathogen (n)	MIC90 (μg/mL)			
	Retapamulin	Cephalothin	Gentamicin	Erythromycin
S. aureus (1,442)	0.12	1	1	$\geqslant$32
MRSA (105)*	0.12	64	$\geqslant$64	$\geqslant$32
USA300 (53)	0.12	4	0.5	$\geqslant$32
Non-USA300 (52)	0.12	128	$\geqslant$64	$\geqslant$32
MSSA (1,333)	0.12	0.5	0.5	$\geqslant$32

*3 MRSA isolates for which meticillin resistance could not be confirmed were removed from this analysis.

O97 Bactericidal activity of iclaprim in rat and human fibrin clots against wild type and thymidine kinase-deficient MRSA

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Background and Objectives: Iclaprim is a novel diaminopyrimidine with potent activity against Gram-positive bacteria, including meticillin-resistant *Staphylococcus aureus* (MRSA). It is known that uptake of thymidine (dT) and its conversion in dT-monophosphate by thymidine kinase (TK) bypass the activity of diaminopyrimidines. dT levels in rodents are very high and antagonize diaminopyrimidine, thus precluding reliable evaluation of their efficacy in vivo. On the contrary, dT levels in humans are low and do not affect their activity. It is hypothesised that iclaprim should be effective against MRSA in a human environment and that its efficacy should be restored in a rodent milieu using TK-deficient strains. We tested this concept in human and rat fibrin clots in vitro, simulating amorphous cardiac vegetations in vivo.
Methods: MICs of iclaprim were tested in MHB and in MHB supplemented with 25% human serum (MHB-HS), 25% rat serum (MHB-RS) or an excess (1 mg/L) of dT (MHB-dT). Fibrin clots were made from human or rat plasma containing the parent MRSA AW6 or its TK-deficient derivative AH1252, and treated for 48h with homologous serum supplemented with either saline (controls), iclaprim (3.5 mg/L), trimethoprim-sulfamethoxazole (SXT) (8–40 mg/L) or vancomycin (40 mg/L). The extent of drug exposure mimics standard treatment length in animal models of endocarditis.
Results: MICs of iclaprim in MHB/MHB-HS/MHB-RS and MHB-dT were 0.06/0.06/>2/>2 for AW6 and 0.03/0.03/0.06/0.03 for AH1252. Median CFU/clot values in controls as well as in clots after drug treatment are shown in the Table.

		CFU/clot values, Human/Rat			
		Controls	Iclaprim	SXT	Vancomycin
AW6	0 h	6.56/6.21			
	48 h	8.81/8.67	3.20†/5.89	2.84†/5.96	2.30†/2.00†‡
AH1252	0 h	6.68/6.32			
	48 h	8.97/8.97	2.15†/3.46†#	2.00†/3.44†#	2.00†/2.00†

† $p < 0.05$ vs controls at 0 h; ‡ $p < 0.05$ vs ICL and SXT; # $p < 0.05$ vs AW6 in rat clots.

Conclusions: Iclaprim was bactericidal in human clots against both wild type AW6 and TK-deficient AH152 strains, and was equipotent to SXT and vancomycin. Iclaprim was bacteriostatic against parent AW6 in rat clots but killed the TK-deficient mutant AH1252 in the same media. The bactericidal activity of iclaprim against MRSA in human clots, a setting mimicking the intravascular milieu, shows the potential of this drug for the treatment of MRSA infections in humans. TK-deficient mutants could also prove useful for studies of diaminopyrimidines in rodents.

O98 Influence of thymidine on bactericidal activity of iclaprim against vancomycin-intermediate and vancomycin-resistant *Staphylococcus aureus* strains

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Background: Iclaprim is a bactericidal diaminopyrimidine antibiotic that exhibits potent and bactericidal activity against Gram-positive pathogens, notably MRSA. Previous studies have shown that excess thymidine (⩾1 μg/ml) in the growth medium abolishes the bactericidal effect of the drug in vitro. Importantly, thymidine levels in humans are extremely low (⩽0.01 μg/ml). This study used time-kill analysis to determine the effects of adding or removing excess thymidine in growth medium on the bactericidal potential of iclaprim against one vancomycin intermediate (VISA) and one vancomycin resistant (VRSA) *S. aureus* isolate.
Methods: Media included 1) Oxoid Mueller-Hinton broth, previously proven to have minimal amounts of thymidine; 2) The latter Oxoid broth + 2 μg/ml thymidine; 3) BBL cation adjusted Mueller-Hinton broth + 0.2 units/ml thymidine phosphorylase; 4) BBL cation-adjusted Mueller-Hinton broth + 2 μg/ml thymidine. MICs were by CLSI macrodilution in each of the four above media. For time-kills organism suspensions (5×10^5–5×10^6 cfu/ml) were incubated overnight with iclaprim at MIC, 2 x MIC and 4 x MIC in all four media, in a shaking water bath at 35ºC. Viability counts were done at 0, 3, 6, 12, and 24 h on BBL trypticase soy agar plates + 5% sheep blood.
Results: Iclaprim MICs for the VRSA and VISA were as follows: 1) Oxoid Mueller-Hinton broth without added thymidine: 0.06 and 0.25 μg/ml, respectively. 2) Cation-adjusted Mueller-Hinton broth with thymidine phosphorylase: 0.12 and 0.5 μg/ml respectively. On BBL medium with no added thymidine or thymidine phosphorylase, iclaprim MICs were 0.125 and 0.25 μg/ml, respectively. Iclaprim MICs were >8.0 μg/ml after 2 μg/ml thymidine was added to either media. Iclaprim at 2 x MIC showed ⩾3 log10 (99.9%) killing after 12 h in Oxoid Mueller-Hinton broth and BBL cation-adjusted Mueller-Hinton broth with thymidine phosphorylase. By contrast, no killing by iclaprim occurred in either media after adding thymidine (2 μg/ml).
Conclusions: Iclaprim yielded low MICs and was bactericidal against VRSA and VISA strains when tested in broth media without significant levels of thymidine. For accurate determination of the bactericidal activity of iclaprim, media without significant levels of thymidine should be used.

O99 In vitro activity of gallium against meticillin-susceptible and meticillin-resistant *Staphylococcus aureus* and *S. epidermidis*

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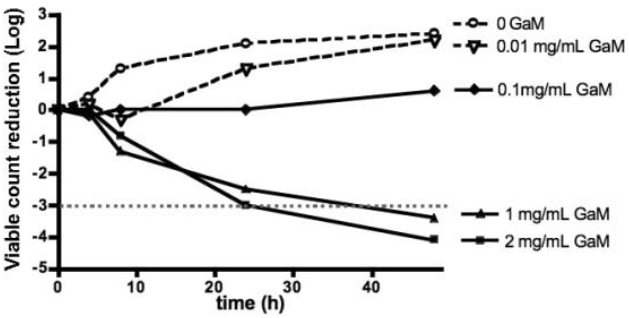
Objectives: Gallium (III) is a semi-metallic element physically similar to iron (III) and competes for iron-binding sites of transporters and enzymes. Since gallium (III) is not reducible like iron (III), it is unable to participate in biologically important redox reactions, which represents the presumed mode of antimicrobial action. We investigated the in vitro anti-staphylococcal activity of gallium maltolate (GaM), an oral formulation with good bioavailability and a favourable safety profile in clinical studies.
Methods: GaM in vitro susceptibility was performed in triplicate by a macrodilution assay with 10^5 CFU/mL in iron-limited RPMI 1640 for determination of MIC and logarithmic MBC (MBClog) or in PBS for determination of stationary MBC (MBCstat). The following standard strains were tested: *S. aureus* ATCC 29213 (MSSA), *S. aureus* ATCC 43300 (MRSA), *S. epidermidis* 1457 (MSSE) and *S. epidermidis* B3972 (MRSE). Time-kill studies were performed with GaM at 10^5 CFU/mL in RPMI 1640 over 24 h (for MSSA) or 48 h (for MSSE) and expressed as reduction of viable counts in log CFU/mL.
Results: GaM MIC/MBClog/MBCstat values were (in mg/mL): 1.5/>6/>6 (for MSSA) and 1.1/6/>6 (for MRSA), 0.10/1.5/4.5 (for MSSE) and 0.14/1.5/1.5 (for MRSE). The ratio MBCstat/MBClog was 3 (for MSSE) and 1 (for MRSE). Figure shows the time-kill curve of MSSE at GaM concentrations at 0.1×, 1×, 10× and 20× MIC. In time-kill studies, GaM at 0, 0.2, 2 and 6 mg/mL reduced after 24 h incubation MSSA bacterial load (in log CFU/mL) of –2.3, –1.5, 0.8 and 1.3, respectively (positive values denote net growth).



Conclusion: GaM inhibits growth of *S. epidermidis* at considerable lower concentrations than of *S. aureus* (0.1 vs. 1 mg/mL). Against *S. aureus*, GaM was only bacteriostatic at concentrations up to 6 mg/mL. In contrast, GaM kills *S. epidermidis* in both logarithmic and stationary growth phases exhibiting a 3-log CFU reduction at 2 mg/mL GaM in 24 h. GaM is a potential anti-staphylococcal drug and may be especially useful in combination with other antimicrobials to prevent development of resistance.

O100 The activity of iclaprim against European *Streptococcus pyogenes* and *S. agalactiae*

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Objectives: Iclaprim (ICL) is a novel diaminopyrimidine within the same class as trimethoprim but with more potent broad-spectrum bactericidal activity in vitro. An intravenous formulation of ICL has just successfully completed phase III trials for complicated skin and skin structure infections and a phase II trial for hospital- and ventilator-associated pneumonia is underway. An oral form of ICL is also being evaluated in phase I trials. This current study ascertained the in vitro activity of ICL and comparators against *S. pyogenes* (GAS) and *S. agalactiae* (GBS).
Methods: MIC was determined by CLSI broth microdilution methodology against 500 recent GAS primarily from respiratory infections and 44 recent GBS from predominantly wound swabs originating

from European countries. Clarithromycin (CLA), clindamycin (CLI), trimethoprim-sulphamethoxazole (SXT), levofloxacin (LVX), linezolid (LNZ), penicillin G (PEN) and vancomycin (VAN) were used as comparators.
Results: 9% of the GAS tested were resistant to CLA and 4% resistant to CLI. For GBS, resistance to CLA and CLI was around 14% and 11%, respectively. Three GAS isolates were intermediate to levofloxacin. Summary MIC data are shown in the Table.

	MIC mg/L			
	MIN MIC	MIC_{50}	MIC_{90}	MAX MIC
GAS (N = 500)				
ICL	⩽0.004	0.015	0.06	0.25
SXT	0.03	0.25	1	2
CLI	⩽0.015	0.06	0.06	⩾64
CLA	⩽0.015	0.03	0.06	⩾64
LVX	0.25	0.5	1	4
LNZ	0.5	1	2	2
PEN	0.004	0.015	0.015	0.03
VAN	0.25	0.5	0.5	1
GBS (N = 44)				
ICL	0.03	0.12	0.25	0.5
SXT	0.12	0.25	0.5	1
CLI	0.03	0.06	⩾64	⩾64
CLA	0.03	0.03	2	⩾64
LVX	0.5	1	1	2
LNZ	1	1	2	2
PEN	0.06	0.06	0.12	0.12
VAN	0.5	0.5	0.5	1

ICL was active against all isolates, being slightly more active against GAS than GBS, including isolates resistant to CLA or CLI. Trailing end-points were observed with SXT against about 9% of GAS producing false-resistance unless read strictly at ⩾80% growth reduction according to CLSI methods. This potentially subjective reading procedure was not necessary with ICL.
Conclusion: ICL showed excellent activity in vitro against GAS and GBS, which supports the use of iclaprim in the treatment of infections caused by these bacteria.

O101 Efflux pump inhibitors may overcome antibiotic resistance in multi-resistant bacteria by increasing intracellular drug concentration

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Introduction: Multi drug resistance (MDR) of bacteria is an increasing problem in clinical practice of antimicrobial therapy. Inhibition of bacterial efflux mechanisms appears to be a promising target in order to restore antimicrobial susceptibility in MDR bacteria. Previous in-vitro studies have shown that inhibitors of bacterial efflux pumps may improve the antimicrobial effect of fluoroquinolons. However, for most substances concentrations necessary to restore antimicrobial susceptibility were too high for clinical use, which might be ascribed to insufficient increase of intracellular concentrations of antimicrobials. The present study set out to investigate the potency of various efflux pump inhibitors (EPI) to overcome MDR and to explore changes of intracellular concentrations of ciprofloxacin for the most potent substances.
Methods: Two previously described EPIs, 1-(1-naphthylmethyl)-piperazine (NMP) and phenyl-arginine-beta-naphthylamide (PAβN) and with two novel, specific p-glycoprotein (PGP) inhibitors, tariquidar and elacridar, were investigated in terms of effects on in vitro antibacterial activity. Antimicrobial susceptibility to ciprofloxacin in the absence and presence of EPIs were tested in the following strains: *Staphylococcus aureus* ATCC 29213 (SA), ciprofloxacin resistant *S. aureus* SA-1199B (rSA), *Pseudomonas aeruginosa* ATCC 27853 (PS) and MDR *Stenotrophomonas maltophilia* ATCC BAA-85 (SM). Changes in intracellular concentrations of ciprofloxacin were determined by use of [14C]Ciprofloxacin.
Results: Inhibition of PGP mediated drug efflux by tariquidar and elacridar reduced MICs of ciprofloxacin for rSA from 16 mg/l to 2 mg/l in a dose depended manner, whereas only minor effects were observed for SA and Gram-negative strains. Addition of MNP and PAβN at high concentrations increased susceptibility towards ciprofloxacin in all resistant strains tested. By addition of tariquidar and elacridar a dose dependent increase of intracellular [14C]Ciprofloxacin was detected.
Conclusions: Our findings suggest that tariquidar and elacridar are potent inhibitors of PGP mediated bacterial efflux. Both substances may restore susceptibility for PGP over expressing pathogens by dose dependent increase of intracellular ciprofloxacin. MDR in *Stenotrophomonas maltophilia* could not be overcome by specific PGP inhibitors but susceptibility could be increased by addition of high doses of MNP and PAβN.

O102 Microbicidal activity of monochloramine and chloramine T compared

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Objectives: Chloramine T (CAT) and monochloramine (NH2Cl) are active chlorine compounds and well-known antiseptics with broad-spectrum microbicidal activity. CAT has stronger oxidative activity than NH2Cl, while the latter one is a smaller molecule and more lipophilic. The question arose if lower oxidative activity can be compensated by higher lipophilicity regarding microbicidal effectiveness.
Methods: Bacterial strains (*Escherichia coli* ATCC 11229, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853) and clinical isolates of *Aspergillus fumigatus*, *Aspergillus flavus* and *Candida albicans* were used for quantitative killing assays. All microorganisms were tested separately in equimolar solutions of CAT and NH2Cl, respectively. Pathogens treated in buffer without antiseptics served as controls.
Result: NH2Cl showed a markedly stronger bactericidal and fungicidal activity than CAT.
At a concentration of 0.011 mM, NH2Cl killed all test bacteria within 30 min, while 0.011 mM CAT did not cause a reduction in colony forming units after that time. At a concentration of 0.036 mM and 0.107 mM, NH2Cl led to a 3–4 log10 reduction of *E. coli* approx. 200 times faster than CAT. The killing of *S. aureus* and *P. aeruginosa* by NH2Cl was 4–6 times faster. NH2Cl (0.355 mM) caused a 3 log10 reduction of *C. albicans* after 1 min compared to a 2 log10 reduction by the same concentration of CAT after 60 min. Conidia of *Aspergillus* were even killed approximately 200 times faster by NH2Cl than by CAT.
Conclusion: The enormous difference between both agents can be attributed to the lipophilicity and lower bulk of NH2Cl which by far overcompensate its lower oxidative activity.

O103 Prevalence and antibiotic susceptibilities of *Yersinia enterocolitica* and other *Yersinia* species recovered from meat and chicken in Tehran, Iran

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Objectives: *Yersinia enterocolitica* is known as a psychrotrophic waterborne and food borne enteropathogen.*Yersinia* can grow to large numbers at refrigeration temperatures, so meat, chicken, milk, cheese contaminated with that organism could become a significant health risk for consumers. The aims of this study addition to investigate the prevalence of *Y. enterocolitica* and other *Yersinia* species in meat and chicken samples in different seasons, were to determine the antimicrobial

susceptibility pattern of *Yersinia enterocolitica* and other *Yersinia* species isolated from meat and chicken in Tehran, Iran.
Methods: 189 peaces of meat and 190 chickens purchased from 28 different local butcher's shops and supermarkets in Tehran that examined for the presence of *Yersinia* species between April 2005 and March 2006.25 gr sample of homogenised food was pre-enriched in PBS medium then it was transferred to cefsulodin-irgasan-novobiocin (CIN) agar. Susceptibility testing of bacterial strains was achieved at 28ºC by the agar diffusion method.
Results: *Yersinia* spp. was recovered from 60 of 379 (15.8%) peaces of meat and chickens samples. *Y. enterocolitica* was found in 48 of 60 (80%) positive samples. The other 3 *Yersinia* spp. were *Y. frederiksenii*, *intermedia*, *kristensenii* in 7 (11%), 4 (6%) and 1 (0.01%) of 60 isolates, respectively. Also the most prevalence of *Yersinia* spp. was in early spring and in mid autumn.
All 4 strains (98%) were susceptible to choloramphenicol and gentamicin and 95%, 86%, 78%, 76%, 63%, 41% were susceptible to trimethoprim and ciprofloxacin, cephotaxim, Tetracycline, nalidixic acid, streptomycin and ampicilin respectively. the most antibiotic resistance belong to cephalothine (98%).
Conclusion: Several factors such as isolation method, season, and geographical location play an important role in reports of increase or decrease in the prevalence of the *Yersinia* spp. *Y. enterocolitica* had the most prevalence among other species. Our results show isolation ratio of *Y. enterocolitica* and other species is higher in colder climates. The majority of isolates were resistant to first generation cephalosporins (cephalothine). The most active pharmacologic agents were choloramphenicol, aminoglycozide and sulfunamdes. Regarding to high Sensivity of *Yersinia* spp. to gentamicin and chloramphenicol, these agents should be effective in the treatment of *Yersinia* spp. when clinically indicated.

The genetics of host susceptibility to infectious diseases

S120 Mannose-binding lectin genetics as an example of single gene influences on host susceptibility towards infection

N. Klein (London, UK)

In 1968 a report appeared in the literature of a patient who had suffered from recurrent upper respiratory tract infections in the first 2 years of life whose only defect appeared to be a failure to phagocytose *Saccharomyces cerevisiae* (Baker's yeast) efficiently. The defect was reversed when serum of other donors was used in the same assay. This opsonic defect could be found in a high proportion (5–8%) of apparently healthy populations, but was found frequently in children with recurrent unexplained infections. It is now known that this 'common opsonic defect' is due to a deficiency of a protein termed mannan or mannose-binding lectin (MBL). It is a member of the collectin sub-family of C-type lectins, and initiates the lectin pathway of complement activation following binding to mannose, N-acetylglucosamine, fucose and glucose residues presented in the orientations and densities commonly found on microorganisms. Once bound, MBL activates the complement system in an antibody and C1-independent manner. This is predominantly mediated through a serine protease, MASP-2, which cleaves C4 and C2 to generate a C3 convertase.
The human collectin genes are located in a cluster on chromosome 10. The MBL-2 gene comprises four exons and it is now known that three single point mutations in exon 1 lead to a functional deficiency of the MBL protein. Several polymorphisms have also been identified in the promoter region of the MBL gene. Largely through a combination of structural gene and promoter polymorphisms, MBL concentration can vary thousand-fold in apparently healthy individuals, and approximately a third of the Caucasian population has genotypes conferring concentrations deemed "insufficient".
Numerous studies have now been performed to determine the role of MBL in a clinical setting. It would appear that individuals who are deficient in this protein are prone to getting a range of infections, particularly in the context of another immune defect. However, there are also situations and diseases in which MBL deficiency may be protective. This talk will outline the current thinking on the role of MBL in susceptibility to infection.

Update on the perils of Gram-negative bacteria (Symposium jointly arranged with the ICAAC Program Committee)

S126 Extended-spectrum β-lactamases spreading in the community

R. Cantón (Madrid, ES)

Since first description in 1983 and during the eighties and nineties, most of the detected extended-spectrum β-lactamases (ESBL) were SHV and TEM types. They were mainly described in *Klebsiella pneumoniae* and associated with nosocomial outbreaks. This situation dramatically changed in the last few years worldwide, and most of the ESBL-producing isolates are now Esherichia coli expressing CTX-M enzymes. The majority of them are now recovered from community patients, mainly from urinary tract infections (UTIs) and new risk factors have been identified. Moreover, an increase of ESBL-producing isolates has been detected in nursing homes and healthcare associated facilities in the community, with the potential influx of ESBLs from the community to the hospital. Also, an increasing prevalence of faecal carriage with ESBL-producing isolates in outpatients and healthy volunteers has been described. ESBL producers are also recognised in other non-hospital compartments, including farm and companion animals and in the environment. The population structure of CTX-M-producing isolates is complex and was initially associated with a polyclonal spread of ESBL-producers (described as allodemia) and/or specific plasmids or other mobile platform genetic elements. Nevertheless, the application of an MLST typing scheme on ESBL-producing *E. coli* isolates in addition to a PFGE based scheme shows a new situation with the description of successful internationally disseminated multiresistant clones harbouring specific (epidemic) plasmids. The case of CTX-M-15 illustrates this situation. The ST131 *E. coli* clone belonging to phylogroup B2 and associated with a multidrug-resistant IncFII plasmid harbouring the blaCTX-M-15 gene has been found in different countries all over the world and mostly isolated in patients with UTIs in the community. Other examples of well dispersed ESBL in the community are CTX-M-14, CTX-M-1 and CTX-M-2. The former has been frequently reported in West Europe and North America with the potential dispersion of an epidemic plasmid in different clones. CTX-M-1 is prevalent all over Europe while CTX-M-2 in South America and Asia. Moreover, ESBLs belonging to classical groups, such as SHV-12 and TEM-52, seem to be also well dispersed in the community compartments. TEM-52 is widespread in Europe and associated with *Salmonella* isolates, and more recently with *E. coli* isolates in UTIs. Although the association of blaESBL genes with successfully mobile genetic platforms has been magnified as an important factor for the increasing prevalence of ESBLs, the presence of specific virulence determinants in ESBL producers might also has facilitated the persistence and subsequent dispersion of ESBLs. In addition, co-resistance to non-β-lactam antibiotics not only decreases therapeutic treatment options in community patients but also might has facilitated co-selection processes and maintenance of ESBL producers. It is remarkable that most of the new plasmid mediated resistance genes, including qnr, aac(6′)-Ib-cr and qepA affecting fluoroquinolones or the methylase genes affecting aminoglycosides (armA and rmtB), have been found in ESBL-producing organisms in several community compartments. With this landscape, reversion of the current pandemic situation of ESBLs in the community seems to be difficult and new strategies should be designed at local and international levels to curtail the spread of these enzymes.

S127 AmpCs, carbapenemases and carbapenem resistance

P. Nordmann (Le Kremlin Bicetre, FR)

Emerging resistance to broad spectrum β-lactams is increasingly observed in the main Gram-negative species that are involved in human infections i.e Enterobacteriaceae, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. Besides the clavulanic-acid inhibited extended-spectrum β-lactamases, the plasmid-mediated clavulanic-acid resistant cephalosporinases are increasingly reported in enterobacterial species that do not express naturally AmpC (*Escherichia coli*, *Proteus mirabilis*, *Salmonella* sp., *Klebsiella pneumoniae*). They confer resistance to expanded-spectrum cephalosporins. Chromosome-encoded cephalosporinases with extended spectrum of activity towards ceftazidime or cefepime have been also reported in *E. coli*, *Serratia marcescens* and *Enterobacter* sp. The carbapenemases that hydrolyse at least imipenem, ertapenem or/and meropenem are of the Ambler molecular classes A, B, and D in Enterobacteriaceae. The emerging carbapenemases in are mostly the plasmid-mediated KPC, VIM/IMP and OXA-48 enzymes in Enterobacteriaceae. Because most of these carbapenemases confer only reduced susceptibility to carbapenems, they may remain underestimated as a consequence of the lack of their detection. In *P. aeruginosa* and *A. baumannii*, β-lactamase mediated resistance to carbapenems is also emerging. In *P. aeruginosa*, the metallo-β-lactamases (IMP, VIM; SPM, GIM) are reported worldwide as a source of nosocomial outbreaks. In *A. baumannii*, although metallo β-lactamases are known, the acquired oxacillinases that are quite specific to *Acinetobacter* sp. (OXA-23, OXA-40, OXA-58-types) are the main source of carbapenem resistance. The natural reservoir of carbapenemases is unknown with the noticeable exception of the blaOXA-23 and the blaOXA-48 genes that we have identified as being *Acinetobacter* radioresistens and Shewanella sp., respectively. Finally, resistance to carbapenems may result in all Gram-negative species from impaired permeability (or/ and overexpression of efflux) together with overexpresssion of β-lactamases with very weak carbapenemase activity.

Nosocomial infections and infection control

O133 Healthcare-associated infection in acute hospitals: what makes a difference? Exploration of national English data

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Objectives: To investigate the practice-related factors linked to Healthcare Associated Infection (HCAI) rates in English acute hospitals.
Methods: A questionnaire tool was developed using expert input to cover what were considered to be important elements related to the management and control of HCAI. Questionnaires sent to all trust directors of infection prevention and control and chief executives in all acute hospital trusts in England for further distribution and completion by the relevant healthcare workers in the trust. Other data were collected for responding trusts from the Patient Environment Action Team (PEAT) and the Clinical Negligence Scheme for Trusts (CNST)). Infection outcomes comprised the mandatory surveillance data for meticillin-resistant *Staphylococcus aureus* (MRSA) bacteraemias and *Clostridium difficile* associated diarrhoea (CDAD. Univariate and multivariate analyses were performed.
Results: Trust level data were received from 155 of the 173 acute NHS trusts in England. A lower MRSA infection rate was linked to hand hygiene performance measures and isolation practices, whereas a lower rate of CDAD was linked to cleanliness (PEAT Scores), good practice in antimicrobial prescribing and surveillance of infections. Lower rates of MRSA and CDAD, were related to strategic, planned interventions such as the inclusion of infection control (IC) in the staff development programme. However, certain interventions, for example increased levels of training, were related to a higher infection rates. There are many aspects of the outcome data that will be described as possible confounding factors to such studies in England.
Conclusions: The associations we have found between lower rates of MRSA and CDAD have "face value" in that they can be supported by evidence from the infection control literature. We have, however, found relationships between interventions and higher infection rates that are counter-intuitive and may represent examples of what we are calling 'reactive practice' to higher rates of infection. Whilst it is interesting to hypothesise that these interventions may be swift and simple to introduce and may not be sustained compared to more strategic and planned interventions linked to lower infection rates, this will have to be confirmed by further studies over time.

O134 Prevalence of nosocomial infections, France, 2006

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Background: A prevalence survey of nosocomial infection (NI) was conducted in French healthcare facilities (HCF) in June 2006 to assess the burden of NI, describe their characteristics and by comparison to the 2001 NI survey assess the impact of the national infection control programme.
Methods: The survey was proposed to public and private HCF and used CDC definitions. Data were collected in a standardised manner by trained personnel in HCF and sent by encrypted e-mail to regional infection control coordinating centres (CClin) and the French Institute for Public Health Surveillance (InVS). The prevalence of patients with a NI (PPNI) was compared between 2001 and 2006 by multiple logistic regression, adjusting for characteristics of HCF, wards and patients.
Results: Among 358 353 patients included from 2 337 HCF (accounting for 95% of all French hospital beds), 17 817 (4.97%) were infected and 19 294 NI were documented; 1 406 (0.39%) patients were infected by meticillin-resistant *Staphylococcus aureus* (MRSA). Urinary tract, lower respiratory tract and surgical site infections accounted for 30, 15 and 14% of NI, respectively. Among 15 800 isolated micro-organisms, the 3 most frequent were *Escherichia coli* (25%), *S. aureus* (19%) and *Pseudomonas aeruginosa* (10%). The PPNI varied with type of HCF or wards and was greater among the elderly, males, patients with severe underlying disease, immunocompromised, undergoing surgery or exposed to invasive devices. Compared to 2001, the prevalence in 2006 was significantly lower for NI (adjusted odds ratio [ORa] = 0.88, 95%CI: 0.85–0.90) and MRSA infection (ORa = 0.60, 95%CI: 0.54–0.66).
Conclusion: The participation of HCF to the survey was a success. The decrease observed, particularly for MRSA infections, suggests a positive impact of the national infection control plans.

O135 Infectious complications of short-term ventricular assist devices

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Background: VAD are mechanical pumps that take over the function of damaged ventricle/s in patients with heart failure until the recovery of myocardial function or as effective bridge before heart transplantation (HT). These critically-ill patients are prone to nosocomial and device-related infections and to non-infectious complications such as bleeding or thromboembolism. Most reports come from countries with long waiting-lists for heart transplantation (HT) with average supports that may exceed 100 d. In Spain, the average waiting time for HT is shorter, and VAD-related infections in this setting have not been sufficiently analysed.
Methods: During the period Jan 1989-March 2007, 58 patients required a VAD in our institution. Clinical and microbiological records were reviewed to determine the incidence, risk factors and outcome of nosocomial infections in these patients. Standard CDC (Centers for Disease Control) criteria were used for syndrome definitions.
Results: Mean age was 52 years and 62% were male. Median VAD support length was 3 days (1–52). Most common reason for VAD was postcardiotomy ventricular failure (56.9%), followed by allograft failure after HT (22.4%) and medical cardiogenic shock (20.7%). An

infection (34 episodes) was diagnosed in 28 patients (50.9%): pneumonia (41%), urinary tract (14.7%), VAD infection (11.7%), wound infection (8.8%), catheter related (5.8%) and others (11.7%). *S. aureus* followed by Enterobacteriaceae, CNS and *P. aeruginosa* were the most common pathogens. In non-transplanted patients 27/32 died (85.1% of them due to a non-infectious cause) and 26 reached transplantation (80.8% despite having suffered an infection). At the end of VAD support, 38% of the patients were alive, 15.5% died due to cardiogenic failure and 15.5% of neurological complications. Infection accounted for 3.4% of the early deaths and for 19% of late demise.
Conclusion: Infections complicate the course of 51% of the patients requiring short-term VAD in our study but they do not preclude heart transplantation.

O136 Ventilator-associated pneumonia and attributable mortality: a systematic review of observational studies

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Objective: To determine the attributable mortality of Ventilator-associated Pneumonia (VAP) based upon the results of observational studies.
Methods: A systematic review and meta-analysis was performed. The studies were identified by performing a systematic search strategy using PubMed, Web of Science and Embase through February 2007. Only studies reporting mortality rates of patients with and without VAP were included. The data were extracted using standardised forms and the quality of all studies was determined by a validated scoring system.
Results: 52 studies with a total of 17.347 patients met the inclusion criteria. Mortality rates of patients with VAP ranged from 14 to 78%. Pooling of all studies resulted in an I^2 statistic of 69%, indicating considerable heterogeneity and, therefore, precluding estimation of an overall effect. A significant association between VAP and mortality, in univariate analyses, was observed in 17 of 52 studies (33%). Yet, nine studies also performed multivariate analyses, and significance of independent association persisted only in four of these studies. The origin of heterogeneity could not be explained by differences in study design, study quality and diagnostic approach. However heterogeneity was limited for studies investigating only trauma patients (I^2=1.3%) or acute respiratory distress syndrome (ARDS) patients (I^2=0%). The estimated relative risk of mortality of patients with VAP in these patient groups were 1.09 (95% CI 0.87–1.37) among trauma patients and 0.86 (95% CI 0.72 to 1.04) among ARDS patients.
Conclusions: The widely held belief that VAP is associated with attributable mortality cannot be based on the available evidence from observational studies. In fact, for two specific patient groups (trauma and ARDS) there is evidence of absence of such an association. Only analyses including more patient specific data, thereby allowing to adjust for possible confounders, might confirm or reject the presumed association for other patient groups.

O137 Effect of 10 years of surveillance on the incidence of surgical site infections in the Netherlands

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Objectives: Surgical site infections (SSI) continue to present a major proportion of adverse events in surgical patients. Many countries have established national surveillance systems, which aim to reduce the patients' risk of infection. This study evaluates the time-trend in SSI rate for five frequently-performed surgical procedures in the Netherlands, between 1996 and 2006.
Methods: Hospitals that participated for at least three consecutive years in the Dutch PREZIES surveillance network were included, and CDC definitions for SSIs were used.
Hospitals receive a feedback report per surgical procedure, including crude and expected SSI rates, which are usually spread and discussed in the hospital with involved personnel. Per surgical procedure, the association between SSI rate and surveillance year was estimated by odds ratios, which were obtained by logistic regression and adjusted for confounders and method of postdischarge surveillance. A random coefficient model (multilevel logistic modelling) was used to adjust the risk estimates for random variation among hospitals.
Results: Among the five surgical procedures, the number of included surveillance years varied between 6 and 10 years, the number of hospitals between 19 and 34, the number of procedures between 3,031 and 31,407, the number of SSIs between 249 and 766, and the overall SSI rate between 1.6% and 12.2%. The results of the multilevel logistic modelling are presented in the Table.

Table Results of multilevel logistic regression analysis. Odds ratio (OR) with 95% confidence interval (95% CI) and p-value of change in SSI rate **per 1-year increase** in surveillance time to operation.

	OR (95% CI)	*P*
Mastectomy[1]	1.04 (0.96–1.08)	0.46
Colectomy[2]	0.92 (0.83–1.02)	0.10
Replacement of the head of the femur[3]	0.94 (0.88–1.00)	0.07
Total hip prosthesis[4]	0.94 (0.90–0.98)	0.01
Knee prosthesis[5]	0.97 (0.91–1.03)	0.32

[1]Adjusted for: postdischarge surveillance (PDS), age, duration of surgery, gender.
[2]Adjusted for: PDS, ASA classification, wound contamination class, duration of surgery, duration of preoperative hospitalisation, emergency procedure.
[3]Adjusted for: PDS.
[4]Adjusted for: PDS, age, ASA classification, duration of preoperative hospitalisation, wound contamination class, duration of surgery.
[5]Adjusted for: PDS, university-affiliated hospital, duration of surgery, gender, age.

A non-significant increase in SSI rate was found for mastectomies. A significant decrease in SSI rate of 6% per surveillance year occurred for total hip prosthesis, indicating a 60% decrease after 10 years. And decreasing trends in SSI rate per 1-year increase in surveillance time appeared of 6% for replacement of the head of the femur, of 8% for colectomy, and of 3% for knee prosthesis, although statistically not significant.
Conclusion: For one of the five surgical procedures the decreasing trend in SSI rate was statistically significant and three procedures showed a non-significant decreasing trend. These encouraging results are most likely a result of an improvement in the quality of care, caused by changes in infection control in the hospitals. This study showed that an active surveillance system with feedback of infection rates to hospital staff might be an effective strategy to reduce the SSI incidence. Additional interventions might further decrease the SSI rate, and sustaining control efforts are necessary to maintain a low SSI level.

O138 Cost effectiveness of employing a TPN surveillance nurse for the prevention of catheter-related bloodstream infections

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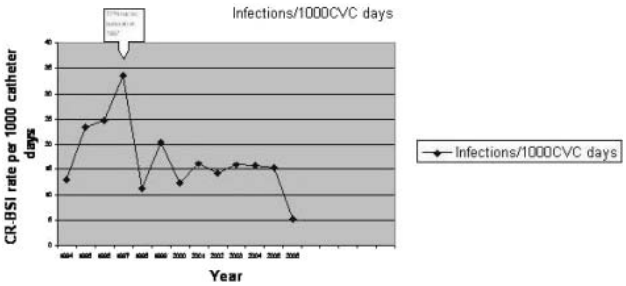
Objectives: The cost of catheter-related bloodstream infection (CR-BSI) is substantial in terms of morbidity, mortality and financial resources. Guidelines recommend dedicated multi-disciplinary teams to reduce the incidence of these infections. Total Parenteral Nutrition (TPN) is a well-recognised risk factor for CR-BSI. In our hospital, a dedicated TPN surveillance nurse was promoted from staff grade in 1997 and quarterly multi-disciplinary meetings were introduced. This study shows the reduction in CR-BSI and annual cost savings.
Methods: This study was performed in a 535-bed tertiary referral university hospital. Data was prospectively collected over a 13-year period using specific TPN records and TPN nurse notes. A total of 21,871

CVC days and 453 CR-BSI's were recorded as per CDC guidelines. All CVCs were non-antibiotic impregnated and other lines such as PICC, hickman, peripheral and home TPN were excluded.
Results: We compared the mean number of infections prior to and after the introduction of a dedicated TPN nurse. The location of patients was highest on general wards (58.1%) followed by ITU (27.9%) and HDU (13.4%). Mean CR-BSI /1000 catheter days was 19.2 prior to 1997 and 14.48 after this date. This resulted in a mean reduction of CR-BSI /1000 catheter days by 4.94. The annual mean of 28.3 infections prior to 1997 and 17.8 after this date resulted in a mean decrease of 10.5 infections per year. The saving made by preventing 10.5 infections/year was calculated using data on cost of bed days obtained from the hospital finance office. We used 12 days as the increased hospital stay attributable to CR-BSI, with 8 of these days being in the ITU and 4 days on the general ward. The cost in hospital days saved as a result of the reduction in infection rate was 127,955 Euro (excluding the cost of antimicrobials and laboratory investigations). Taking into account the salary of a TPN nurse, this resulted in a saving to the hospital of 71,255 Euro per annum.



Conclusion: This study shows that there was a decrease in the CR-BSI rate after the introduction of a dedicated TPN nurse and quarterly multi-disciplinary meetings. By calculating the saving made in reduction in CR-BSI we conclude that it is clinically and economically viable to employ a dedicated TPN nurse. These figures do not take into account other savings such as cost of antimicrobial treatment and other medical and laboratory investigations

O139 Building a benchmark through active surveillance: the Italian network SPIN-UTI

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Objectives: The Italian Nosocomial Infections Surveillance in Intensive Care Units (ICUs) (SPIN-UTI) project of the Italian Study Group of Hospital Hygiene (GISIO) – Italian Society of Hygiene, Preventive Medicine and Public Health (SItI), was implemented to ensure standardisation of definitions, data collection and reporting procedures coherently with the HELICS-ICU benchmark.
Methods: Hospitals were invited to join the SPIN-UTI project by GISIO members. Before starting surveillance, participant ICUs were gathered in order to involve the key stakeholders in the project through participated planning. A first phase consisted in a training session to share and further refine the protocol and the data collection tools. Four electronic data forms, designed using SPSS Data Entry Enterprise Server (SPSS Inc.) for web-based data collection, were presented and discussed. After a monitoring phase, the final SPIN-UTI protocol and tools were produced integrating as much as possible the conclusions of debates and discussions and the analysis of the methods used in the existing national surveillance protocols. The six-months patient-based prospective survey was performed from November 2006 to May 2007, preceded by a one-month surveillance pilot study to assess the overall feasibility of the programme, and to determine the needed time and resources for participant hospitals.
Results: The SPIN-UTI project included 53 ICUs, 3,046 patients with length of stay longer than two days and 35,152 patient-days. A total of 619 infections were reported accounting for an incidence rate of 20.3 per 100 patients and an incidence density of 17.6 per 1000 patient-days. The most frequently encountered infection site was pneumonia and "*Pseudomonas aeruginosa*" the most frequent infection-associated microorganism, followed by "*Staphylococcus aureus*" and "*Acinetobacter baumannii*". Site-specific infection rates, for pneumonia, bloodstream infections, central venous catheter-related bloodstream infections and urinary tract infections, stratified according to patient risk factors, were below the 75th percentile reported by the HELICS network benchmark.
Conclusion: The SPIN-UTI project showed that introduction of ongoing surveillance does seem to be possible in many Italian hospitals. The study provided the opportunity to participate in the HELICS project using benchmark data for comparison and for better understanding of factors that impact on associated risks.

O140 *E. faecalis* and *P. aeruginosa* are useful epidemiological markers for the analysis of transmission events on intensive care units

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Objectives: At least 15% of nosocomial infections in intensive care units are due to the cross-transmission of causative organisms between patients. In contrast to endogenous infections, these exogenous infections are more likely to be prevented by infection control measures. Therefore, knowledge of surrogate organisms for the analysis of patient-to-patient transmissions would be useful.
Methods: As known from KISS, the German hospital infection surveillance system, *A. baumannii*, *E. faecium*, *E. faecalis*, *K. pneumoniae*, *P. aeruginosa* and *S. aureus* are amongst the most common pathogens responsible for nosomial infections in ICUs. Over a two years period, primary isolates of these six "indicator" organisms cultured from clinical samples of patients who stayed at 11 ICUs at two University hospitals were genotyped by PFGE (pulsed field gel electrophoresis). Genetically indistinguishable isolates from different patients were considered as cross-transmissions if the patients were hospitalised on the same ICU in a temporal proximity of at least 9 days. The association between the isolation of each of the six pathogens and cross transmission (transmission events per 1000 patient days and per 100 cultured organisms) was analysed.
Results: During 100 781 patient days, 100 829 microbiological specimens from 24 362 patients were sampled (average investigation density of 1.0 sample per patient * day) and 3 370 indicator organisms were cultured (29.1% *S. aureus*, 22.6% *E. faecalis*, 22.2% *P. aeruginosa*, 14.6% *E. faecium*, 8.3% *K. pneumoniae*, 3.1% *A. baumannii*). Altogether, 416 cross-transmissions (incidence density of 4.1 transmissions per 1 000 patient days, ranging between 1.4–8.4 per 1 000 patient days depending on the respective ICU) were discerned. Of these, 19% were due to *E. faecalis*, 14% to *E. faecium*, 11% to *P. aeruginosa* resp. *S. aureus*, and 6.4% to *A. baumannii*. There was a significant correlation between the isolation of *E. faecalis* and *P. aeruginosa*, and transmissions per 1000 patient days resp. per 100 cultured organisms (Spearman correlation coefficient >0.8, $p < 0.01$).
Conclusion: *E. faecalis* and *P. aeruginosa* are useful epidemiological markers for the analysis of patient-to-patient transmissions on ICUs in non-outbreak settings. As cross-transmissions are indicative of poor clinical care, transmission analysis of *E. faecalis* and *P. aeruginosa* may be used to monitor adherence to standard infection control precautions.

O141 Is screening for colonisation of extended-spectrum β-lactamase producing *Klebsiella pneumoniae* in intensive care unit necessary in the absence of an outbreak?

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Objectives: Extended-spectrum β-lactamase producing Klebiella pnemoniae (ESBL-KP) is a concern of importance for nosocomial infections

and responsible for outbreaks particularly in intensive care units (ICUs). Colonisation is a prerequisite for infection by ESBL-KP. The aim of this study was to evaluate the impact of routine detection for colonisation with ESBL-KP in the absence of an outbreak and to detect the rate of infections related to colonisation or transmission.
Methods: This prospective study was conducted in a medical intensive care unit (MICU) with nine beds at Hacettepe University Hospital, a tertiary care centre in Ankara, Turkey. The study was approved by the local ethical comittee. Patients admitted to MICU between August 2002-March 2003 were screened for ESBL-KP by obtaining pharyngeal and rectal swab cultures upon admission, 48th hour of admission and weekly until discharge. The staff of the MICU were not informed about the colonisation status of the patients. Standard infection control practices continued during the study period. All lactose positive and oxidase negative colonies isolated from swabs and cultures, taken when there is a suspicion of infection, were tested for ESBL production and antimicrobial susceptibility testing were performed for ESBL positive isolates according to CLSI recommendations. Enterobacterial repetitive intergenic consensus PCR (ERIC-PCR) method was performed for the evaluation of the genetic diversity.
Results: A total of 100 patients were included in the study and 332 rectal, 332 oropharyngeal swabs were performed from these patients. Eight patients were found to have rectal colonisation with ESBL-KP after 48 hours of ICU admission. ERIC-PCR revealed six different genotypes. One of the colonised patient developed a catheter infection with ESBL-KP. The isolate that was recovered from the catheter infection was found genotypically identical with the colonised strain. The strains that were isolated from three other patients who shared MICU in the same period were all in an unique ERIC-PCR pattern.
Conclusion: Performing routine surveillance cultures for detection of colonisation with ESBL-KP is a hard work for clinical microbiology laboratories. This study indicates that the addition of microbiological screening does not improve the detection or prevention of ESBL infection in the absence of an outbreak.

O142 Impact of routine surgical and intensive care units admission surveillance cultures on hospital-wide nosocomial MRSA infections in a university hospital

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Objectives: Despite the existence of an established infection control programme to combat MRSA transmission that includes effective barrier precautions, an alert system for readmitted MRSA patients and screening of roommates, a steady increase in nosocomial MRSA infections was observed at Hannover Medical School – a 1400-bed university hospital – in 2004. In reaction to this, an extended admission screening policy was established in mid-2004 which included surgical wards and intensive care units (ICUs). The aim of this study was to assess the impact of this intervention.
Methods: The study used a single-centre prospective quasi-experimental design to evaluate the effect of MRSA admission screening policy on the incidence density of nosocomial MRSA infected patients per 1000 patient-days (MRSA-infpat/1000pd) in the whole hospital. The screening policy was implemented during a six month period (July to December 2004). The effect on MRSA-infpat/1000 pd was calculated by segmented regression analysis of interrupted time series with 30 months prior and 24 months after the implementation period.
Results: As a consequence of the implementation of admission screening, the number of surveillance cultures increased nine-fold increase, from 2.3 in 2002 to 20.4 nares cultures per 1000 patient-days in 2006. The intervention had a highly significant hospital-wide effect on the incidence density of MRSA infections. Segmented regression analysis showed both a significant change in level (–0.122 MRSA-infpat/1000pd, 95% CI, –0.205 to –0.004) and a significant change in slope (–0.008 MRSA-infpat/1000pd per month, 95% CI, –0.013 to –0.003) after the intervention. A decrease of MRSA infections by 63% is a conservative estimate of the reduction between the last month before intervention (0.259 MRSA-infpat/1000pd) and the last month in the analysed post-intervention period (0.096 MRSA-infpat/1000pd).
Conclusion: This is the first hospital-wide study which investigates the impact of introducing admission screening in ICUs and non-ICU wards as single intervention to prevent nosocomial MRSA infections performed with a time series regression analysis. Admission screening is a potent tool in controlling the spread of MRSA infections in hospitals.

Pharmacokinetics and pharmacodynamics

O143 Intracellular activity of antibiotics against *Staphylococcus aureus* internalised human skin keratinocytes: comparison with THP-1 macrophages

S. Lemaire, F. Van Bambeke, J.P. Pirnay, G. Verween, P. De Corte, D. De Vos, P.M. Tulkens (Brussels, BE)

Objectives: Relapsing and chronic *S. aureus* infections has been ascribed to intracellular persistence. While the activity of antibiotics against *S. aureus* in macrophages has already been extensively studied, little is known in keratinocytes. We examine here the activity of antibiotics commonly used for the treatment of SSSI infections (oxacillin, vancomycin, linezolid, rifampicin) and of more recently approved ones (quinupristin-dalfopristin, daptomycin, moxifloxacin) in a model of human keratinocytes in comparison to human macrophages.
Methods: We used a fully susceptible *S. aureus* strain (ATCC 25923). MICs were determined by broth microdilution. Infection of human skin keratinocytes and THP-1 macrophages was performed following published methods (Br. J. Dermatol. 2002; 146:943–51; AAC 2006;50:841–51). Activity was measured after 24 h of exposure to a wide range of drug extracellular concentrations and data [change in log CFU vs. log of extracell. conc.] analysed using a pharmacological dose-response model (Hill equation).
Results: The table shows MICs values. Sigmoidal dose-responses were seen for al drugs ($R^2 > 0.9$) and allowed to calculate static concentrations (SC) and Emax values.

Antibiotic	MIC (mg/L)	Intracellular activity in			
		THP-1 macrophages		Keratinocytes	
		Static concentr. $(mg \cdot L^{-1})/\times MIC^a$	Emax[b]	Static concentr. $(mg \cdot L^{-1})/\times MIC^a$	Emax[b]
Oxacillin	0.25	0.60/2.4	–0.8±0.1	0.70/2.8	–0.9±0.1
Vancomycin	0.5–1	3.26/3.2–6.5	–0.8±0.1	3.10/3.1–6.2	–1.1±0.3
Linezolid	0.5	4.74/9.5	–1.2±0.4	2.40/4.8	–1.3±0.2
Rifampicin	0.03	0.06/2	–1.4±0.1	0.001/0.03	–2.8±0.1
Daptomycin	0.125	1.63/13	–1.4±0.3	1.74/13.9	–1.4±0.2
Moxifloxacin	0.06	0.20/3.3	–2.2±0.1	0.08/1.3	–1.7±0.1
Quinupristin-Dalfopristin	0.5	0.50/1	–2.4±0.2	0.70/1.4	–1.9±0.4

[a]extracellular concentration of antibiotics resulting in no apparent bacterial intracellular growth (no change of CFU); [b]maximal effect for drug concentration at infinity.

Conclusions: Pharmacological parameters were similar in both cell types for all drugs (except rifampicin, which showed higher Emax and lower static concentration in keratinocytes). The data extend to keratinocytes the poor activity of oxacillin, vancomycin and linezolid seen against intracellular *S. aureus* in macrophages. They also suggest to further assess the potential advantages offered in this context by daptomycin, moxifloxacin, quinupristin/dalfopristin, and rifampicin.

O144 Ceftobiprole and Levofloxacin are synergistic against an isolate of *Pseudomonas aeruginosa* as evaluated in a neutropenic mouse thigh infection model

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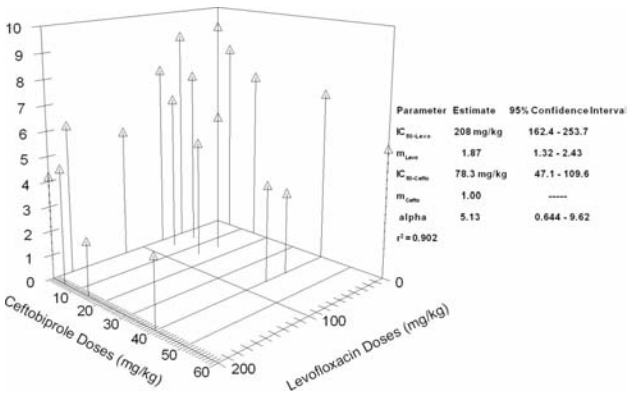
Objectives: The pharmacodynamic interaction of Ceftobiprole (Cefto) plus Levofloxacin (Levo) was examined in a mouse thigh infection model, as judged by the Greco Interaction Model.
Methods: The mouse thigh infection model described by Craig was employed. Mice were made neutropenic by cyclophosphamide, 150 mg/kg day −4 and 100 mg/kg day −1. *P. aeruginosa* ATCC 27853 was injected into the posterior thighs of mice at a burden of 10^6 CFU. A preliminary study was performed as a dose range of Cefto alone and Levo alone. Sigmoid Emax analysis was employed to identify areas of greatest Fisher Information. As this model has 3 parameters, each preliminary experiment allowed identification of 3 doses most informative as to linking exposure to response. This allowed a second experiment to be performed (16 cohorts): no-treatment control; 3 Cefto alone; 3 Levo alone and 9 combination therapy cohorts (3X3). At 24 h, mice were sacrificed, the posterior thighs dissected, homogenised and plated after serial dilution on plates without drug (estimation of total bacterial load) and on plates containing 3 x baseline MIC of either Cefto or Levo (isolates resistant to one of the drugs). The Greco URSA model was fit to the data using ADAPT II. Weighting was the inverse of the observation variance.
Results: For Cefto as a single drug, Emax (cell kill) was 4.1 log (CFU/g) and EC50 was 12.6 mg/kg. For Levo as a single drug, Emax was 6.9 log (CFU/g) and EC50 was 145.4 mg/kg. Combination therapy was statistically significantly synergistic (α – interaction parameter 5.13; 95%CI 0.644–9.62). The cell kill is demonstrated in the Figure. Perhaps more importantly, the combination efficiently suppressed resistance emergence to either of the two drugs. Doses of Levo greater than 50 mg/kg, combined with Cefto doses greater than 15 mg/kg generally suppressed all resistant bacteria.



Ceftobiprole-levofloxacin combination therapy against *P. aeruginosa* ATCC27853.

Conclusion: Combining two agents may be preferred as empiric therapy for patients with serious infections suspected to be caused by *P. aeruginosa*. In this in vivo model, Cefto-Levo combination therapy was synergistic against this *P. aeruginosa* isolate and suppressed emergence of resistance. The importance of this finding warrants further evaluation with other isolates of *P. aeruginosa* including those with derepression of the AmpC β-lactamase.

O145 Pharmacodynamics of amikacin against *Acinetobacter baumannii*: modelling bacterial response to drug-selective pressures

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Objective: We have previously developed a mathematical model predicting the response of *P. aeruginosa* to meropenem (J Antimicrob Chemother 07) and levofloxacin (Ann Biomed Eng 07). However, the applicability of the model to other pathogens is unknown. We extended our model to predict the effect of various fluctuating amikacin (AMK) exposures on *A. baumannii* (AB), an emerging bacterium associated with multidrug resistance.
Methods: Time-kill studies (TKS) with 10^7 CFU/ml of AB ATCC BAA 747 at baseline were performed. AMK at 0–32× MIC was used for 24h (MIC = 4 mg/l). The experimental data were used to derive estimates of the best-fit model parameters, and AB response to various AMK exposures over 72h was predicted via a 3-dimensional response surface. The computer model predictions were subsequently validated using an in-vitro hollow fiber infection model (HFIM); AMK profiles (t1/2 = 2.5h) corresponding to 7.5 mg/kg every 12h and 30 mg/kg every 24h were investigated over 72 hours.
Results: TKS data were satisfactorily captured by the mathematical model (r^2 = 0.93). A significant initial reduction in bacterial burden was predicted for both AMK exposures examined. Regrowth over time due to resistance emergence was predicted for 7.5 mg/kg every 12h, while eradication of bacterial population was predicted with 30 mg/kg every 24h. These predictions correlated well with our experimental data in HFIM.
Conclusions: The mathematical model was reasonable in predicting extended AB response to various fluctuating AMK exposures qualitatively, based on limited input data from TKS. In view of its robustness and efficiency, our mathematical modeling approach holds great promise as a high throughput screening tool for dosing regimen selection in antimicrobial (pre)-clinical development.

O146 Temocillin protein binding is concentration-dependent and not restricted to albumin

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Objectives: Temocillin (TMO), a 6-alpha-methoxy penicillin, is highly protein bound in healthy volunteer (85%). However, we recently observed that the level of protein binding (PB) is lower in critically ill patients receiving TMO during continuous infusion (down to less than 70% for some individual patients). This variation could have a major therapeutic impact as it is generally presumed that only the free form of β-lactams is active. We have therefore studied the binding properties of temocillin to whole human serum and purified human serum albumin (HSA).
Method: Solutions of HSA in water (1, 2.5, and 5%), whole serum, and PBS-diluted serum solutions were spiked with known concentrations of TMO (25 to 1000 mg/L). Free fractions of TMO were measured by HPLC after separation from proteins by ultracentrifugation through 30 kDa cut-off membranes (Centrifree®, Millipore, MA).
Results: PB of TMO in HSA and serum was concentration-dependent and decreased with increasing concentrations of TMO. A sigmoid function (Hill equation) could be fitted to all PB-binding data (see parameters estimates in the table).
In HSA, EC50 values were proportional to the HSA concentration and residual binding increased with the concentration. In serum, the maximal PB observed was 86% (consistent with the observations made in healthy volunteers), and the Hill's slope was much less steep than for HSA. The serum PB decreased gradually to 24% at 1000 μg/ml. Interestingly, PB remained unchanged (about 20%) when serum spiked with high TMO concentrations (1000 mg/L) were diluted with PBS, except for high dilutions (>40×).

Matrix	Max binding %	Min binding* %	EC50 μg/ml	Hill's slope
HSA 1%	96	3	116	–2.14
HSA 2.5%	96	11	286	–3.28
HSA 5%	96	38	514	–4.35
Serum	86	3	329	–1.05

*Calculated residual binding.

Conclusion: TMO PB is concentration-dependent. Considering the normal concentration of HSA in serum (around 4%), the shapes and values of the binding curves, and the maximum binding observed in serum, our data suggests that other factors are involved in TMO protein binding and does not bind exclusively to albumin. Moreover, our data may partially explain the observations made in critically ill patient (an increased free fraction) because these patients often have low serum proteins levels.

O147 MDR1 (P-glycoprotein) and MRP1 (multidrug resistance-related protein 1) eukaryotic efflux transporters do not affect the cellular accumulation and intracellular activity of tigecycline towards intraphagocytic *Staphylococcus aureus*

S. Lemaire, F. Van Bambeke, M.P. Mingeot-Leclercq, P.M. Tulkens (Brussels, BE)

Objectives: Efflux pumps expressed by eucaryotic cells can reduce antibiotic accumulation, impairing thereby their activity towards intracellular bacteria (JAC 2003, 51:1167–73). We have examined whether this could apply to tigecycline, a new glycylcycline for which intracellular activity against *S. aureus* has been evidenced in infected polymorphonuclear leucocytes (JAC 2005, 56:498–501).
Methods: The cellular accumulation of tigecycline was assessed by microbiological assay after 5 h of incubation in 3 cell lines differing by their level of expression of multi-drug efflux transporters (human THP-1 [expressing MDR1]; Madin-Darby Canine Kidney Cells [MDCK]: wild type cells and variants overexpressing either MDR1 or MRP-1). Intracellular activity was determined against *S. aureus* (ATCC 25923) phagocytosed by THP-1 macrophages after 24 h exposure to increasing concentrations of tigecycline and fixed concentrations of inhibitors of P-gp (verapamil) or MRP (gemfibrozil).
Results: Tigecycline accumulated 3–4 times in all 3 cell types, disregarding their level of expression of MDR1 or MRP-1 transporters. Against phagocytosed *S. aureus*, tigecycline exerted a concentration-dependent activity (sigmoidal concentration-effect relationship obeying to the Hill equation) with static effect for an extracell. conc. of 0.15 mg/L, and maximal effect (Emax) of -0.76 ± 0.05 log CFU. These parameters were not modified in the presence of efflux pump inhibitors.
Conclusions: In contrast to daptomycin (AAC 2007, 51:2748–57) and azithromycin (JAC 2003, 51:1167–73), for wich accumulation and intracellular activity are reduced by MDR1, tigecycline is substrate of neither MDR1 or MRP-1 efflux transporters, two well characterised multidrug efflux pumps widely expressed in many eucaryotic cells.

O148 Selection of daptomycin (DAP)-resistant *Staphylococcus aureus* mutants with DAP alone and in combination with rifampicin at subtherapeutic concentrations: simulations using an in vitro dynamic model

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Objective: A bell-shaped relationship between the ratio of area under the curve (AUC) to the MIC and the enrichment of daptomycin (DAP)-resistant *S. aureus* have been reported in our simulations of multiple-dose DAP pharmacokinetics. AUC/MICs near 200 h (that are lower than the therapeutic AUC/MICs) protected against the selection of resistant mutants whereas they were maximally enriched at AUC/MICs of 32–64 h. To determine if such selection could be prevented with DAP in combination with other agents, the pharmacodynamics of DAP alone and combined with rifampicin (RIF) at the subtherapeutic AUC/MICs were studied in an in vitro model.
Methods: *S. aureus* ATCC 43300 and a clinical isolate *S. aureus* 866 with MICDAPs of 0.2 and 0.4 mg/L, respectively, and MICRIF of 0.012 mg/L were exposed to five-day dosing of DAP (AUC/MIC 64 h) alone and in combination with RIF (AUC/MIC 100 and 500 h). Bacterial growth on agar plates containing 0x, 2x and 4xMICDAP was examined daily. The cumulative effect of each simulated treatment on susceptible *S. aureus* sub-populations was expressed by area under the time-kill curve (AUBC) measured from time zero to 120 h.
Results: Both simulated DAP+RIF regimens were more efficient against the two organisms and the DAP-resistant mutants. With *S. aureus* ATCC 43300, RIF100 and RIF500 lowered the AUBC 1.4- and 1.9-fold relative to DAP alone. With *S. aureus* 866, 1.6- and 1.8-fold reductions in AUBC were observed, respectively. With both organisms, mutants resistant to 2x and, to a lesser extent, 4xMICDAP were enriched in the mono-treatments with DAP but not in the DAP+RIF500 treatments. RIF500 resulted in a 1.3 (*S. aureus* ATCC 43300) and 3.4 (*S. aureus* 866) fold reduction in the area under the bacterial mutant curve (AUBMC) for mutants grown in the presence of 2xMICDAP. The protective abilities of DAP+RIF100 were similar to those of DAP+RIF500 for *S. aureus* 866 but were weaker for *S. aureus* ATCC 43300. The DAP+RIF100 combination postponed but did not prevent the production of DAP-resistant mutants.
Conclusions: This study suggests that DAP+RIF combinations can reduce the selection of DAP-resistant *S. aureus* mutants.

O149 Efficacy of antibiotics in cerebrospinal fluid

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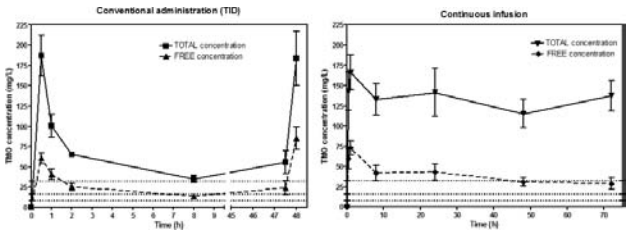
Objectives: Treatment of shunt-associated ventriculitis (SAV) requires administration of an effective antibiotic regimen to avoid life-threatening sequelae. Due to their antimicrobial spectrum and penetration properties fosfomycin, cefepime and rifampicin are frequently considered for treatment of SAV. The present study aimed at describing killing kinetics of fosfomycin, cefepime and rifampicin in cerebrospinal fluid (CSF) compared to conventional growth medium. In addition, the general applicability of human CSF as growth medium for bacteriologic experiments was explored.
Methods: CSF was collected from over 700 patients who did not receive antibiotics. Time-kill curves over 24 hours were performed using drug concentrations of 0.25, 0.5, 1, 2, 4, and 8-fold the MIC of the *Staphylococcus aureus* test strain. Time-kill curves were preformed in Muller-Hinton-broth (MHB), in CSF, and in CSF incubated with 5% CO2. Ambient CO2 served to adjust the pH of CSF to physiological values.
Results: As expected drug concentrations above the MIC led to effective bacterial killing in MHB. Addition of fosfomycin to CSF did not induce any inhibition of bacterial growth if incubated at ambient air. Experiments performed in CSF with physiological pH achieved by 5% CO2 incubation showed sustained growth inhibition only at fosfomycin concentrations of 8-fold the MIC, while re-growth was observed for all lower fosfomycin concentrations. For rifampicin killing patterns similar to those of fosfomycin were observed. In contrast, cefepime exerted higher killing in CSF with CO2 incubation than in MHB, while being less effective in CSF at ambient air than in MHB.
Conclusions: CSF is eligible for performing bacteriologic experiments. CSF and MHB differ substantially with regard to bacterial growth and killing. In CSF with CO2 incubation bacterial re-growth occurs at fosfomycin or rifampicin levels above the MIC, while cefepime concentrations below the MIC exert inhibitory effects. We conclude that dependent of the investigated antibiotic the use of MHB might overestimate or underestimate the efficacy of antibiotics in CSF. Thus, CSF should be considered as medium for evaluation of the efficacy of antimicrobial drugs for intracerebral infections in order to better reflect the clinical setting.

O150 Temocillin 6g daily in critically ill patients: continuous infusion vs. conventional administration

P.F. Laterre, T. Dugernier, X. Wittebole, N. Couwenbergh, P.M. Tulkens, S. Carryn (Brussels, Ottignies, BE)

Objectives: Temocillin (TMO) is currently used in critically patients but PK data in this population are scarce. TMO has recently been proven to be suitable for continuous infusion (De Jongh et al., JAC in press). However, serum free concentrations (SFC) achieved with the conventional posology (2g q12h) may be close to the MIC in critically ill due to increased volume of distribution and altered PK/PD. In order to achieve acceptable SFC we have therefore administered 6g of TMO daily either as 2g q8h (TID) or in continuous infusion (CI).
Method: ICU patients with documented Gram-negative infection susceptible to TMO were included and randomised in both groups. Patients with renal replacement therapy were excluded. Blood samples were collected after the 1st and 7th dose for the TID group and daily for 4 days for the CI group and assessed by HPLC. SFC of TMO were measured after separation from proteins by ultracentrifugation through 30 kDa cut-off membranes (Centrifree®, Millipore, MA). Safety was assessed through adverse events monitoring.
Results: 8 patients were included in each group (8 intra-abdominal infections, 6 LRTI, and 2 UTI; 4 patients had a positive bloodculture). No significant differences were observed between groups for age (SEM) [TID: 70 (4), CI: 64 (5)], SOFA scores (SEM) [TID: 8.4 (1.3), CI 6.5 (1.1)], or creatinine clearance (SEM) [TID: 52 (10), CI: 71 (14)]. TMO total and free concentrations for both dosing regimen are shown in the figure (value are represented as mean±SEM).
For TID the time above the Belgian susceptibility breakpoint (i.e. 16 mg/L) for the SFC reached 86% on average. For CI, the SFC remained 100% of the time above the breakpoint and even reached 2-times this concentration for more than 60% of the time (lowest mean free concentration: 28.8 mg/L). As expected in critically ill patients the alpha half-life in the TID group was highly decreased during the first injection (less than 1h) while the terminal half-life remained close to 3h. Finally, despite the high serum concentrations reached, no adverse events related to TMO were observed



Conclusion: TMO is safe at the posology of 6g per day. If TID seems sufficient to achieve PK/PD goal for β-lactam efficacy, CI allows a better margin of security compared to the breakpoint. These data suggests that the optimal dose for TMO in critically ill without renal replacement therapy should be increased to 6g daily.

O151 An exploratory analysis of the relationships between voriconazole plasma concentrations and clinical efficacy

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Objective: To explore possible relationships between voriconazole concentrations in plasma and efficacy in the Phase II/III clinical studies.
Methods: Plasma samples taken randomly during voriconazole therapy, in 1008 ITT patients from 9 Phase II/III clinical studies of fungal infection, were analysed using a validated hplc assay. Data were summarised as weekly mean concentrations with one/weekly window. The relationship between plasma concentration and efficacy was investigated by logistic regression (using one overall mean plasma concentration/patient) upon the binary outcome variable – success or failure – as determined by the investigator at end of therapy. Success was defined as a complete or partial response and failure as any other. Polynomial models were used to investigate linearity and curvature. The impact of covariates was assessed. MICs were determined using CLSI methodology (M27A-2 for yeasts and M38A for moulds).
Results: There were 529 yeast (70% success) and 479 mould (59% success) infections identified at clinical baseline. Mean voriconazole plasma concentrations were divided into 11 bands. The relationship between response and mean plasma level was non-linear and best fitted to a quadratic polynomial model ($p = 0.001$). Mean plasma levels <0.5mg/L or >6.0mg/L were associated with clinical responses of <60%. Individually significant covariates were: study ($p < 0.001$), protocol ($p < 0.001$), primary/salvage therapy ($p < 0.001$), region ($p = 0.0165$), yeast/mould ($p = 0.004$), duration of therapy ($p < 0.001$), site of infection ($p = 0.003$), underlying disease ($p < 0.001$) and mean levels < or >6mg/L ($p < 0.001$). When combined in the quadratic model none of these covariates altered the non-linearity. In 469/1009 (46%) patients where a baseline pathogen MIC was available there was a linear relationship between mean voriconazole free drug level/MIC ratio and clinical response ($p = 0.014$).
Conclusions: There is a significant, non-linear relationship between voriconazole mean plasma concentration and efficacy. Covariates with a significant impact on their own, when combined do not change the curvilinear relationship. There is a significant and linear relationship between mean voriconazole free drug/MIC ratio and clinical response.

O152 Use of pharmacokinetic-pharmacodynamic principles for decision support for short-course oritavancin dosing regimens for complicated skin and skin structure infections

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Objectives: Optimal dose selection for Phase 2/3 clinical trials is critical for successful drug development. Application of pharmacokinetic-pharmacodynamic (PK-PD) principles provides a scientific basis for optimising both dose and duration of therapy. Dosing regimens for short-course therapy were evaluated for oritavancin (ORI), a novel glycopeptide currently in development for complicated skin and skin structure infections (cSSSI).
Methods: Using PK data from 20 intensively-sampled subjects who received ORI 800 mg IV Q24h x 5 days, a population PK model was developed. PK parameter estimates based on this model were used to conduct simulations to evaluate daily and cumulative free-drug plasma AUC values following front-loaded ORI regimens (i.e., the majority of the AUC is delivered on Day 1) of 800 to 1200 mg. Both single-and multiple-dose regimens were evaluated. Multiple-dose regimens involved a loading dose followed by a booster dose (400 or 800 mg) on Day 4, 5 or 6. Population PK analysis and simulations were performed using S-ADAPT 1.53. Free-drug AUC values for each regimen were assessed relative to those associated with a 1 and 2 log10 CFU decrease in *S. aureus* using data from a neutropenic murine-thigh infection model (Craig WA, Andres DR. ICAAC 2004, Abstr. A-1863). Single-dose free-drug AUC values in mice corresponding to a 1 and 2 log10 CFU decrease were 61 and 159, respectively. Free-drug AUC values were also assessed relative to those successfully studied for previous cSSSI studies (200 mg Q24h X 3–7 days) and *S. aureus* MIC population statistics.
Results: A 3-compartment model with zero-order input and linear clearance best described the PK data. Plasma concentrations were well fit by the model ($r2 = 0.966$). ORI 1200 mg X 1 dose or 800 mg on Day 1 followed by 400 mg on Day 5 provided mean cumulative free-drug AUC values which exceeded non-clinical free-drug AUC targets and which were similar to those values for the previously studied 3–7 day 200 mg Q24h regimen (see Table).
Discussion/Conclusion: The approach to support selection of short-course dosing regimens described herein is especially useful for agents such as ORI, which display a concentration-dependent pattern of bactericidal activity and a long half-life. It is predicted that front-loaded ORI regimens will result in improved response rates for patients with

cSSSI relative to those regimens previously studied. These data were used to support dose selection for a Phase 2 cSSSI study.

Regimen (Infusion duration in minutes)	Mean cumulative free-drug AUC in plasma			
	Day 4	Day 5	Day 6	Day 7
200 mg Q24h×3 days (45)	180	196	206	214
200 mg Q24h×4 days (45)	218	249	267	279
200 mg Q24h×5 days (45)	218	287	320	340
200 mg Q24h×6 days (45)	218	287	358	393
200 mg Q24h×7 days (45)	218	287	358	432
800 mg on Day 1 (90)	263	276	286	293
800 mg on Day 1, 400 mg on Day 4 (90/60)	339	382	407	424
800 mg on Day 1, 400 mg on Day 5 (90/60)	263	352	391	415
800 mg on Day 1, 400 mg on Day 6 (90/60)	263	276	362	399
800 mg on Day 1, 800 mg on Day 4 (90)	415	487	529	556
800 mg on Day 1, 800 mg on Day 5 (90)	263	428	497	536
800 mg on Day 1, 800 mg on Day 6 (90)	263	276	438	504
1000 mg on Day 1 (120)	328	345	357	366
1200 mg on Day 1 (120)	394	414	428	439

Challenges from the rise of resistant pathogens: Part 1 (Symposium organised by Janssen Cilag)

S157 Changing landscape of antimicrobial resistance in Europe: focus on Gram-positives

M. Wilcox (Leeds, UK)

Antimicrobial resistance patterns are constantly changing throughout the world. Starkly different resistance patterns have arisen among countries within Europe, and between Europe and North America. The distinction between hospital-acquired and community-acquired infections is becoming blurred, with strains that once were prevalent in the hospital now found in the community and vice versa. For example, the virulence determinant Panton-Valentine leukocidin (PVL) can no longer be considered a marker of just community-acquired staphylococcal infections. For both meticillin-resistant *Staphylococcus aureus* (MRSA) and meticillin-sensitive *S. aureus* (MSSA), the traditional opinion on the role of PVL as a virulence determinant is being questioned.

The success of a particular organism does not depend on where it arose initially but rather on whether it can enter and prosper in specific niches. Successful control of a pathogen depends upon eliminating the niche or preventing transmission. A notable example is MRSA in hospitals in England, where the prevalence of MRSA-related infections rose to among the highest in Europe. The incidence of MRSA bacteraemia has decreased markedly in response to major evidence-based healthcare initiatives and infection targets that were introduced to combat the spread of the resistant pathogen. Reservoirs of MRSA are still prevalent, notably among the elderly in care homes in the United Kingdom.

Resistance development by one organism can be an unintended response to measures taken against other organisms. In Canada, for example, the prevalence of vancomycin-resistant enterococci (VRE) markedly increased with the change in use of antimicrobial agents (from metronidazole to vancomycin) to treat *Clostridium difficile* infections. Data indicate that usage of some antibiotics correlates with the nosocomial prevalence of MRSA. However, compared with data on Gram-negative bacterial infections, information is limited regarding the effect of the changing use of antibiotics for Gram-positive bacterial infections within the hospital setting. Hospitals should take measures to ensure that resistance does not continue to spread among Gram-positive organisms. Increasing therapeutic options for Gram-positive infections open up new opportunities to alter antibiotic prescribing practices, with the potential to impact on the prevalence of endemic resistant pathogens.

S158 Critical success factors for the management of complicated skin infections

J. Solomkin (Cincinnati, US)

To guide the development of agents to treat skin and skin structure infections (SSSIs), the FDA has created two categories: uncomplicated infections (eg, simple abscesses, impetiginous lesions, furuncles, cellulitis) and complicated infections (deeper tissue involvement, often requiring significant surgical intervention, eg, abscesses, extensive cellulitis, diabetic foot infections, and necrotising infection). The latter infections, often with associated underlying disease, carry an increased risk of Gram-positive and Gram-negative co-pathogens. These infections, occurring in hospitalised patients, are unlikely to rapidly resolve with surgical measures alone; assessment of severity may help determine when emergency surgery and antibiotics are required. Severity assessments for SSSIs include clinical signs of systemic inflammation and local findings, particularly the size of abscesses or cellulitis, or signs of necrotising infection, including bullae and subcutaneous haemorrhage. In these more severe cSSSIs, empiric therapy effective against the organisms subsequently identified on culture is important in limiting the extent of tissue loss and more rapidly resolving the acute illness. In the US and some European areas, the frequency of MRSA associated with SSSIs has risen dramatically in the past 5 years, reaching up to 60%. Because of the high prevalence of resistant strains in the community (community-acquired MRSA), recommendations in the US are to treat *Staphylococcus aureus* infections with agents active against MRSA and to assess microbiologic sensitivities when available.

Vancomycin has been the standard parenteral treatment for MRSA, but concerns exist regarding toxicity and increasing hetero- and other forms of resistance. New cephalosporins with activity against MRSA may provide clinicians with useful options for the treatment of cSSSIs. Ceftobiprole, an investigational broad-spectrum anti-MRSA cephalosporin, has demonstrated efficacy in two multi-centre, double-blind, active controlled trials in various types and severity of skin and soft tissue infections, including diabetic foot infections.

S159 From CAP to HCAP to HAP: new considerations and approaches

T. Welte (Hannover, DE)

Pneumonia acquired outside the hospital or identified within 48 hours of admission (with presumed acquisition prior to hospitalisation) is defined as community-acquired pneumonia (CAP). Pneumonia acquired in the hospital or up to 1 week after discharge (with presumed acquisition during hospitalisation) is defined as hospital-acquired pneumonia (HAP). Healthcare-associated pneumonia (HCAP) is acquired by patients in frequent contact with the healthcare system (eg, haemodialysis patients, nursing home patients). HCAP deserves separate recognition, as treatment issues are more similar to HAP than CAP.

Scoring systems may aid the decision of where (eg, outpatient setting or in hospital) and how aggressively to treat pneumonia patients. Appropriate sampling with sufficient sputum for culture can be obtained in only half of pneumonia patients, so antibiotic therapy is often based on suspected infection and knowledge of probable pathogens. Most prevalent in CAP is *Streptococcus pneumoniae* followed by *Haemophilus influenzae*, while Gram-negative bacilli and *Staphylococcus aureus* are most common in HAP, with risk of *Pseudomonas aeruginosa* and *S. aureus* highest in pneumonia that develops from 2 days to a week after hospitalisation.

Pneumonia in nursing home patients, the elderly, and multi-morbid patients is commonly due to Gram-negative bacilli, *S. aureus* or *S. pneumoniae*, including multi-drug resistant *S. pneumoniae* (MDRSP). Prior antibiotic treatment increases the risk of meticillin-resistant *S. aureus* (MRSA) and *P. aeruginosa* in pneumonia in all settings. *Staphylococcus aureus* may cause severe CAP, especially after influenza infection.
Resistance can increase both mortality and costs. Reports of community acquired (CA)-MRSA are increasing in the United States and are already high in some European countries (eg, Greece, the UK). However, European countries with lower resistance patterns have also reported cases of CA-MRSA, suggesting that this pathogen will be more important in the future.

S160 The role of MRSA in pneumonia

R.G. Wunderink (Chicago, US)

The spectrum of meticillin-resistant *Staphylococcus aureus* (MRSA) pneumonia now extends across all categories of pneumonia, including CAP, HAP, HCAP, and pneumonia in immunocompromised patients. Origin may not be as important as formerly; distinctions between CAP, HCAP, and HAP are blurring as strains move from institutions to the community and vice versa. In general, resistance in HCAP in Europe is less common than in the US due to differences in nursing home populations, or because definitions of HCAP differ. In the US, nursing home patients may be sicker, with a greater frequency of comorbidities such as chronic tracheotomies, multiple decubiti, and impaired consciousness.
Risk factors for MRSA HAP and VAP are similar to other multidrug resistant pathogens. The exception is that the reservoir for MRSA is often the nares and nasopharynx. For this reason, screening for MRSA on admission for patients at high risk for pneumonia or skin and soft-tissue infections is increasingly common. This has important implications for VAP prevention strategies as none of the components of most VAP prevention "bundles" specifically address this issue. Our research also suggests that conventionally-accepted risk factors for CA-MRSA CAP may not be good predictors.
Time to the first antibiotic dose has been a measure of quality of care in pneumonia. Delays of several days in initiating appropriate therapy, as may occur while awaiting culture results, are associated with worse outcomes. Given the difficulties, delays, and low yield inherent in diagnosing the aetiology of pneumonia, antibiotic treatment often is empiric. As MRSA is a common pathogen, empirical anti-MRSA coverage is increasingly common. The negative predictive value for MRSA in an adequate lower respiratory tract sample is very high. However, despite microbiologic results, clinicians are often reluctant to narrow antibiotic therapy. Delayed response to therapy even when appropriate antibiotics are used is common.
Vancomycin is the most commonly used antibiotic for MRSA but success rates range from only 35% to 57% in MRSA VAP. Treatment failures due to vancomycin MICs in the upper range of sensitive may not be overcome without pushing vancomycin levels above the tolerated dose. Vancomycin also does not affect exotoxin production, an important aspect of CA-MRSA. Currently available agents or those in development may offer advantages over older agents for the treatment of MRSA pneumonia.

Community MRSA: hyper virulent or just hype?

K172 Community meticillin-resistant *Staphylococcus aureus*: hypervirulent or just hype?

F. Tenover (Atlanta, US)

Reports of community-associated meticillin-resistant *Staphylococcus aureus* (CA-MRSA) infections among healthy persons who have had no recent contact with healthcare and who have none of the traditional risk factors for MRSA infection have been increasing at a substantial rate in the last 7 years. Children in daycare, sports participants, military recruits, prisoners in institutional settings, pig farmers, and men who have sex with men are among the many diverse groups who appear to be at risk for CA-MRSA infections. While these are primarily skin and soft tissue infections, severe and rapidly fatal syndromes, including necrotising pneumonia, also have been reported, particularly among young adults. The pulsed-field gel electrophoresis type USA300 (ST-8; SCCmec type IV) is prominent among CA-MRSA infections in the United States, and recent evidence confirms that USA300 has spread to Europe, South America, and the Pacific Islands. Genomic sequencing data from multiple USA300 isolates confirm the dramatic clonal expansion of the USA300 MRSA strain, which has been the predominant strain type isolated both from patients with invasive CA-MRSA infections and from patients presenting to emergency departments with uncomplicated skin infections. However, data on the virulence of USA300 strains and the outcomes of CA-MRSA infections are quite divergent. Is this really a fearful new epidemic strain, or is CA-MRSA more a media event than a medical problem?

The myth and realities of decision making in national vaccination programmes

S173 Funding drugs based on cost-effectiveness: do vaccines warrant a different approach?

P. Beutels (Antwerp, BE)

Vaccines and vaccination programmes have features that require special consideration when assessing their effectiveness and cost-effectiveness for public funding. Special features of vaccines are related to herd immunity, quality of life losses in very young children, parental care and work loss, time preference, uncertainty, eradication, macroeconomics and tiered pricing. These features are discussed, and related to specific topical childhood vaccinations against rotavirus, human papillomavirus, varicella-zoster virus, influenza and pneumococcus.
Advisory committees on public funding for vaccines (which often give advice on all pharmaceuticals in a country) should be knowledgeable about the nature and the impact of these special features, and flexible enough to allow these features to be explored in cost-effectiveness calculations presented to them.

Molecular diagnostics

O175 A novel method for the rapid detection of *Mycobacterium tuberculosis* complex in respiratory and non-respiratory specimens

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Objective: Rapid detection of *Mycobacterium tuberculosis* complex (MTB) directly on clinical respiratory and non-respiratory specimens is essential for an efficient management of patients suspected of tuberculosis. A new method, based on the Transcription Reverse-transcription Concerted reaction (TRC; Tosoh Corporation, Japan) enables one step amplification and real-time detection of the MTB 16S rRNA target directly on respiratory and non-respiratory specimens. The performance of the EXTRAGEN M.TB kit, TRCRapid M.TB kit and TRCRapid-160 analyser was evaluated in three laboratories.
Method: An analytical sensitivity assay was performed by adding MTB reference strains at known concentrations to an MTB negative sputum sample and assayed in triplicate. The TRC results were compared to smear and culture and the exact number of bacilli was determined by counting the colony forming units (CFU) in solid media.
In addition, 633 respiratory and non-respiratory specimens were tested and all results were compared to smear and culture. All specimens were

subjected to standard procedures and 500 µl of the processed samples were used for RNA extraction following the TRCRapid-160 protocol.
Finally, 126 patients (Paris cohort only) were followed-up to evaluate clinical performances of the TRC-method.
Results: The analytical sensitivity assay results showed that the TRC method can detect as little as 50 CFU/ml.
Of the 633 clinical specimens evaluated, 22 were excluded from the evaluation (1 contaminated, 11 showed inhibition and 10 were identified as Mycobacteria Other Than Tuberculosis (MOTT)), thus a total of 552 respiratory specimens and 59 extra-pulmonary specimens were included. Overall sensitivity and specificity of TRC for pulmonary specimens was 86.8% and 96.3% respectively. Sensitivity and specificity of TRC for extrapulmonary specimens was 83.3% and 95.7% respectively.
Of the 126 patients analysed, 15 patients presented and were treated for pulmonary tuberculosis. Twelve out of 15 treated patients had a positive culture and 13 out of 15 treated patients had a positive TRC. This gave a sensitivity of culture and TRC of 80% and 86.7% respectively. Specificity of culture and TRC were 100% and 93.9% respectively.
Conclusion: Theses results showed that a rapid detection of MTB was possible (less than 2 processing hours for 14 specimens) using ready to use reagents for real time detection of MTB in clinical samples with a high sensitivity and specificity.

O176 The impact of a PCR assay for detection of candidaemia on antifungal drug prescribing in critically ill adults

R. McMullan, L. Metwally, P. Coyle, S. Hedderwick, B. McCloskey, H. O'Neill, H. Webb, R. Hay (Belfast, UK)

Objectives: Molecular assays for the diagnosis of fungal infection from clinical specimens are increasingly described. However, the extent to which these are adopted by clinical staff and, critically, the extent to which their results impact therapeutic decisions is not well described. Such information is important to justify the cost of developing and delivering novel diagnostic tests. The aim of this study was to evaluate the extent to which a clinically validated PCR assay for candidaemia impacted antifungal drug prescribing.
Method: A prospective observational study was conducted in a mixed 17-bed ICU in which the assay, with turn-around-time of 24-hours, was made available for a period of six months. Physicians were encouraged to use the assay particularly when considering prescribing an antifungal drug in the context of probable *Candida* infection. Each assay request was recorded, as well as whether the patient received an antifungal drug within 72hr of the test request and whether this prescribing decision reflected the test result. When the prescribing decision and assay result were discordant, a reason for this was sought.
Results: In all, 55 PCR tests were requested on 45 patients. Overall, the PCR test appeared to strongly influence the prescribing decision in 44 (80%) instances whether negative or positive. An antifungal drug was prescribed within 72hr of PCR requesting in 14 (25%) instances; of these PCR requests, 4 were positive and a further one was from a patient who had a positive blood culture within 72hr of a negative PCR. For the remaining nine PCR-negative patients who received an antifungal drug, various factors were considered to over-rule the PCR test; these included drug prescribed prior to test ordering, compliance with the ward prophylaxis policy and high likelihood of infection as determined by extant pre-emptive prescribing practice.
Conclusions: While the availability of the rapid molecular assay displayed substantial capacity to modify antifungal drug prescribing in certain circumstances, it is clear that physician behaviour cannot be completely influenced by a single test result regardless of other factors. Indeed it is unlikely that this is desirable, given that no test has been shown to perform with 100% reliability. Nonetheless, rapid diagnostic methods may help to improve antifungal conservation and their contribution to the 'model' therapeutic algorithm merits further evaluation.

O177 Performance of an automated platform

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Objectives: Polymerase chain reaction (PCR) is commonly used to detect microbial nucleic acids in clinical samples. However, significant risk of PCR contamination, high reagents costs, and significant workload precluded the wide use of PCR for the aetiological diagnosis of infectious diseases. We thus established a high throughput versatile automated molecular platform and analysed its performance including the critical aspects of troubleshooting.
Methods: DNA and RNA were extracted with MagNApure (Roche) and EasyMAG (BioMérieux), respectively. RNA was converted to cDNA prior to PCR. A TECAN liquid handling system (LHS) was used for 384 well PCR plate setup. Real-time PCR was performed with an ABI 7900HT.
Results: A total of 5953 analyses and 3037 runs have been done during the study period. The median time from reception of the sample to results was overall of 28.3 hours. Time to results were significantly longer when the pathogen to be detected was a RNA virus (because of the retrotranscription step), a fungus (lysis step before DNA extraction) or a CMV/EBV quantitative PCR (runs performed only three time a week; $p < 0.001$). Longer time to results were also due to invalid runs, with need of repetition of the PCR reactions in a subsequent run: the median time to results was 27 hours for 5097 analyses that did not need to be repeated, 54 hours for 646 analyses repeated twice and 100 hours for 154 analyses repeated three times. Among all 3037 runs, 264 runs were invalid (8.7%). Runs were mainly considered invalid because of the negativity of the ten copies positive control (7.1% of a total of 3037 runs). Major causes of invalid runs were hardware problems with the liquid handling system (2.3% of all runs), use of a defective real time PCR master mix (1.8%), human procedural errors (1.8%), progressive degradation of the positive control (1.4%) and defective primer and probes (0.49%). Contaminations were detected in only 21 runs (0.7%).
Conclusions: Our platform allows to perform a comprehensive set of microbiological diagnostic tests with a high success rate (>91%). Improvements in terms of time to result and reduction of repeats are being addressed thanks to this evaluation.

O178 A mass spectrometry-based approach for *Neisseria gonorrhoeae* species identification

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Objectives: Correct species identification of *N. gonorrhoeae* remains an actual problem of sexual transmitted diseases monitoring. A large collection of gonococci isolated in Russian clinics was studied by the MALDI BioTyper system which allows to perform rapid microorganism identification based on mass spectrometry (MS) profiles.
Methods: Gonococci were grown under standard conditions (ASM, IHO). Single colonies of fresh bacterial cultures were transferred into a 1.5 ml tube (Eppendorf, Germany) with 300 µl of water (Fluka, Switzerland) and suspended well. Then, 1 ml of 96% ethanol was added. The pellet obtained by centrifugation (12000 rpm, 5 min) was suspended in 35% formic acid/50% acetonitrile and centrifuged again. Supernatant was used for further MS analysis using saturated solution of alpha-cyano-4-hydroxy cinnamic acid in 50% acetonitrile/2.5% TFA as matrix. Mass spectra were collected on a Microflex LT MALDI-TOF mass spectrometer, followed by analysis with the BioTyper 1.1 software (Bruker Daltonics, Germany).
Results: Totally, 293 bacterial isolates identified as *N. gonorrhoeae* in clinical bacteriology laboratories were investigated. For 280 (95.6%) samples the mass spectrometry profiles were matched to *N. gonorrhoeae* ATCC strain 49226 with excellent scores (2.3–2.9 with species threshold sets at 2.0). Comparative analysis of spectra showed good reproducibility within *N. gonorrhoeae* species with slight differences between isolates. The majority of peaks were constant; only three ones with m/z 4473,

5051, and 8165 changed their m/z value to 4487, 5081, and 8146, respectively, for several strains. The remaining isolates (n = 13) obtained similar mass spectra which were rather different from *N. gonorrhoeae* ATCC strain 49226 as well as from main spectra generated for 13 *N. meningitidis*, 15 non-pathogenic *Neisseria* strains and further 1671 different microorganisms stored in the BioTyper library. The initial misidentification was confirmed by Crystal *Haemophilus*/*Neisseria* (BBL) test which allowed to identify these bacteria as *Oligella urethralis*. Although *O. urethralis* is a commensal inhabitant of urogenital tract in rare cases it can be infectious. The main spectra of *O. urethralis* have been included in the new generation of BioTyper library.

Conclusion: The MALDI BioTyper system is an easy to use and highly suitable tool for the correct *N. gonorrhoeae* species identification in routine settings.

O179 Rapid screening of the human microbiome by bacterial profiling

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Objectives: It is well known that the human microbiota plays an important role in health and disease. However, the exact role of the commensal bacteria and the ways in which they influence our wellbeing remains elusive. One of the major obstacles in solving these and related questions is the analysis of the microbiome. New insights have recently been gained with high throughput sequence analysis but, although valuable, these methods are not suited for large scale screening. Here we present a rapid and straightforward assay for the profiling of the human microbiota.

Methods: The molecular method involves two features: the length of the interspace (IS) region between the 16s and the 23s rDNA and specific primer sequences discriminating Firmicuta and Bacteroidetes, the two major phyla in the human colon. The IS region is conserved within a species, but varies between species. Thus bacteria can in principle be identified by the length of their IS region. Unknown species can be recognised using the specific primer sequences. The primers were combined with non-specific reverse primers in a double label multiplex PCR. Size and colour sorting of fragments was performed in an ABI 3130XL sequencer.

For validation purposes an in silico profile of 5 bacteria was constructed based on known sequence information and tested with the cultured bacteria. Then, colonic mucosal biopsies from 20 patients were analysed using this method. Intra- and interpatient composition of colonic microbiota was analysed in 5 biopsies per patient, from coecum, flexura hepatica, flexura lienalis, sigmoid and rectum.

Results: All cultured bacteria showed a profile identical to the in silico profile. Subsequently, excellent bacterial profiles were obtained for the clinical samples. Each patient had a unique bacterial profile, with only a few fragments being identical between patients. These fragments corresponded to well known colonic bacteria such as Faecalibacterium prausnitzii and Bacteroides thetaiotaomicron. Patterns obtained from the different sites of the colon of a single patient were almost identical.

Conclusions: The bacterial profiling method proved to be identical to expected profiles based on sequence data. In addition, the method was suited for characterisation of the complex human microbiome. With this method, profiles of the human colonic microbiota can be obtained in a straightforward fashion. The method is universal, fast, reproducible and ideally suited for analysis of large sample sets.

Hepatitis

O180 HBV lamivudine resistance mutations in a cohort of mono-infected and HIV-co-infected patients

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Objectives: Pattern and prevalence of mutations between HIV-HBV coinfected (HIVpos) and HBV monoinfected (HIVneg) individuals on lamivudine (LAM) therapy have not been extensively documented. We investigated the frequency of mutations and variables potentially associated with an increased risk of HBV mutations in HBsAg+ individuals.

Methods: Among 128 patients tested for LAM resistance, after the exclusion of inappropriate tests (performed <90 days from LAM initiation or >180 days from last LAM treatment) we analysed 102 pts (64 HIVneg and 38 HIVpos). pol gene mutations were assessed by direct sequencing reverse transcriptase fragment 125–213aa, while HBV genotype by comparison with reference sequences. Association of pol mutations with selected factors was assessed by means of odds ratios fitting multiple logistic regression (MLR) equations and their 95% confidence intervals (CI), adjusting for sex, age, HIV- and HCV-coinfection, LAM exposure, HBeAg positivity and HBV genotype.

Results: Average LAM exposure was higher in HIVpos than in HIVneg (39 vs. 29 months, $p = 0.054$) as well as genotype A prevalence (58% vs. 14%, $p < 0.0001$). LAM-resistance mutations were detected in 71/102 individuals (69.6%): 26/38 (68%) HIVpos and 45/64 (70%) HIVneg patients. Mutation was significantly associated with prolonged LAM exposure (MLR-OR=4.9 CI:1.2–20.3, >3 vs <1 years) and older age (MLR-OR=1.2 CI:1.0–1.6, for each 5 years increase). M204V mutation was observed in 42 patients (60%); interestingly this mutation was found in almost all HIVpos (24/25, 92%) and only in 18/45 (40%) HIVneg patients ($p < 0.0001$). Prevalence of triple mutations (M204V+L180M+V173L) was higher in HIVpos (31% vs. 2%, $p < 0.001$). Independently to HIV-infection, HBeAg expression is more likely to be associated with M204V than M204I mutation (MLR-OR=7.9 CI:1.5–42.8).

Conclusions: In conclusion, main predictor for pol mutations is prolonged LAM exposure. In HIVpos patients, M204V and multiple mutation were more prevalent; independently from HIV positivity, M204V mutation was associated with HBeAg expression. Further studies are needed, to clarify the kinetics and significance of different mutation patterns observed in monoinfected and HIV-HBV-coinfected pts.

O181 MicroRNA deregulation in HCV-associated hepatocellular carcinoma

A. Sinigaglia, L. Barzon, E. Lavezzo, M. Trevisan, F. Farinati, G. Palù (Padua, IT)

Objectives: Recent evidences on the role of microRNAs (miRNAs) in human cancers and their possible effects on HCV replication led us to investigate miRNAs expression profile in HCV-related hepatocellular carcinoma (HCC) as compared to adjacent non-neoplastic liver and its relationship with the presence of HCV infection.

Methods: Tissue samples from 22 HCCs and adjacent non-tumoral tissue were obtained from surgical specimens removed during HCC resection. RNA extraction from samples was performed using miRNA-compatible protocols and reagents. miRNAs expression profile was investigated by microarray analysis and confirmed by real-time RT-PCR. HCV detection and typing was performed by RT-PCR-sequencing by using PCR primer pairs targeting different regions of viral genome.

Results: HCV RNA was detected in 17 out of 22 HCCs; HCV genotype 1b accounted for 60% positive cases and HCV-1a and 2 for 20% each. Analysis of miRNA expression profile in HCCs showed deregulation of a group of miRNAs, including miR-122a, which was down-regulated in 15 (76%) cases as compared with non-neoplastic adjacent liver samples; miR-195, miR-199a, and miR-199b, which were consistently under-expressed in about 60% of HCCs, and miR-145, under-expressed in 40% of cases. At variance, miR-222 was up-regulated in 50% HCCs. MiR-145, miR-222, and miR-199a showed a stronger deregulation in HCV-positive HCCs than in HCV-negative cancers. These miRNAs could play an important role in the development of HCC since they target many genes involved in the control of cell cycle and cell proliferation, as predicted by the Sanger miRBASE Sequences Database. Among target genes, there are cyclin G1 (regulated by miR-122a), cyclin E1 (potentially regulated by miR-195), map3k4 and map3k11 (targeted by miR-199a and miR-199b, respectively), junB (targeted by miR-199b),

bcl-w and cdc42 (targeted by miR-195), and ras-related protein rreb1 (targeted by miR-199a).
Conclusions: While investigating a larger number of HCC samples, these results indicate that HCC, and in particular HCV-related HCC, is characterised by deregulation of a set of miRNAs involved in the control of the cell cycle.

O182 Comparison of the COBAS Taqman PCR and b-DNA PCR in patients treated for hepatitis C genotype 1, partly co-infected with HIV

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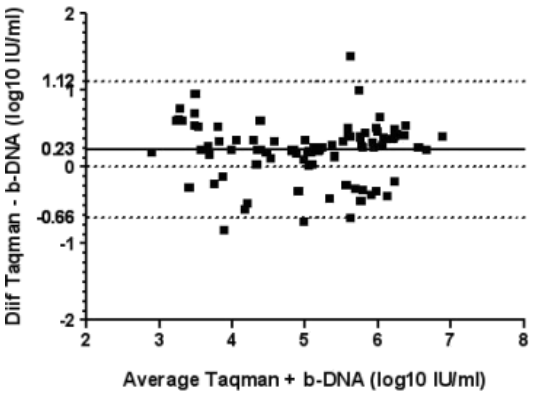
Background/Aim: Treatment decisions (e.g. discontinuation or shortening of therapy) are based on plasma hepatitis C (HVC) RNA measurements. Both quantitative and qualitative detection systems are commercially available. These systems rely either on target (Roche Cobas Taqman RT-PCR) or signal amplification (Bayer Versant b-DNA V3). Differences in linear amplification, sensitivity and specificity, lower limit of detection and standard of automated testing makes comparison between these assays mandatory. The aim of this study is to correlate plasma HCV RNA values of both the COBAS Taqman PCR and the Versant b-DNA PCR assay.
Methods: From 20 HCV genotype 1 positive patients treated with peginterferon alfa-2a and weight-based ribavirin, plasma samples at baseline, day 2 and at weeks 1, 2, 4, 8, 12, 48 of treatment and 24 weeks after therapy are collected for measurements of plasma HCV-RNA loads. Totally, paired samples with a quantitative range from undetectable to 1.6 10E7 IU/mL are compared, excluding those values that were undetectable. For both the COBAS Taqman (lower limit of detection 10 IU/mL) and the Versant b-DNA (lower limit of detection 615 IU/ml) 50 uL of plasma is used.



Results: A total of 86 paired samples are analysed with a median value for the Taqman assay of 5.05 log IU/mL versus a median value of 4.93 log IU/mL for the Versant b-DNA assay. In 69% of the measurements the Taqman PCR shows a higher HCV viral load than the b-DNA PCR. This is mainly for measurements in the higher viral loads, i.e. above 5log10 IU/mL. There is an excellent correlation between both assays (Spearman ranks coefficient 0.902, $p < 0.01$). A Bland-Altman analysis, shows a bias of 0.23 log10 IU/mL with a standard deviation of 0.45 (95% CI of –0.66 to 1.12 log10 IU/mL).
Conclusion: There is an excellent correlation between the COBAS Taqman and the Versant b-DNA assay. Overall, compared to the bDNA assay, the COBAS Taqman PCR measurements are higher, mainly within the higher viral loads.

O183 The first 48 hours of viral response during therapy differentiate between rapid viral response and null response in hepatitis C genotype 1 patients

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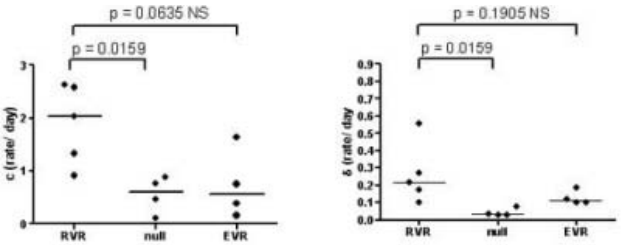
Background / Aim: During peginterferon/ribavirin therapy, decrease of plasma HCV RNA occurs in a biphasic pattern, consisting of a rapid first phase (48 hours) of eliminating free virus and a slower second phase of clearing infected hepatocytes. Previous models showed no difference in rapid first phase between patients with an early viral response (EVR) versus a null response. Recently, a rapid viral response (RVR) proved important in shortening therapy. Because viral decay rates in these patients have not been analysed, our aim is to compare the first and second phase decay in patients with a RVR versus those with an EVR or a null response.
Methods: From HCV genotype 1 positive patients treated with peginterferon alfa-2a and weight-based ribavirin, plasma samples at baseline, day 2 (48 hours) and at weeks 1, 2, 4, 8, 12, 48 of treatment and 24 weeks after therapy, are collected. The viral decay rates/ day are calculated for both the rapid first phase after 48 hours (rate c) and the slower second phase from day 2 until week 12 (rate d). Standard accepted definitions for both EVR and null response are used whereas RVR is defined as a qualitatively undetectable HCV-RNA at week 4 of treatment.
Results: A total of 13 patients are analysed with 5 patients showing a RVR, 4 a null response and 4 an EVR. The viral decay rates for both the first and second phases are statistically significant between RVR and null responders; pc = 0.0159 with c = 2.04 vs. 0.61 and pd = 0.0159 with d = 0.22 vs. 0.03 respectively (see figure). Between patients with a RVR and an EVR there is a trend toward a faster response in the first phase decay but no difference in second the phase decline. Baseline viral load (below or above 800,000 IU/ml) and body weight at baseline were of no influence on viral kinetics in both the first and second phase between clinical patient groups.



Conclusion: A faster decline of plasma HCV RNA during the first 48 hours of therapy differentiates between patients with a RVR and null responders.

O184 Comparison of quality of life in inactive hepatitis B virus carriers versus chronic hepatitis B patients versus normal Turkish population

M. Tasbakan, O. Sertoz, H. Pullukcu, S. Calik, O. Sipahi, T. Yamazhan (Izmir, TR)

Objective: It was aimed to evaluate the QOL in inactive HBsAg carriers with a control group who had a diagnosis of chronic hepatitis B and had not received any antiviral therapy, yet.
Methods: The study sample was consisted of two groups. First group was comprised of inactive HBsAg carriers recruited from individuals who were regularly followed-up in Infectious Diseases & Clinical Microbiology outpatient clinic of our setting due to inactive HBV infection [HBV DNA levels $<10^3$ copies/mL, Robogene Hepatitis B virus quantitation kit, Roboscreen, Germany) and normal biochemical parameters (Aspartat amino transferase-AST, alanine amino transferase-ALT, bilirubin, no HIV or HCV infection, no drug abuse.)]. Second

group was recruited among patients who had a diagnosis of chronic HBV infection [Hepatic activity index ⩾4 in liver biopsy, HBV DNA >20,000 IU/ml and AST, ALT ⩾2× normal] and who were not undergoing active treatment yet. Both groups were requested to fill health related quality of life by means of short form 36 (HRQOL-SF-36) questionnaire and a form asking data of age, gender and education. The data of two groups were compared between each other and both groups were compared with QOL data of normal Turkish population.

Results: Of 131 inactive HBsAg carriers and 38 patients with chronic HBV diesease 128 (50 females, 78 males, mean age 41.4±12.3) and 28 (9 males, 19 females, mean age 36.68 ±10.56) accepted to be included in the study. The QOL data of group 1 and 2 are shown in Table 1. The groups did not show any significant difference by means of age and gender, though a significant difference was observed in educational level. HRQOL of patients showed a profile similar to that of the general Turkish population, with normal physical functioning, and vitality scores, but statistically significant lower role limitations due to emotional problems, bodily pain, general health perceptions, social functioning, role limitations due to physical health problems, and mental health domain scores ($p < 0.001$). HRQOL of patients with chronic HBV disease was also significantly lower in role limitations due to emotional problems, bodily pain, general health perceptions, social functioning, and role limitations due to emotional problems scores ($p < 0.001$).

Table 1: Comparison of quality of life between inactive HBsAg carriers and patients with chronic HBV infection and not undergoing active treatment

SF-36 domains	Inactive HBsAg carriers n = 128	Chronic HBV infection n = 28	Covariance analysis
Physical functioning	86.05±15.01*	80.71 (22.8)	F = 1.55 Df = 1.153 P = 0.22
Role limitation – physical*	77.15±35.49	54.46 (37.3)	F = 9.52 Df = 1.153 P = 0.002
Bodily pain	79.43±21.47*	70.25 (22.2)	F = 2.47 Df = 1.153 P = 0.11
General health perception	61.82±19.01*	59.59 (22.7)	F = 0.29 Df = 1.153 P = 0.59
Vitality	67.89±19.74	64.58 (22.7)	F = 0.29 Df = 1.153 P = 0.59
Social functioning	79.29±19.43*	71.42 (27.6)	F = 2.27 Df = 1.153 P = 0.13
Role limitation – emotional	76.04±34.73*	58.33 (42.2)	F = 3.64 Df = 1.153 P = 0.06
Mental health	66.58±16.89*	65.85 (22.5)	F = 0.00 Df = 1.153 P = 0.9

Means (with standard deviations), *$p < 0.05$.

Conclusion: QOL in inactive HBsAg carriers was quite similar to that of patients with chronic HBV disease and both inactive HBsAg carriers and patients with chronic disease had lower QOL than normal Turkish population.

Bacterial virulence factors: does in vitro activity reflect in vivo relevance?

S188 The Panton-Valentin leukocidin as an example of a bacterial toxin underestimated clinical relevance

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The history of Panton Valentine leucocidin (PVL), one of the toxins of *Staphylococcus aureus*, began in 1894. The torch of PVL research was then handed down over the decades between small groups of scientists fascinated by *S. aureus* toxins. Knowledge advanced in fits and starts, notably with the advent of new research tools.

The first studies focused on the effects of *S. aureus* on leukocytes, while the latest, 100 years on, involves genetic characterisation of the leucocidin. In addition, highly epidemic PVL positive meticillin-resistant *S. aureus* strains have recently emerged as a worldwide health issue, leading to a further upsurge of interest in PVL. Confusion has been recently introduced in the PVL literature because one group of scientists recently failed to express PVL in their experimental models. However epidemiological, clinical and biological data obtained from staphylococcal infections in human concur to demonstrate that PVL is a virulence factor in human involved in human primary skin infection, necrotising pneumonia and bone and joint infection but not in other diseases. The relationship between PVL and virulence is independent of meticillin resistance phenotype.

Is the case for antifungal prophylaxis in the ICU now established beyond doubt?

S189 Is the case for antifungal prophylaxis in the ICU now established beyong doubt? PRO

P. Eggimann (Lausanne, CH)

Fungal infection are suspected in many critically ill patients who failed to respond to empirical treatment of nosocomial infections. However, if a substantial proportion of patients become colonised with *Candida* during ICU stay, only a minority subsequently develop an invasive candidiasis. Clinical signs of invasive candidiasis manifest only late, representing a particular challenge for diagnosis, and remains characterised by a mortality similar to septic shock (40% to 60%). An improved knowledge of the pathogenesis of candidiasis and the availability of new compounds for pre-emptive and prophylactic therapy have contributed to improve the prognosis of severe *Candida* infections, to the possible cost of the emergence of non-*albicans Candida* strains with reduced susceptibility to imidazoles. Despite growing evidence in the literature, guidelines do not integrate systematic antifungal prophylaxisfor patients at risk and empiric treatment for those who are septic with major risk factors and without documented source of infection. The Figure propose a practical approach (Adapted from: P. Eggimann, J. Garbino, D. Pittet. Management of *Candida* species infections in critically ill Patients Lancet Infectious Diseases 2003; 3:772–85). In order to avoid exposure of patients at lower initial risk, pre-emptive antifungal treatment should be based on the combination of the presence of risk factors with the dynamics of *Candida* colonisation. Finally, in non-immunocompromised patients critically ill patients, prophylaxis should be strictly restricted to highly-selected groups of patients in whom it's efficacy is proven.

S190 The case against antifungal prophylaxis in the ICU

R.A. Barnes (Cardiff, UK)

Up to half of all invasive fungal infections (IFIs)occur in non-neutropenic ICU patients and the majority are due to *Candida* species. Despite this, incidence of IFIs on most ICUs is low and ranges from 1–5%

in most European units. Many candidal infections are nosocomial and preventable.
Several RCTs have looked at prophylaxis, predominantly with fluconazole in ICU patients and three systematic reviews have been performed. Meta-analyses reportedly demonstrates a reduction in proven IFI. However, ICU populations are heterogenous:there are marked differences between Europe and North America, diagnostic criteria are not defined and prophylaxis and targeted therapy are not distinguished. Widespread use of azole drugs promotes resistance, pathogen shifts and adverse drug related events. It is necessary to establish methods to identify patients at greatest risk of IFI who will benefit most from antifungal drugs.
Numerous risk factors have been identified including length of stay, colonisation, antibiotics, surgery, central venous catheters, total parenteral nutrition, gastric acid suppression, bacterial sepsis etc. but none accurately predict IFI or fungal related mortality. Several prediction rules and scoring systems have been proposed to identify subpopulations of ICU patients who would benefit from prophylaxis. These rules identify different percentages of patients – up to 85% in some centres. Many have been created and validated within the same database and none are practical or have proven robustness across all ICU populations. Cost effectiveness has never been evaluated and numbers needed to treat to prevent a single case remain high in centres with a prevalence of less than 20%. Centres reporting high prevalence of candidal infection should address infection control issues before resorting to prophylactic drugs.
Diagnosis still relies on conventional microbiological techniques of culture from sterile sites but the role of biomarkers (antigen and PCR testing) merit further investigation in ICU populations. The high negative predictive value of these newer assays has the potential to influence antifungal strategy by identifying patients who do not have fungal infection. Combined with a robust and simple model to predict the risk of developing invasive *Candida* infection, these tests that can rapidly exclude fungal infection in high-risk groups and allow a targeted (pre-emptive strategy) to be developed and supersede unnecessary prophylactic treatment.

Genetic diversity and mechanisms of genetic diversification in bacteria

O195 Characteristics of *Neisseria meningitidis* isolates causing fatal disease

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Neisseria meningitidis, named meningococci (Mc), is a major cause of acute bacterial meningitis and septicaemia worldwide. The spectrum of human meningococcal relation goes from asymptomatic carriage to fatal infection. Despite modern medical treatment about 10% of the cases with invasive meningococcal disease are fatal. The reason why some Mc causes invasive disease while others are harmless is basically unknown. Part of the explanation refers to factors within the patient, for example, the status of innate and adaptive immunity and part of the explanation is probably found within in the bacterium as such.
Objectives: The objectives of the present study were to compile and describe a selection of characteristics of fatal meningococcal isolates (n = 62) as compared to invasive non-fatal ones (n = 474) collected in Sweden during 1995 to 2004. The coverage of the fatal serogroup B isolates by four different outer membrane vesicle (OMV) vaccines was also estimated.
Methods: The isolates were characterised by serogroup, serotype, genosubtype, multilocus sequence type and antibiogram. Basic epidemiological data were also gathered.
Results and Conclusion: The results for the 62 fatal isolates (fatality rate 12%) comprised of 34 serogroup B (11%), 17 C (12%), 9 Y (17%) and 2 W-135 (8%). Characteristics that were associated with a higher mortality were age, gender, serogroup Y, serotype 14 and 15, genosubtypes P1.7,16-29,35 and P1.5-1,10-4,36–2. On the contrary were non14/non15 serotypes, the genosubtypes P1.5-1,10-8,36-2; P1.7-2,4,37 and P1.7,16,35, and reduced sensitivity for penicillin G associated with a decreased mortality. The presently discussed OMV vaccines could, based solely on the complete genosubtype, theoretically cover up to 44% of the fatal serogroup B cases and up to 100% if every variable region by itself is capable to induce protection. Alarming is however the fact that among the invasive but non-fatal ones three isolates did not express a functional PorA protein and will thereby not be covered by any PorA components in these vaccines.
Our present 62 fatal respectively 474 non-fatal isolates form a well-defined basis that can be further characterised with supplementary methods and for example investigated concerning new vaccine antigens and potential pathogen-specific genes.

O196 Identification of a "hot spot" for integration of mobile elements in *Enterococcus faecium*

J. Top, W. van Schaik, M. Bonten, R. Willems (Utrecht, NL)

Objectives: The aim of the current study was to determine the integration site and exact size of the esp containing putative pathogenicity island (PAI) of *Enterococcus faecium* strain E1162 and to investigate sequence heterogeneity adjoining the PAI integration site in PAI positive and negative isolates.
Methods: The genome sequences of the PAI-negative strain E1071 and the PAI-positive strain E1162 have been determined in our laboratory (Van Schaik et al., in preparation). From the genome sequences, regions that were homologous to the flanking sequence of the esp containing PAI in E1162 were identified in E1071 and in another PAI negative strain, *E. faecium* DO, which has a publicly available genome sequence. This allowed us to identify the PAI integration site. Subsequently, PAI integration and sequence heterogeneity adjoining the integration site was determined using normal and long-range PCR in 17 PAI-positive and 34 PAI-negative strains using combinations of PAI specific primers and primers specific for the flanking genes.
Results: DNA alignments between E1162 and DO revealed integration in E1162 of a 61-kb large DNA fragment, containing the esp gene, in the 3′ end of an open reading frame with high identical to orf1671 of *E. faecium* DO. This integration resulted in a 54-bp duplication. At the 5′ end of the E1162 PAI a 22-kb region with high similarity (up to 100%) to an *E. faecalis* mobile element was found, while the 3′-end is homologous to orf13 to orf23 of Tn916. Interestingly, in strain E1071 another 8.3-kb element with no homology to the PAI of E1162 was integrated at the same position. PCR demonstrated that in all 17 PAI-positive isolates the PAI was integrated in the DO orf1671 homolog. In 23/33 PAI-negative strains, PCR confirmed no integration at this site, however, in one isolate PCR indicated integration of a fragment of at least 13-kb. In 9 strains no PCR product using primers spanning the integration site was obtained suggesting either integration of DNA elements too large to amplify or polymorphisms at the primer annealing site.
Conclusions: The striking variety of mobile DNA inserted into the DO orf1671 homolog suggests that this site may be a "hot spot" for integration of exogenously acquired genetic elements in *E. faecium*.

O197 Variability of Inc group of conjugative plasmids and CTX-M gene environments in Enterobacteriaceae nosocomial strains isolated from Russia

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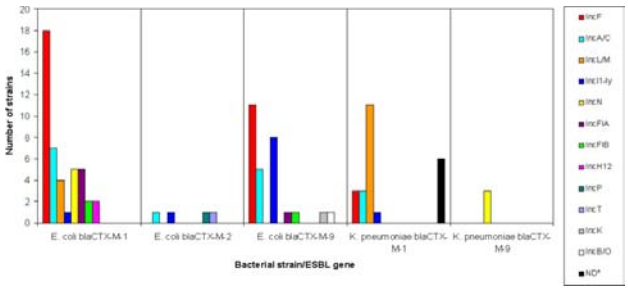
Objectives: CTX-M is the most prevalent type of extended-spectrum β-lactamase (ESBL) among clinical isolates [85% including *Escherichia coli* (n = 205) and *Klebsiella pneumoniae* (n = 151)] collected from 2003 to 2007 in Russia. Subtypes of blaCTX-M genes were identified: blaCTX-M-1 (92%), blaCTX-M-9 (6%), blaCTX-M-2 (1%), and a combination of blaCTX-M-1 and blaCTX-M-9 (1%). Plasmid localisation of blaCTX-M genes and a description of their gene environments are the focus of this study.

Methods: PCR mapping and DNA sequencing were used for detection, localisation, and identification of the genes under study. Conjugation was performed in broth using *E. coli* C600 (RifRAzR) as the recipient strain. Antimicrobial resistance phenotypes were determined by broth microdilution. Plasmid DNA was extracted by the method of Kado & Liu (1981) and plasmid incompatibility (Inc) group identification was determined by PCR-based replicon typing (Carattoli et al., 2005).
Results: All isolates examined carried blaCTX-M genes on large plasmids (~70–160 kb). Sixty plasmids were confirmed to be conjugative. Restriction fragment length polymorphism (RFLP) analysis indicated several different subgroups among plasmids of equivalent size. Plasmids appeared to belong to different Inc groups varying by genus and blaCTX-M allele (Fig.1). IncF, a major conjugative plasmid group in *E. coli* producing CTX-M1-like enzymes, included ISEcp1, IS26, and mucA structures. IncL/M, a major plasmid group in *K. pneumoniae* CTX-M1-positive strains demonstrated the same gene arrangement, but with more variants because of additional combinations and small insertions. Gene environments of CTX-M9-like genes include ISEcp1, IS26, and IS903 structures on IncI1, IncF, IncA/C (*E. coli*), and IncN (*K. pneumoniae*) plasmids. A unique *E. coli* strain had ORF153 upstream of a CTX-M2-like gene on an IncI1/IncP plasmid.

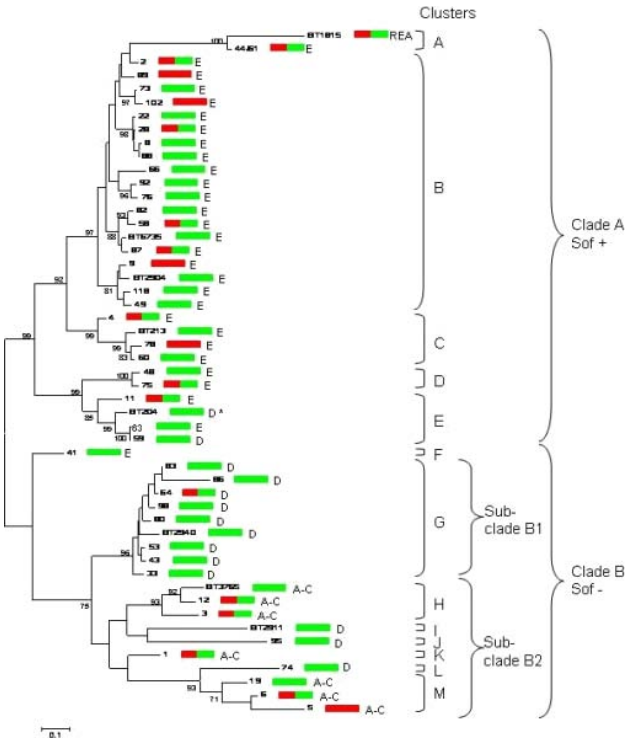


Conclusion: Nosocomial *E. coli* and *K. pneumoniae* isolates from different regions of Russia carry blaCTX-M genes on high-molecular weight plasmids belonging to various Inc groups. CTX-M gene environments are different for blaCTX-M subgroups and bacterial genera indicating differences in gene transmission mechanisms.
Acknowledgements: This study was done on the frame of the ISTS#2913/BTEP#62 Project.

O198 Genetic diversity and evolution of group A *Streptococcus* M protein

P.R. Smeesters, P. Mardulyn, A. Vergison, R. Leplae, L. Van Melderen (Gosselies, Brussels, BE)

Objective: Group A Streptococci (GAS) are divided in 150 emm-types based on the sequence of the N-terminal 50-amino acids (aa) hypervariable region of the M protein. M is a major virulence factor, anchored in the membrane through a conserved C-terminal region. M is composed of 4 regions (A, B, C and D) formed of repeat blocks. Such structures are substrates for intragenic homologous recombination which may lead to antigenic variation. Epidemiological studies performed in developed countries underlined the small number of different emm-types. However, a larger diversity of emm-types was found in developing countries, notably Brazil. To gain insights into the molecular bases for such difference, the genetic relatedness of the whole surface exposed part of M proteins of Belgian and Brazilian isolates was investigated.
Methods: Multiple alignments of the M sequences (from the N-terminus to a conserved sequence in the D2 repeat; 200 to 435 aa) from 51 representative emm-types (selected among a well-characterised Belgian and Brazilian epidemiological collection by a clustering method) were performed. A phylogenetic tree was constructed using the neighbour-joining algorithm. Several methods were assayed on the same dataset with congruent results.
Results: The prevalence of the Belgian and Brazilian isolates was similar in clades A and B. Clade B contains two monophyletic groups showing geographical preference, sub-clade B1 was mainly composed of Brazilian isolates while sub-clade B2 contained 55% of the Belgian isolates and only 14% of the Brazilian ones. B2 presented the particularity of being composed of isolates that are distantly related. Multiple alignments revealed that (i) the genetic diversity is mostly generated by a high degree of sequence variation in the C repeat region and (ii) by an increase of the number of A and B repeat leading to an overall increase of the size of the M proteins. The Dn/Ds analysis showed that purifying selection drives the evolution of M.
Conclusion: Despite a small number of different emm-types and consequently an apparent low diversity, the overall genetic diversity of the M proteins from the Belgian isolates was comparable to that of the Brazilian ones. The evaluation of the whole surface exposed sequence variations will shed light on the molecular mechanisms involved in virulence and host colonisation as well as the selective pressure driving the antigenic variation and evolution of the M protein.



Genetic diversity of M protein among Belgian and Brazilian GAS isolates. The *emm*-type of each sequence is indicated next to the bar. Green and red squares indicate Brazilian and Belgian strains respectively. REA indicate a rearranged *emm* pattern. The evolutionary history was inferred using the Neighbor-Joining method. Bootstrap value higher than 70% are shown next to the branches (500 replicates). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used for the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method and are in units of the number of amino acid substitutions per site. The rate variation among sites was modeled with a gamma distribution (shape parameter = 0.5). Thirteen clusters (A–M) were defined based on an arbitrary defined maximal genetic distance of 0.37 substitutions per site in each cluster. Clade A and B group Sof-positive and -negative *emm*-types respectively. Sof activity is unknown (*).

O199 **The *Mycoplasma pneumoniae* MPN229 gene encodes a protein that selectively binds single-stranded DNA and promotes *Escherichia coli* RecA-promoted homologous DNA recombination**

M. Sluijter, T. Hoogenboezem, N.G. Hartwig, C. Vink (Rotterdam, NL)

Objectives: *Mycoplasma pneumoniae* has previously been characterised as a micro-organism that is genetically highly stable. In spite of this genetic stability, homologous DNA recombination has been hypothesised to lie at the basis of antigenic variation of the major surface protein, P1, of *M. pneumoniae*. In order to identify the proteins that may be involved in homologous DNA recombination in *M. pneumoniae*, we set out to characterise the MPN229 open reading frame (ORF). This ORF bears sequence similarity to the gene encoding the single-stranded DNA-binding (SSB) protein of *Escherichia coli*, which is known to play a vital role in DNA recombination as well as DNA replication and repair.
Methods: The MPN229 ORF was amplified by PCR on genomic DNA from *M. pneumoniae* strain MAC, cloned into a protein expression vector, and expressed to high levels in *E. coli*. The resulting protein, which was termed Mpn SSB, was purified, and its activity was determined in various DNA binding and recombination assays.
Results: The MPN229 ORF has the capacity to encode a 166-amino acid protein with a calculated molecular mass of 18.4 kDa. The amino acid sequence of this protein (Mpn SSB) is most closely related to that of the protein predicted to be encoded by the MG091 gene from *Mycoplasma genitalium* (61% identity). Mpn SSB was expressed in *E. coli* and purified to >95% homogeneity. The purified protein was found to: (i) exist primarily as dimer in solution, (ii) strongly and selectively bind single-stranded DNA (ssDNA) in a divalent cation- and DNA substrate sequence-independent manner, and (iii) stimulate *E. coli* RecA-promoted DNA strand exchange.
Conclusion: The Mpn SSB protein represents the *M. pneumoniae* counterpart of SSB proteins from other bacteria. The protein efficiently binds ssDNA and stimulates *E. coli* RecA-promoted homologous DNA recombination. As a consequence of these activities, the Mpn SSB protein may play a crucial role in DNA recombinatorial pathways in *M. pneumoniae*. The results from this study will pave the way for unraveling these pathways and assess their role in antigenic variation of *M. pneumoniae*.

Community-acquired bacterial infections

O200 **PTX3 and C-reactive protein in severe meningococcal disease**

T. Sprong, P. Giuseppe, C. Neeleman, A. Mantovani, S. Signorini, J.W.M. van der Meer, M. van Deuren (Nijmegen, NL; Rozano, Milan, IT)

The long pentraxin PTX3 is an important element of the innate immune system and has potential as a diagnostic tool in inflammatory conditions. We studied PTX3 in patients admitted to an ICU with severe meningococcal disease and compared it with the short pentraxin CRP.
26 patients with meningococcal disease were studied, 17 patients presented with meningococcal septic shock (shock group) and 9 patients with meningococcal meningitis or bacteraemia (no shock group). PTX3 and CRP were measured by ELISA.
High plasma concentrations of PTX3 (median 579 mg/L) were seen at admission in patients with meningococcal disease. Concentrations were significantly higher in patients with shock compared to patients without shock (median 801 mg/L and median 256 mg/L, P = 0.006, respectively). In contrast, CRP at admission was lower in the shock group as compared to the no shock group (median 58 mg/L and median 165 mg/L, P = 0.008, respectively). Time course of PTX3 and CRP showed that in patients with shock, PTX3 concentration was highest at admission, whereas CRP peaked 48 hours after admission. In patients with meningitis however, CRP peaked within the first 24 hours after admission (Figure 1).

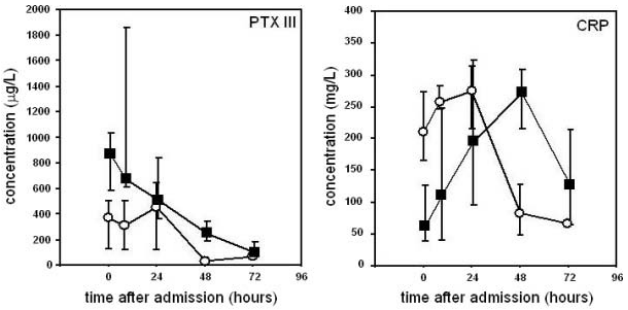


Figure 1. Time course of PTX3 and CRP in meningococcal disease. Closed squares indicate patients with shock, open circles patients with meningitis. Medians and interquartile ranges are shown.

High PTX3 and low CRP concentration at admission discriminated between presence and absence of shock (area under the ROC curve 0.85, P = 0.007 for PTX3 and 0.84 P = 0.01 for CRP). PTX3 was not correlated with disease severity (PRISM) and days spent in ICU. CRP concentration at admission showed a strong negative correlation with parameters of disease severity.
In conclusion, PTX3 was an early indicator of shock in patients with severe meningococcal disease that followed a pattern of induction distinct from CRP. High PTX3 and low CRP plasma concentrations discriminated between the presence or absence of shock. Thus, a high PTX3 level at admission may alert the clinician for imminent deterioration and shock.

O201 **Short versus long course antibiotic therapy for acute pyelonephritis in adults: a meta-analysis of randomised controlled trials**

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Objective: Despite the high incidence of acute pyelonephritis in the community setting, there is no consensus on the optimal duration of treatment. To our knowledge, no quantitative synthesis has been previously performed investigating the issue of shortening the prevailing therapeutic schemes for the treatment of this disease.
Methods: We searched PubMed and Cochrane Central Register of Controlled Trials to identify and extract data from relevant RCTs for a meta-analysis comparing the effectiveness and toxicity of short versus long regimens for the treatment of acute pyelonephritis. Inclusion criteria were: RCTs involving adult patients with acute pyelonephritis and comparing regimens with the same antibiotic, in the same daily dosage but administered for a different duration of time (a short-course and a long-course).
Results According to our initial search, 205 potentially relevant articles were retrieved from PubMed and 136 from the Cochrane Central Register of Controlled Trials. Finally, 4 RCTs were eligible for inclusion in our meta-analysis. There was no difference between the short course treatment (7–10 days) and the long course treatment (14–21 days) for acute pyelonephritis regarding clinical success [odds ratio (OR)=1.27, 95% confidence interval (CI) 0.59–2.7] and microbiological eradication (OR=0.80, 95% CI 0.13–4.95), and relapse (OR=0.92, 95% CI 0.29–2.88). Also there was no difference between the short course and the long course treatment regarding adverse events (OR=0.57, 95% CI 0.29–1.11) and withdrawals due to adverse events (OR=0.37 95% CI 0.09–1.45)
Conclusions: The findings of our meta-analysis suggest that short course regimens are as effective and safe as long course regimens for the treatment of acute pyelonephritis. However, due to the relative scarcity of data, more RCTs focused on this important clinical question are needed in order to come to a definitive conclusion.

O202 Procalcitonin and C-reactive protein as markers for infection and mortality in patients with severe sepsis and septic shock

J. Koeze, E. Metz, R.M.L. Brouwer (Enschede, NL)

Introduction. Procalcitonin (PCT) is used as a marker to differentiate sepsis from other non-infectious causes of the systemic inflammatory response syndrome (SIRS). C-reactive protein (CRP) is also used as a marker for sepsis and severity of disease. The Surviving Sepsis Campaign (SSC) has been developed to improve the management, diagnosis, and treatment of sepsis. In March 2007 the SSC guidelines were introduced in the ICU and the departments of Internal Medicine and Surgery of our hospital. We studied the clinical application of PCT and CRP plasma concentrations in the detection of severe sepsis /septic shock and the assessment of severity of disease.
Hypothesis. PCT and CRP are useful in discriminating bacterial infections from other causes of SIRS and can be used as markers for mortality.
Methods. Prospective observation study in patients admitted to the departments of internal medicine, surgery and the ICU of a large non-academic teaching hospital who were enrolled in the SSC. PCT and CRP plasma levels were determined at inclusion (0) and 24 hour (24) after inclusion in de SSC registration. Patients were classified according to outcome (hospital discharge / mortality), presence of infection confirmed by microbiological culture and PCT / CPR levels. Data were analysed using non-parametric statistical methods (Pearson Chi-Square and Kruskal-Wallis test).
Results. Ninety one patients (age 65±1.6 mean±s.e.m., 70% male) were included. 46 (50%) were admitted to the ICU, 45 patients (50%) were treated in the wards. Infection was confirmed by cultures in 50% of patients. There was no difference in PCT (0/24) between patients with and without microbiological culture proven infection. 45 patients (50%) were classified as severe sepsis, 46 (50%) as septic shock. Overall hospital mortality was 21% and there was no difference in PCT (0/24) and CRP (0/24) between survivors and non-survivors.
Conclusion. PCT does not differentiate patients with culture confirmed infection and severe sepsis/septic shock from patients with severe SIRS and suspected but no proven infection. PCT and CRP are no indicators of outcome in patients with severe sepsis and septic shock.

O203 *Streptococcus suis* infection and risk factors for death in northern Thailand

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Objectives: To describe clinical characteristics of *Streptococcus suis* infection, a re-emerging zoonotic disease in Northern Thailand, and to determine the risk factors for death of this disease.
Methods: The retrospective cohort study was conducted among patients who were diagnosed culture-confirmed *S. suis* infection in Sawanpracharak Hospital, a tertiary care centre in Nakornsawan Province, Northern Thailand, between January 2005 and October 2007. The medical records were reviewed and clinical data was extracted. Risk factors for death were determined by multivariate analysis.
Results: There were 66 patients with a mean (SD) age of 52.9 (11.5) years and 68% were male. The most common risk of *S. suis* infection was eating of unwell-cooked pork or internal organs (87%). Clinical presentations included acute meningitis (52%), sepsis without localised infection (27%), septic shock (12%), endocarditis (8%), and septic arthritis (1%). Hearing loss was observed in 35% and significantly associated with meningitis. Positive cultures of *S. suis* were recovered from blood (92%) and cerebrospinal fluid (CSF, 73%). All strains were susceptible to penicillin, cefotaxime and levofloxacin. CSF profiles (median values) among patients with meningitis were as follows: white blood cell, 450 cells/mm^3; PMN 48%; L 45%; protein 309 mg/dl; glucose 3 mg/dl; and 40% had positive Gram stain. The mortality rate was 17% and 64% of death occurred in the first 24 hours. Patients who died were more likely to have occupational contact with raw pork ($p = 0.016$), headache ($p = 0.021$), gastroenteritis ($p = 0.008$), septic shock ($p < 0.001$), low platelet ($p = 0.025$), low serum bicarbonate ($p < 0.001$), low albumin ($p < 0.001$), high ALT ($p = 0.040$), and high total bilirubin ($p = 0.001$). Multivariate analysis revealed that only occupational contact with raw pork ($p = 0.008$) and high ALT ($p = 0.001$) were the only significant risk factors for death.
Conclusion: *S. suis* infection commonly presented with acute meningitis or sepsis without localised infection. The most common risk of *S. suis* infection is eating unwell-cooked pork. The risk factors for death are occupational contact with raw pork and high ALT. Education for people in Northern Thailand to eat well-cooked pork and parts and safely contact with raw pork is crucial. Patients who present with high ALT need intensive care and closed monitoring. A prospective interventional study is needed to minimize the incidence of disease and death from *S. suis* infection.

O204 Association between group A beta-haemolytic streptococci and vulvovaginitis in adult women: a case control study

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Objectives: Vulvovaginitis including fluor vaginalis accounts for approximately 250,000 visits in the Netherlands to general practitioners (GPs) each year. Guidelines for managing vaginal discharge mention *Candida albicans*, *Trichomonas vaginalis*, bacterial vaginosis, *Chlamydia trachomatis* and *Neisseria gonorrhoeae* as the usual suspects. Culturing for other agents is not recommended, unless symptoms recur or persist despite treatment. Lately, we noted a high isolation rate of non-group B (A, C, F, G) beta-haemolytic streptococci from fluor vaginalis samples from women with recurrent vulvovaginitis in our region. Group A streptococci are known as a cause of vulvovaginitis in children, but evidence of infection in adult women is limited to a few case reports and two methodologically flawed studies lacking well-defined control groups. The significance of group C, F and G streptococci in vaginal flora is unclear. To investigate the association between non-group B beta-haemolytic streptococci and vulvovaginitis in adult women, we conducted a case control study.
Methods: Cases ($n = 1010$) were women consulting their GP with an abnormal non-bloody vaginal discharge with or without itch, irritation, redness or pain from whom a fluor sample was cultured. Controls ($n = 206$) were asymptomatic women consulting their GP to have a smear taken for the cervical cancer screening programme, who consented to having a vaginal swab taken. Additionally, asymptomatic volunteers among hospital personnel submitted a self-obtained vaginal swab. Ages of cases as well as controls ranged from 30–60 years.
Results: Non-group B streptococci were isolated from 86 (8.5%) cases and from 6 (2.9%) controls (OR 3.1, 95% CI 1.4–7.2; $P < 0.01$). The significant difference was caused by group A streptococci, that were isolated from 49 (4.9%) cases and not from any of the controls ($P < 0.01$). Isolation rates of group C, F and G streptococci from cases were low (1.2, 0.1 and 2.4% respectively) and did not differ statistically from those from controls (1.0, 0.0 and 1.9% respectively).
Conclusion: Group A beta-haemolytic streptococci are associated with persistent vaginal discharge in women aged between 30–60 and should be diagnosed and reported as a pathogen in vulvovaginitis. The role of other non-group B streptococci requires more study because of the low numbers isolated. For adequate management of vaginal discharge culturing is necessary if initial treatment fails. Guidelines should be adapted to this effect.

New resistance mechanisms

O205 Hyperexpression of MexXY-OprM and MexCD-OprJ in clinical isolates of *Pseudomonas aeruginosa* with decreased susceptibility to cefepime

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Background: In *P. aeruginosa* ceftazidime (CAZ) and cefepime (FEP) show a similar susceptibility pattern but in some isolates FEP is less active than CAZ, which has been related to active efflux or production of some oxacillinases. We have studied the level of expression of two efflux pumps in clinical *P. aeruginosa* isolates with MICs of FEP higher than those of CAZ.
Methods: Nine *P. aeruginosa* isolates from different patients for which the MICs of FEP were $\geqslant$ 1 dilution higher than those of CAZ and where production of oxacillinases was excluded by multiplex polymerase chain reaction (PCR) were selected. MICs of CAZ and FEP were determined in triplicate in three different days by microdilution (CLSI guidelines). Clonal relationship was determined by REP-PCR. Quantitative reverse transcriptase-PCR was performed to identify expression of MexXY-OprM and MexCD-OprJ. Pump overexpression was considered when the level of specific mRNA of mexD or mexY in the tested strains was >4 times compared with the PAO-1 strain (real-time PCR). Mutations in nfxB (negative regulator of MexCD-OprJ) were studied in all the strains.
Results: All isolates were clonally unrelated. Three isolates were susceptible to FEP (MICs: 4–8 mg/L), 4 intermediate (16 mg/L), and 2 resistant (32–64 mg/L). Eight isolates were susceptible to CAZ (MICs: 1–8 mg/L) and 1 intermediate (16 mg/L). In all strains mexY expression levels ranged from 18.32 to 186.7 fold relative to the reference PAO-1 strain; three isolates had mexD expression levels of 4.05 to 150.45 fold relative to PAO-1. MICs of FEP were not higher for strains overexpressing both mexY and mexD than for strains with mexY overexpression alone. In one strain (MICs FEP/CAZ: 16/4 mg/L; mexD hyperexpression 150.45 fold) a mutation in N180 of nfxB modified the protein structure and changed its conformation in the C-terminal region.
Conclusions: Hyperexpression of MexXY-OprM in clinical isolates of *P. aeruginosa* is related to higher MICs of FEP than of CAZ. Additional overexpression of MexCD-OprJ does not contribute to this phenotype.

O206 Evolution of quinolone resistance mediated by genetic changes in qnrA, qnrB and qnrS genes

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Objectives: Plasmid-mediated quinolone resistance is increasing worldwide in Enterobacteriaceae. Pentapeptide repeat proteins QnrA, QnrB and QnrS confer reduced susceptibility to quinolones. In this study the genetic evolution of quinolone resistance mediated by changes in the coding sequences and promoter regions of qnrA1, qnrS1 and qnrB1 genes are analysed in vitro.
Methods: qnr genes, containing coding sequences and putative promoter regions, were cloned and expressed in *E. coli* DH10B. Random mutagenesis assays were performed and the mutants obtained were selected on plates with or without quinolones. Comparing the aminoacid sequences of these and other pentapeptide proteins with activity against quinolones several conserved positions were found. The role of these positions in the activity of these proteins against quinolones was evaluated by site directed mutagenesis. The residues analysed were G56, C72, C92, G96, F114, C115, S116, A117 and L159, according to the sequence of QnrA1. Further, the quinolone susceptibility of the mutants obtained by random or site directed mutagenesis were analysed by disk diffusion, E-test or microdilution methods.
Results: Three different phenotypes were obtained in the random mutagenesis assays compare with the wild-type phenotypes: (i) similar activity against nalidixic acid and ciprofloxacin, (ii) higher activity against nalidixic acid and ciprofloxacin and (iii) lower activity against nalidixic acid or ciprofloxacin. Only two mutants increased the quinolone resistance: QnrA1 containing R103C+K111M (two folds for nalidixic acid); and QnrS1 containing D185Y (four folds for ciprofloxacin). By site directed mutagenesis, only one change in several conserved positions (G56-, G56D, C72Y, C92Y or L159D) produced complete lost of activity for QnrA1, QnrB1 or QnrS1. The effect on the activity of these proteins was not identical in different positions.
Conclusions: Aminoacid sequences of pentapeptide repeat proteins QnrA1, QnrB1 and QnrS1 could be optimised in its activity against quinolones. One or several changes seem to be insufficient to obtain variants producing fluoroquinolones clinical resistance. In spite of the high aminoacid variability of these pentapeptide proteins, several of the conserved residues analysed are critical in the activity of these proteins against quinolones.

O207 A new plasmid-mediated gene for quinolone resistance, qnrC

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Objectives: Since the discovery of qnrA in 1998 two additional qnr genes, qnrB and qnrS, have been described. These 3 plasmid-mediated genes contribute to quinolone resistance in Gram-negative pathogens worldwide. A new gene, qnrC, was cloned from a transferable plasmid pHS9.
Methods: Plasmid pHS9 came from a clinical strain of *Proteus mirabilis*, which transferred low-level quinolone resistance but was negative by PCR for the known qnr genes. Plasmid pHS9 was transferred to azide-resistant *E. coli* J53 by conjugation. The plasmid was digested by HindIII and a fragment containing the new gene was cloned into plasmid puc18 and sequenced. The ciprofloxacin MICs for clinical and transconjugant strains were determined by Etest.
Results: The strain of *P. mirabilis* was isolated from an outpatient with a urinary tract infection. It was susceptible to most antimicrobials, but resistant to ampicillin and trimethoprim-sulfamethoxazole. Ciprofloxacin MICs for the clinical strain, J53 R-, and a J53 pHS9 transconjugant were 0.25, 0.008, and 0.125 μg/ml, respectively. A 4.49-kb HindIII fragment of pHS9 was cloned into puc18, and recombinants were transformed into *E. coli* DH5alpha. Sequencing showed that the responsible 537-bp gene, designated qnrC, encoded a 178 amino acid protein. QnrC shared 67.6%, 48.0% and 63.1% amino acid identity with QnrA1, Qnr B1 and Qnr S1, respectively. An integrase-like gene and an amidase gene were found upstream and downstream from qnrC.
Conclusion: A new quinolone resistance gene, qnrC, was characterised from plasmid pHS9 carried by a clinical isolate of *P. mirabilis*.

O208 The overexpression of SdiA and SoxS is associated with the development of multiple antibiotic resistance induced by diazepam and haloperidol in *Escherichia coli* AG100

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Objectives: The previously described multidrug antibiotic resistance (MAR) phenotype, which is induced in *Escherichia coli* AG100 by diazepam and haloperidol (drugs used in surgery) is comparable to MAR phenotype resulting from marAB operon activation by salicylate (OmpF loss and enhanced active efflux). Nevertheless, MAR phenotype can also result from the overexpression of other transcriptional regulators such as SoxS. This work studied some transcriptional activators that could be involved in the induction of MAR phenotype by diazepam or haloperidol in the susceptible *E. coli* AG100 strain.
Methods: The effect of several subinhibitory concentrations of diazepam and haloperidol in the AG100 strain was evaluated on outer membrane protein (OMP) expression, cyclohexane tolerance and the expression of the marA, soxS, rob and sdiA genes. OMP expression was analysed by SDS-PAGE. Expression of the marA, soxS, rob and sdiA genes was studied by reverse transcription of total RNA and PCR of cDNA (RT-PCR), using gapA gene as internal control of expression. PCR products were separated in SDS-PAGE and silver stained, quantifying the levels

of gene expression by using ImageQuant TL. AG100 (induced or non-induced with 5 mM salicylate or 0.2 mM paraquat) was the control strain.

Results: MAR phenotype, which was induced by growing concentrations of diazepam or haloperidol, was accompanied by a significant increased expression of sdiA and soxS genes, coinciding with previous results on MAR and increased active efflux induced by subinhibitory concentrations of any of both drugs. A large up-regulation of 8.9-fold for sdiA gene and 12.8-fold for soxS gene was induced by 0.05–0.1 mM haloperidol. The induced decreased expression of OmpF, increased cyclohexane tolerance as well as increased expression of sdiA and soxS genes occurred in a reversible and concentration-dependent manner. AG100 induced by diazepam or haloperidol did not show a significant increment in the expression level of MarA or Rob.

Conclusions:

i. SdiA, a homologous to the LuxR family of quorum-sensing transcription factors, as well as SoxS were involved in MAR phenotype induced by diazepam or haloperidol.
ii. The MAR phenotype, increased cyclohexane tolerance, and the up-regulation of sdiA and soxS genes were always induced by any of the two studied drugs in a reversible and concentration-dependent manner.
iii. The decreased expression of OmpF induced by diazepam or haloperidol could be related to SoxS overexpression.

O209 Genetic structure at the origin of acquisition of blaOXA-18 gene, encoding an emerging class D clavulanic-acid inhibited extended-spectrum β-lactamase

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Objectives: *Pseudomonas aeruginosa* (Pa) is an important nosocomial pathogen that may acquire resistance to expanded-spectrum cephalosporins mostly by overproducing its cephalosporinase and/or acquiring Ambler class A, B and D β-lactamases. Several clavulanic acid-inhibited Ambler class A extended-spectrum β-lactamases (ESBLs) and extended-spectrum class D enzymes (OXA-ESBLs) have been isolated in Pa. OXA-18 is a peculiar OXA-ESBL, since it is not a point mutant derivative of broad-spectrum OXA enzymes and is well inhibited by clavulanic acid. It was initially reported along with OXA-20 in Pa MUS from France in 1996, and very recently in several Pa isolates involved in an outbreak in Tunisia.

Methods: Genetic structures surrounding the blaOXA-18 gene of the prototype Pa MUS strain were characterised by cloning, PCR analysis and sequencing and compared with those found in three Pa isolates from Belgium. The strains were analysed by plasmid extraction, conjugation and electroporation assays, and by PFGE.

Results: The three Belgian isolates originated from sputum and blood specimens of three patients hospitalised in different wards over a six-month period. The presence of ESBLs detected by double-disk diffusion tests on cloxacillin-containing plates, and PCR followed by sequencing revealed the presence of blaOXA-18 and blaOXA-20 genes. Both were chromosomally-encoded and PCR mapping revealed identical genetic environments for Pa MUS and the Belgian isolates. While most oxacillinases are integron located, blaOXA-18 lacked gene cassette specific sequences but was inserted into an aac6′ Ib gene cassette. In addition, it was bracketed by two novel insertion sequences of ISCR family. It is likely that these ISs were at the origin of the blaOXA-18 gene mobilisation. Furthermore, detailed analysis of an 8.5-kb cloned genomic fragment containing blaOXA-18 gene revealed co-linearity of the blaOXA-18 gene and the integron containing blaOXA-20 gene. PFGE defined genetic relatedness between the three Belgian Pa isolates and Pa MUS strain. The recently identified OXA-18-producing Pa isolates from Tunisia were blaOXA-20-negative (but positive for either TEM-1 or SHV-1), genetically different from Pa MUS, thus suggesting that at least two OXA-18-producing Pa clones are currently spreading.

Conclusion: This report characterised the genetic elements at the origin of blaOXA-18 ESBL in Pa and suggests the emergence of this type of ESBLs in Pa in Belgium.

Paediatric infections

O210 Emergence of a single ST-8 clone of Panton-Valentine leukocidin-positive meticillin-resistant *Staphylococcus aureus* in an ambulatory paediatric population, Madrid, Spain

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Objectives: Community-acquired, meticillin-resistant *Staphylococcus aureus* (CA-MRSA) infections in children are increasing in frequency. The aim of this study was to report the frequency of CA-MRSA and the prevalence of Panton-Valentine leukocidine (PVL) among the children population as well as to determine the clinical, epidemiologic and molecular characteristics of these patients with community-acquired infections.

Patients and Methods: We conducted a prospective study from January to November 2007. The study included all patients attended in the Paediatric Emergency Department (PED) with a *S. aureus* infection. All isolates underwent polymerase chain reaction analysis for the LPV gene. Molecular characterisation of LPV(+) isolates was performed using PFGE following DNA extraction with SmaI and multilocus sequence typing (MLST).

Results: During the study period, *S. aureus* was detected in 58 samples taken from 53 children.

They had a median age of 42 months (0–170 months), and 70% were boys. An MRSA was detected in 7 patients (13.2%). Forty six patients were tested for PVL genes, and a positive result was obtained in 12 (26%).The prevalence of LPV was 71% in the group of MRSA (5/7)and 18% in the MSSA group (7/39) Final diagnosis of patients comprised superficial infections 57%, cellulitis or abscess 31%, and deep infections 10%. A 33% of the children were admitted in the hospital and a 30% required drainage. Although no significant differences in clinical characteristics or classic risk factors were observed between CA-MRSA and CA-MSSA, we found significant differences related to the country of origin of the children: CA-MRSA isolates were more frequent isolated from children with an origin in Ecuador (44% vs. 7%; $P = 0.002$) and all of them belonged to the same ST 8 IV clone according to the PFGE and the results of MLST, whereas all the MSSA PVL(+) isolates belonged to different clones. Regarding to the epidemiologic characteristics, infections by isolates with and without PVL gene did not show significant differences but it is to mention that isolates with PVL gene were clearly associated with need of drainage (75% vs. 27%; $P = 0.001$).

Conclusions: There is an emergence of a single clone (ST 8 IV) of the CA-MRSA infections among children in Madrid and is clearly associated with children with an origin in Ecuador. Infections with isolates PVL(+) required a more aggressive treatment with drainage as a result of the isolates' increased virulence.

O211 Diagnosing congenital cytomegalovirus infection: will the universal Guthrie card (dried blood spot) testing hinder more than help?

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Objectives: Previous population studies have identified Ireland as having a low prevalence of CMV infection. However, the changing demographic has resulted in an increase in CMV seroprevalence; this may result in an increased risk of infection in susceptible pregnant women. The aim of this study is to determine if laboratory investigation of neonatal dried blood spots (DBS) collected on Guthrie cards can be used as the sole diagnostic approach to accurately identify cases of congenital CMV (cCMV) infection.

Diagnosis of cCMV infection requires detection of CMV in the neonate in the first three weeks of life. Culture of CMV from urine remains the gold standard for the detection of live virus, although a definitive diagnosis by traditional culture may take up to three weeks. More rapid results can be obtained through the complementary use of urinary DEAFF (detection of early antigenic fluorescent foci) testing. Advances in molecular methods have resulted in a move away from DEAFF/culture to the detection of CMV DNA by PCR in urine samples or DBS. PCR testing may be supplemented with serology for CMV specific IgM.
Methods: We conducted a retrospective review of all neonates from the three Dublin maternity hospitals clinically diagnosed with cCMV infection over a three year period, 2004 to 2007. All testing was performed in a single specialist virology centre, the National Virus Reference Lab (NVRL), which provides serological, molecular (PCR) and cell culture methods (traditional & DEAFF) for the diagnosis of cCMV in the Republic of Ireland. Of note, there is no national screening programme for cCMV.
Results: Thirty-two children were diagnosed with cCMV in Dublin over the period studied. Thirty-one of 32 (97%) children were urinary DEAFF test positive; 17/31 (55%) were culture positive; 12/26 (46%) were IgM positive; 8/13 (62%) were CMV DNA positive in blood. Eighteen of 28 (64%) neonates were positive in blood for either CMV DNA or IgM; 2/11 (18%) were positive for both DNA and IgM. If the only test offered were PCR for CMV DNA in DBS, 38% of children would not have been diagnosed.
Conclusions: This study illustrates that DBS testing alone is not an adequate strategy for the clinical diagnosis of, or as a screening tool for, cCMV infection in a low seroprevalence population. DBS testing provides virological data of prognostic value in those neonates known to be CMV infected, but it lacks the sensitivity required to be a satisfactory screening test.

O212 Human Bocavirus quantitative DNA detection and phylogenetic analysis in children hospitalised for acute bronchiolitis

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Objective: Human Bocavirus (HBoV) is a newly discovered parvovirus, but its role as causative agent of respiratory disease remains unclear.
Methods: We investigated the presence of HBoV by quantitative PCR of nasopharyngeal samples of 192 French consecutive children hospitalised for acute bronchiolitis. The detection of the other common respiratory viruses was performed using classical immunofluorescence antigens, cell culture detection, or RT-PCR assays.
Results: HBoV was the unique viral pathogen detected in 14 (7%) and was associated with another viral respiratory pathogen in 10 (5%) of 192 study children. This virus was identified as the third aetiological cause of bronchiolitis after respiratory syncytial virus and rhinovirus (45 (23%) and 24 (12%) of 192 cases, respectively), occurring more often in infants aged 1–12 months ($P = 0.002$). The median levels of the HBoV DNA genomes in respiratory samples appeared to be significantly higher in patients with single HBoV infection than those observed in patients with a mixed respiratory viral infection with HBoV (4.10^8 copies/ml vs. 2.10^3 copies/ml, $P < 0.001$).
Conclusion: Our data suggest that HBoV at a high viral load could be an aetiologic agent of respiratory tract disease, whereas the exact role of HBoV at a low viral load, as aetiological cause or as pathophysiological co-factor of respiratory diseases, remains to be determined.

O213 Chronic in utero exposition to nevirapine does not cause hepatotoxicity in HIV-uninfected healthy infants

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Objective: Liver function abnormalities and clinical hepatitis have been associated with NVP use, also in the paediatric age. NVP crosses the placenta and achieves neonatal blood concentrations equivalent to that in the mother (cord-to-maternal blood ratio 0.90). NVP elimination is prolonged in infants. While single-dose NVP regimens at delivery have proved safe, little is known about chronic in utero exposure to NVP and hepatotoxicity in these otherwise healthy HIV-uninfected infants.
Methods: Prospective observational study on a cohort of HIV-uninfected healthy infants born to HIV-infected mothers and exposed in utero to either NVP-based or non-NVP-based HAART regimens. Infants perinatally infected with HIV and/or HCV, and those who received neonatal NVP were excluded. Plasmatic alanine aminotransferase (ALT, normal values up to 40 IU/l) levels obtained up to the age of 12wk were compared between groups; in Exposed, total time of in utero HAART exposure (12wk or less) was also taken into account. Student's t-test and other parametric tests were used.
Results: Overall, 170 infants (76 females, 44.7%) were included, 80 (47.1%) of them (33 females, 41.2%) exposed in utero to a NVP-based HAART regimen. ZDV+3TC ($n = 100$) and 3TC+d4T ($n = 39$) were the most commonly used nucleoside analogue backbones; mothers of the Non-exposed group mostly received a PI-based therapy during gestation (NFV, $n = 57$; LPV/r, $n = 19$). Mean duration of HAART during pregnancy was 28wk (range: 3–40wk). Mean gestational age and weight at birth were 37wk and 2767g, respectively. At delivery, 98.2% of the mothers received intravenous ZDV; all infants received a 4 to 6-wk course of oral ZDV.
None of the patients showed clinical symptoms consistent with hepatitis during follow-up, except for 8 patients (3 Exposed, 5 Non-exposed) who developed self-limited neonatal jaundice. ALT plasmatic levels were obtained at a median age of 26 days (range: 1–84 days). No differences are reported in ALT levels between Exposed (mean value: 19.8, range: 5–31 IU/l) and Non-exposed (mean value: 20.0, range: 1–79 IU/l); in the Non-exposed group, differences were neither observed when length of exposure (12 weeks or less) was considered. Three infants in the Non-exposed group developed non-symptomatic elevation of ALT values that had spontaneously normalised by the age of 6 months.
Conclusions: In this study, chronic in utero exposure to NVP did not cause elevation of ALT levels in HIV-uninfected infants.

O214 Pneumococcal carriage in a geographically isolated indigenous community in Venezuela before, during and after an outbreak of acute respiratory tract infection

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Background: We evaluated pneumococcal carriage rates in children from a geographically very isolated indigenous population, only reachable by plane, that was struck by an outbreak with the clinical criteria that met a bacterial acute respiratory tract infection (ARTI), affecting more than 70% of all the members of the community.
Objective: To evaluate the carriage rates and the serotype/genotype distribution of *S. pneumoniae* strains isolated before, during and after the ARTI outbreak.
Methods: The community consists of about 900 people with 150 younger than 5 years old. Contact with other indigenous communities is rare. Nasopharyngeal swabs were cultured from 321 children aged 3–84 months before (October 2004: $n = 118$), during (November 2005; $n = 123$) and after (March 2006: $n = 80$) the outbreak. The *S. pneumoniae* isolates were serotyped with the Quellung reaction, genotyped by restriction fragment end labelling (RFEL) analysis, and their susceptibility to antibiotics was assessed by disk diffusion.
Result: In total, 117 pneumococcal strains were isolated and carriage rates in the three time periods before, during and after the ARTI outbreak were 16%, 60% and 32%, respectively. 83% (95/115) of the children under 5 years old was diagnosed with ARTI in the second time period of which 66% (63/95) was clinically classified as pneumonia. Before the outbreak the prevalent serotype was 6B (80%). The most prevalent capsular serotypes during outbreak were 6B (40.3%), 9V (13.4%) and 15B (13.4%), and 18C (10.5%). The latter 3 serotypes were not present before the outbreak. After the outbreak, in the third sample period serotype 9V and 15B disappeared completely, and the other serotypes

remained. RFEL analysis clustered all the serotypes isolated during the outbreak in very related genotypes; per serotype 1 cluster or a clade with at least 85% homology. All isolates were susceptible for the antibiotics tested with exception of TMPS harbouring 20.5% resistance.
Conclusions: The increase in carriage rate during disease was mainly due to serotypes not isolated before the outbreak. Hence, we consider that the introduction of the new serotypes in the community might have been related to the outbreak of lower respiratory tract infections. The highly related genotypes found for all the serotypes indicate limited introduction of new serotypes and genotypes in this community. Future investigation should monitor the impact of a pneumococcal vaccine on disease incidence and strain substitution in this isolated community.

Molecular approaches to biofilm biology

O215 *Candida albicans* signalling alcohols as players of cellular cross-talk with *Candida tropicalis*

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Objective: *Candida* species are the most common agents of opportunistic mycoses, which are often associated with biofilms. *Candida albicans* and *Candida tropicalis* biofilms develop most frequently in intracardiac prosthetic devices and voice prostheses with an infection risk of 1–3% and 50–100%, respectively. In natural environments polymicrobial biofilms are observed, but interactions between organisms are not well understood. This study focused on the evaluation of the effect of recently characterised *C. albicans* alcohols signalling molecules, in *C. tropicalis* regarding two hypothetical clinical scenarios: *C. tropicalis*, adhered and within a mature biofilm.
Methods: All experiments were performed in RPMI medium and cells (initial cell density of 1×10^6 cells/ml) grown at 37ºC, 130 rpm. For all experiments, endpoints were determined at the end of 24 h. *C. tropicalis* ATCC 750 biofilms were developed on the surface of microtriter plates. *C. albicans* CECT 1472 secreted alcohols were added after 3 h of adhesion or to 48 h grown biofilms. Isoamylalcohol, 2-phenylethanol, 1-dodecanol, nerolidol, farnesol and tyrosol were independently assayed at physiological levels. Biofilm quantification was performed using two different approaches: total biomass by crystal violet staining and quantification of biofilm cells activity by the reduction of a tetrazolium salt (XTT).
Results: At initial biofilm stages *C. tropicalis* metabolic cell activity was significantly decreased by isoamylalcohol, 2-phenylethanol, E-nerolidol and tyrosol treatments, while cellular mass was positively affected by isoamylalcohol but negatively by E-nerolidol. Nevertheless, mature biofilms were more resistant to the action of these alcohols. Specifically, only isoamylalcohol induced alterations, with the same response pattern observed at earlier stages.
Conclusion: Results obtained show that *C. albicans* extracellular alcohols regulate *C. tropicalis* behaviour, depending on biofilm growth time, suggesting that *C. tropicalis* mature biofilms are more resistance to *C. albicans* signalling crosstalk.

O216 A novel *Staphylococcus aureus* biofilm phenotype mediated by the fibronectin-binding proteins, FnBPA and FnBPB

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Device-associated infections involving biofilm remain a persistent clinical problem. Our research group has recently reported that meticillin-resistant *Staphylococcus aureus* (MRSA) strains can form biofilm independent of the icaADBC-encoded exopolysaccharide. Here we report that mutation of sortase, which anchors LPXTG-containing proteins to peptidoglycan, impaired MRSA biofilm development. Furthermore deletion of fnbA and fnbB, which encode the LPXTG-anchored multifunctional fibrinogen and fibronectin-binding proteins, FnBPA and FnBPB impaired MRSA but not meticillin-sensitive *S. aureus* (MSSA) biofilm development, apparently at the level of intercellular accumulation and not primary attachment. The MRSA fnbAB biofilm defect was complemented by fnbA or fnbB alone. Correspondingly, mutation of fnbA or fnbB alone did not substantially affect biofilm and overexpression of either gene alone in MRSA and MSSA activated biofilm. Interestingly FnBP-promoted biofilm was dependent on SarA, but not at the level of fnbA or fnbB transcription. Using plasmid constructs lacking regions of FnBPA to complement an fnbAB mutant revealed that the A domain alone and not the BCD domain required for fibronectin binding could promote biofilm. Additionally, an A domain N304A substitution which abolished fibrinogen binding did not affect biofilm. These data identify a novel *S. aureus* biofilm phenotype promoted by FnBPA and FnBPB, which is apparently independent of the known ligand-binding activities of these multifunctional surface proteins. Given that implanted surgical and medical biomaterials are rapidly coated by a conditioning film composed primarily of extracellular matrix proteins, our findings suggest that these cell-wall-anchored proteins are important virulence factors in device-related *S. aureus* infections, particularly those caused by MRSA strains, and may represent attractive therapeutic targets.

O217 Roles of flagella and effect of curcumin against biofilm formation by *Helicobacter pylori*

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Objectives: The gastric pathogen *Helicobacter pylori* is shown to have alternate life style as a biofilm. In human infection, a presence of mature biofilm attached to cell surface has been shown and hypothesised that *H. pylori* infections resulting in gastric ulcers may be a manifestation of biofilm. Flagella have been considered to have a possible role during initial step of biofilm formation. Here we have investigated roles of three flagellar genes, including the flaA gene encoding flagellin protein, the flgR regulatory gene and the fliQ export apparatus gene, in the production of biofilm and control of adherence in *H. pylori*. The effect of curcumin against *H. pylori* biofilm formation and adherence to the human cancer cells has also been investigated.
Methods: Isogenic flaA, flgR, and fliQ mutants were constructed from *H. pylori* wild-type 26695 and N6 by inverse PCR mutagenesis. A minimum inhibitory concentration (MIC) of curcumin (diferuloymethane) against *H. pylori* was determined using agar dilution technique. The ability to form biofilm in liquid culture and on glass surface, and adhere to the HEp-2 cells was examined in vitro in a presence of curcumin compared to the controls.
Results: Two different types of biofilm were observed in this study, including pellicle and attached biofilm. The wild-type *H. pylori* 26695 and N6 strains formed biofilm and adhered to HEp-2 cells more efficiently than the isogenic mutants, including flaA mutants; PA315 and NA2, flgR mutants; PR611and NR, and fliQ mutants; PQ and NQ. These results indicate that these genes are involved in the biofilm formation and adhesion of *H. pylori*. Our results showed that curcumin not only inhibited bacterial growth, but also greatly decreased the number of biofilm formation and the ability to adhere to the HEP-2 cells.
Conclusion: We have demonstrated that *H. pylori* in monoculture can form biofilm. It attached to a glass surface and formed a pellicle at air-liquid interface. Flagellar components were found to be important for biofilm formation on abiotic glass surface, pellicle formation in liquid culture, and adherence to HEp-2 cells. Curcumin has been demonstrated an effect against to *H. pylori* biofilm and shown a significant inhibitory action on adherence of *H. pylori* to HEp-2 cells.

O218 agr/RNAIII expression in biofilms in vivo

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Objectives: To assess the role of agr in biofilm formation, we studied gene expression of agr in well-established biofilms in an in vivo rat model.
Methods: agrA and RNAIII expression was evaluated during in vivo foreign body colonisation over a period of 2 weeks. Catheter fragments (n = 180), inoculated with a low inoculum of *S. epidermidis*, were

implanted subcutaneously in rats as described (Vandecasteele et al. BBRC. 2002; 291: 528). After explantation, gDNA and RNA was isolated from adherent (biofilm-associated) bacteria (n = 108), detachable bacteria (bacteria loosely adherent to the catheter and removed by washing) (n = 36) and from bacteria which colonised the surrounding tissue (n = 36). Gene expression was quantified by real-time PCR. The amount of bacteria was calculated by absolute quantification of gDNA copies of the housekeeping gene gmk, recalculated to bacteria/mm^2 (with catheter surface of 79.17 mm^2).

Results: Bacterial density decreased till 6 h post-implantation, again increased in the 1-day old biofilm and subsequently slowly decreased. However it was still higher than in planktonic cultures. In spite of this, expression of the quorum-sensing system genes argA and RNAIII was low 1 day after implantation. RNAIII expression decreased to a minimum in the 2 weeks old biofilm, whereas agrA expression remained at a low level during the whole implantation period.

The number of bacteria that detached and colonised the surrounding tissue significantly decreased from 1 towards 2 weeks post implantation. The number of biofilm-forming bacteria was stable, suggesting a more stable and well-formed biofilm after 2 weeks of implantation. agrA and RNAIII expression were significantly higher in detachable bacteria than in tissue-colonising bacteria for which agrA and RNAIII expression was comparable to that of biofilm-associated bacteria.

Conclusion: Expression of agr is low in biofilms, but high in detachable bacteria. This correlates with a role for agr in active tissue invasion but not in biofilm formation.

O219 Biofilm models for *Streptococcus pneumoniae*

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Objectives: Recent data indicate that pneumococci during otitis media, pneumonia and meningitis may form biofilms in vivo. In order to better characterise pneumococcal biofilm formation we evaluated different models in which to analyse the various phases of biofilm development.

Methods: Static biofilm models were developed in microtiter plates both using low inocula in rich media and high inocula in poor media. Pneumococcal cells adhering to solid support were characterised for viability, extracellular matrix production and responsiveness to quorum sensing signals.

Results: Adherent pneumococci could be recovered few hours after incubation. When using low incula biofilm was found to increase during exponential phase and, after a decline of about half a log, remain stable over time. This stability in stationary culture was dependent on addition of CSP to growth medium. The addition to the growth medium of BLP, the second type of cell-cell signalling peptide of *S. pneumoniae*, had no effect on biofilm formation or stability. Mutants for the cps locus (capsule), comC (CSP), comD (CSP receptor), blpH (receptor of BLP bacteriocin), luxS (quorum sensing molecule) and further 10 TCS were assayed for their ability to form biofilm. Only for the comD mutant was found to be impaired in the formation of mature biofilm, while independence form capsule expression and any type of cell-cell signalling mechanism was shown for the other phases of pneumococcal biofilm. The model relying on high inocula in poor media showed stable biofilm formation for over a week independently from addition of exogenous CSP. Despite difference in CSP dependence in both biofilm model systems the comD mutant shows identical deficiency in extracellular matrix production, indicating the most probable reason for the instability of biofilm. Quantitative analysis of gene expression for a panel of 30 genes did not show significant variation between the two model systems.

Conclusions: Pneumococcal biofilm formation can be assayed in different systems which provide alternative models for the analysis of this complex phenotype. The cell-cell signalling system related to competence development is significantly involved in the stabilisation of pneumococcal biofilms, most probably through involvement of extracellular matrix production.

Foreign body infections: diagnostic and therapeutic implications of basic research

S220 Serological tests to detect antibodies against biofilm specific antigens

T. Elliot (Birmingham, UK)

Staphylococci produce a wide range of antigenic components including macromolecules found in the cell wall (peptidoglycan, teichoic acids and surface proteins), wall-associated capsular and slime polysaccharides as well as a range of potent exported toxins. Knowledge of the antigenic properties of staphylococci in health and disease is important for the development of protective vaccines and antibodies for passive therapy. Design of kits for sensitive and specific serodiagnosis of infection, detection of microbial contaminants and microbial identification also requires detailed understanding of the antigenic properties of staphylococci.

Serological methods have been applied to the study of staphylococci over many years and indicate the complexity and diversity of antibody responses following exposure to staphylococci, either during infection, response to commensal organisms or administration of exploratory vaccines. Variations occur in antibody levels to the staphylococcal peptidoglycan, ribitol (wall) teichoic acid, and lipo (membrane) teichoic acid in sera from both blood donors and patients with verified or suspected staphylococcal infections. Of these antigens, a short chain excreted form of cellular lipoteichoic acid (called lipid S) has been shown to be of some value in the diagnosis of infections associated with orthopaedic prostheses, native and prosthetic valve endocarditis and sepsis associated with central venous catheters.

The experience with StaphVax (a *S. aureus* capsular polysaccharide conjugate vaccine) and Veronate (a polyclonal antibody product directed against protein virulence factors) also suggest that active or passive protection against staphylococcal infection is complex. A number of other products are under clinical evaluation including: AuroGrab (antibody targeting the ABC transporter of *S. aureus*); Altastaph (capsular polysaccharide vaccine); Aurexis (monoclonal antibody fragment targeting *S. aureus* clumping factor) and Pagibaximab (targeting lipoteichoic acid). These may also give a clearer indication of possible antigens for use in serological tests. Many commercial serological kits are already available for identification of staphylococci. These include rapid latex agglutination tests for example the Slidex Staph Plus (bioMérieux), Staphaurex Plus (Murex Diagnostics), Staphytect (Oxoid), Bacti Staph (Remel) and Pastorex Staph-Plus (Sanofi Diagnostics Pasteur). All these tests detect clumping factor and staphylococcal protein A, in addition, the Slidex Staph Plus and Staphaurex Plus tests detect group-specific antigens on the *S. aureus* cell surface, and Pastorex Staph-Plus detects capsular polysaccharides.

S222 Suppression of MRSA by the quorum sensing inhibitor RIP

N. Balaban (North Grafton, US)

Wound, or biofilm-related infections can become chronic, leading to economic loss secondary to patient morbidity and possible mortality. Many of these infections involve Staphylococci spp. which are capable of developing antibiotic resistance. Community or nosocomial acquired microbial antibiotic resistance is eroding the miracle of antibiotics and jeopardising both human and animal welfare. With evidence of increased frequency of resistance to vancomycin, our current most potent antibiotic, the drive is on to develop novel therapeutic agents. RNAIII inhibiting peptide (RIP) therapy represents an exciting alternative.

Staphylococci (like *S. aureus* and *S. epidermidis*) can become virulent and cause diseases through the formation of biofilms and production of toxins. This process is regulated by cell-to cell communication mechanisms, termed quorum sensing (QS). RIP is a heptapeptide (YSPWTNF-NH2) that inhibits QS, and in its presence, the bacteria are no longer virulent and do not cause disease. RIP has been shown

to be extremely effective in preventing and treating drug resistant staphylococcal infections in multiple experimental infection models (tested in mice, rats, rabbits, cows) against various drug resistant strains like MRSA, VISA, MRSE, and VISE. RIP has also been demonstrated as very effective in treating clinical human chronic wound infections in a polymicrobial setting involving multiple types of bacteria. RIP is synergistic to antibiotics and thus can be used (alone or in combination) systemically, topically, or by coating medical devices to suppress infections, including those caused by antibiotic resistant strains.

Molecular epidemiology update on antimicrobial-resistant *Streptococcus pneumoniae* in Europe

S225 Macrolide resistance in *Streptococcus pneumoniae*: from phenotype to genotype

A. Pantosti (Rome, IT)

In recent years, resistance to macrolides in *Streptococcus pneumoniae* has increased in many areas of the world, including Europe. According to the European Antibiotic Resistance Surveillance System, that collects data from invasive isolates, in 2006 most European countries reported a proportion of erythromycin-resistant pneumococcal isolates between 10 and 25% with 6 countries, including Italy, reporting a proportion higher than 30%. The bimodal distribution of the erythromycin minimal inhibitory concentrations of resistant isolates reflects the well-known resistant phenotypes and the corresponding genotypes: high-level macrolide resistance associated with resistance to lincosamides and streptogramin B (the MLSB phenotype) conferred by the Erm(B) ribosomal methylase, or low-level resistance to macrolides only (M phenotype) due to an efflux pump encoded by the mef-msr(D) genes. The relative proportion of isolates carrying either mechanism varies between the different countries and is changing with time. In addition, isolates carrying both resistance mechanisms are becoming increasingly common. Other uncommon genotypes, generally associated with low to moderate-level erythromycin resistance, are represented by point mutations in domain V of the 23S rRNA genes or in the ribosomal proteins, and by the erm(A) gene, encoding for an inducible methylase. Although the main genotypes responsible for erythromycin resistance in *S. pneumoniae* are well known, the genetic elements on which the resistance genes reside and that represent the vehicle for their transmission are only recently starting to be elucidated. The elements carrying the macrolide efflux pumps in *S. pneumoniae* are defective transposons: mef(A) is carried by Tn1207.1 and mef(E) is carried by mega. These two elements are similar, but their distribution and properties are very different. mefA/Tn1207.1 is characteristic of a serotype 14 international clone (England14–9) and its insertion in a competence gene makes the isolates unable to be transformed. Therefore mefA-carrying isolates are usually susceptible to penicillin and most other non-macrolide antibiotics. Isolates carrying mef(E)/mega are heterogeneous in serotype and genetic background and tend to be multidrug-resistant, due to the capability of mega to associate with other antibiotic-resistance transposons, notably with Tn916, carrying tetracycline resistance. Transposons of the Tn916 family are vehicle also for high-level macrolide resistance due to erm(B). Recent studies have shown that erm(B) is often associated with Tn916-like transposons, although tetracycline resistance can be phenotypically unexpressed. The composite transposons have a modular structure, where Tn916 represents the backbone and the modules include one or more macrolide resistance elements, such as mega, Tn917, or the erm(B) element. Although these composite transposons are not conjugative in *S. pneumoniae*, the natural transformability of the pneumococcus ensures their transmission within the species. The study of the resistance elements carried by particular clones can contribute to the understanding of the evolution of macrolide resistance in *S. pneumoniae*: for instance, the low-level erythromycin-resistant international clone Taiwan19F-14 has evolved into the double-macrolide gene isolates of the CC271 complex.

S226 Fluoroquinolone resistance in *Streptococcus pneumoniae* and competitive cost

A. González de la Campa, L. Balsalobre (Majadahonda, ES)

Resistance to fluoroquinolones (Fq) in *S. pneumoniae* (SPN) occurs mainly by alteration of their targets, the DNA topoisomerase IV (ParC2ParE2) and DNA gyrase (GyrA2GyrB2) essential enzymes. Resistance mutations are found in the quinolone-resistance determining regions (QRDRs) of ParC, ParE or GyrA and can be acquired by point mutation, by intraspecific recombination or by interspecific recombination with the *viridans* streptococci of the mitis group (SMG). The current prevalence of Fq-resistant (FqR) SPN is lower than 3% while in the SMG is higher than 13%. Acquisition of resistance by interspecific recombination could be much more common than by point mutation, considering the frequencies of these events in laboratory conditions (10^{-3} and 10^{-9}, respectively). However, recombinants account for less than 11% of the SPN FqR isolates. Besides other factors, such as the availability of DNA in the natural environment and the competence state of the cells, one cause that could account for the low frequency of the FqR recombinant isolates is the fitness cost imposed by the DNA interchange that often implies the acquisition of larger parE-parC intergenic regions. A parallel study of transcription of parE-parC and of the fitness cost of 24 isogenic FqR strains derived from R6 was performed. Six first-level transformants were obtained either with PCR-products containing parCQRDRs of SPN FqR point mutants or with a PCR-product carrying parEQRDR-ant-parCQRDR from a FqR SMG isolate. The latter yielded two strains, T6 and T11, carrying parCQRDR and parEQRDR-ant-parCQRDR, respectively. The first-level transformants were used as recipients in further transformations with gyrAQRDRs PCR products to obtain 18 second-level transformants. RT-PCR experiments showed cotranscription for parE and parC in R6, T6 and T11, and a single promoter located 5′ of parE was identified in R6 by primer extension. Fitness of transformants were estimated by pairwise competition with R6 in both 1-cycle and 2-cycle experiments. In 1-cycle experiments, only strains carrying the GyrAE85K change showed fitness cost, with the exception of recombinant T14. In 2-cycle experiments, cost was observed in first-level transformants carrying FqR changes S79F, S79Y, D83Y and GyrA E85K, with the exception of recombinants T6 and T11. Results suggest that there is no impediment due to fitness cost for the spreading of recombinant FqR SPN isolates, since some recombinants exhibited a compensation of the cost.

Difficult-to-treat infections

S229 Severe diabetic foot osteomyelitis

B.A. Lipsky (Seattle, US)

Infections of bones in the foot in patients with diabetes occur in 20–60% of patients who develop a foot wound. These infections usually occur by contiguous spread from soft tissue infection, and markedly increase the risk for hospitalisation and amputation. Most infections are caused by staphylococci, but other organisms can be involved, sometimes in a polymicrobial infection. The first task for the treating clinician is to diagnose bone infection accurately. This is best done by obtaining a bone specimen (either percutaneously or at surgery) for both culture and histology. Among the available imaging tests, MRI is clearly the best; bone scans are too non-specific. Clinical signs and symptoms are unreliable, but the probe-to-bone test is easy and relatively helpful. Recently, the International Working Group on the Diabetic Foot proposed a diagnostic scheme that estimates the likelihood of osteomyelitis based on combinations of various clinical and laboratory test results.

After confirming a bone infection, the clinician must plan its treatment. Most authorities recommend resecting any necrotic (and in many cases grossly infected) bone, but evidence supporting this advice is sparse. Recently, retrospective case series have shown that some cases of presumed osteomyelitis can be put into long-term remission with antibiotic therapy alone, usually with highly bioavailable antibiotics (e.g.,

fluoroquinolones) given for a prolonged period (3 months or longer). If one removes all infected and necrotic bone, the duration of treatment can be considerably shorter (days to weeks).
No available data support parenteral over oral antibiotic therapy, nor has one antibiotic agent has been found to be superior to others. Some evidence suggests that including rifampin (combined with at least one other anti-staphylococcal antibiotic) improves outcome; clindamycin and β-lactam agents are also frequently used. No adjunctive treatment (e.g., hyperbaric oxygen, granulocyte colony stimulating factor, larval biotherapy) has been proven beneficial. When treatment of osteomyelitis fails, surgeons should opt for the most minor amputation compatible with good residual foot function. Clinicians should monitor patients for at least a year to ensure that they have achieved apparent resolution of infection.

S230 MRSA endocarditis

F. Gudiol (Barcelona, ES)

Nowadays, *S. aureus* is the leading cause of infective endocarditis (IE) in most regions of the developed world. This is a consequence of the increasing rates of healthcare-associated staphylococcal bacteraemias and also of the increasing number of patients with implanted medical devices.
In non-addicts, infection primarily involves the left side of the heart and is associated with mortality rates ranging from 25% to 40%, whereas in addicts often involves the tricuspid valve, and mortality rates are much lower.
An increasing percentage of *S. aureus* strains in both hospital and community settings are meticillin-resistant. In recent series of *S. aureus* endocarditis, MRSA accounts for around 30% of cases. Patients with MRSA-IE are more likely to have chronic conditions, healthcare-associated infections, persistent bacteraemia and higher mortality rates. In those with left-sided endocarditis (especially among patients in maintenance hemodialysis) mortality may be as high as 80%.
According to the current guidelines, vancomycin remains the reference standard for the treatment of both prosthetic and native right-sided and left-sided MRSA-IE. Both slow bactericidal activity and poor penetration into vegetations have been advocated as the main reasons to explain the limited efficacy of the drug. More recently, the emergence of isolates with reduced susceptibility to vancomycin, and the observation of an excess of failures among patients with MRSA bacteraemia caused by "susceptible" strains with MICs $\geqslant 2\,\mu g/ml$, have emphasised the need for alternative therapies.
The addition of aminoglycosides (MRSA usually are non-susceptible) or rifampin, has not been associated with better outcomes.
Among older antibiotics, trimethoprim-sulfamethoxazol and combinations of fosfomycin with carbapenems, which are highly synergistic against most strains, might be reasonable alternatives for selected patients.
New therapeutic options include quinupristin/dalfopristin, linezolid, tigecicline and daptomycin. All these drugs have shown an efficacy equal or better than vancomycin in experimental models of MRSA, h-GISA and GISA endocarditis, but clinical experience is scarce. Among them, daptomycin – which has been approved for use in *S. aureus* right-sided endocarditis – is probably the best alternative due to its rapid bactericidal effect. However, the best dose regimen of daptomycin in this setting, the risk for developing resistance during therapy and the potential for combinations with other agents are largely unknown.
Additionally, a number of new anti-MRSA compounds with good "in vitro" activity and promising results in experimental models are in development, including novel glycopeptides (dalbavancin, telavancin and oritavancin), ceftobiprole, and iclaprim.
An early identification of patients with persistent MRSA bacteraemia and an aggressive multidisciplinary management of those with endocarditis may help to improve prognosis. Especially important, surgical valve replacement may play a relevant role in maximising outcomes in MRSA left-sided and prosthetic valve endocarditis. Patients who fail vancomycin therapy should have a prompt expert evaluation and receive alternative compassionate drugs.

Emerging issues of *Clostridium difficile*-associated disease (Symposium jointly arranged with IDSA and ESGCD)

S233 Antibiotic susceptibility patterns in Europe

J. Brazier (Cardiff, UK)

A selection of isolates of *C. difficile* have been tested for their susceptibilities to a range of antibiotics using both agar dilution and the E test methods. Isolates were of known PCR ribotypes mainly originating from a surveillance study of over 1,000 isolates from symptomatic patients in hospitals in England.
The agents tested included; metronidazole, vancomycin, erythromycin, imipenem, moxifloxacin, levofloxacin, co-amoxyclav, penicillin, piperacillin–-tazobactam.
A separate selection of European isolates of *C. difficile* present in the culture collection of the Anaerobe Reference Laboratory of the UK in Cardiff, were also studied for their susceptibilities to these drugs. Representatives from Austria, Germany, Italy, Poland, Hungary, The Netherlands, Sweden and Spain were included and the data will be presented.
For the UK isolates, a strong relationship was noted between resistance to certain agents and PCR ribotype, particularly amongst the common strains known as Types 027, 106 and 001. This raises the question as to if resistance mechanisms harboured by strains of *C. difficile* offer a selective advantage over strains that do not. The susceptibility of older isolates of a given PCR ribotype was compared to contemporary isolates to investigate if resistance is increasing and this will be discussed.

S234 *Clostridium difficile*: new and old treatment options

E. Bouza (Madrid, ES)

A high percentage of *C. difficile* strains are resistant to antimicrobials such as cephalosporins, clindamycin, macrolides, aminoglycosides, tetracyclines, cotrimoxazole, ertapenem, imipenem, and chloramphenicol. On the contrary, the microorganism shows “in vitro” susceptibility to ampicillin, meropenem, metronidazole, penicillin, piperacillin, teicoplanin and vancomycin. Until recently, the activity of both first-line drugs for the therapy of CDAD, vancomycin and metronidazole, was not argued and susceptibility testing was not even routinely recommended. At present, metronidazole resistance, uncommon but present, is an heterogeneous resistance with clinical consequences that have yet to be elucidated.
Metronidazole and vancomycin remain the drugs of choice for the treatment of CDAD but, both have important limitations and adverse effects. Metronidazole has been linked to a high rate of non-responses and relapses mainly in patients infected with the 027 epidemic strain. Patients with severe or recurrent disease require Vancomycin in higher doses and during longer periods of time.
New antimicrobial agents active “in vitro” against *C. difficile* include: teicoplanin, ramoplanin, daptomycin, telavancin, linezolid, nitazoxanide, tiacumicins B and C and rifaximin. Their role as alternative antimicrobial agents against *C. difficile* is still being defined.
Nitazoxanide is an antihelminthic and antiprotozoal agent that is at least as effective as metronidazole in treating *C. difficile* colitis.
Intravenous immunoglobulins have been used in patients with severe disease or multiple recurrences but there is not any prospective and comparative study to establish their role in the treatment of this disease.
A hyperimmune bovine gammaglobulin that neutralizes the effects of *C. difficile* toxins is under development
Data regarding the role of oligofructose in the prevention of relapses of CDAD are still conflicting. Tolevamer (GT160–246) is a polyanionic polymer chain with a high molecular weight that has clinical cure rates similar to oral Vancomycin when administered to humans at 6 g/day but

failed to achieve non-inferiority when used at 9g/day in a recent clinical trial.
Local bacteriotherapy is the name for the lavage of the lumen of the colon or for the administration of enemas prepared with fresh faeces from healthy volunteers. Reports are almost always of isolated cases or short series and there is no relevant study that allows for giving any recommendations on this method, with obvious drawbacks. This therapy has the additional risk of the transmission of other infectious agents.
Exchange resins such as colestipol or colestyramine that are able to bind to *C. difficile* toxin, may also bind to antimicrobials used to treat CDAD; therefore, their clinical use is not recommended..
Surgery is a last resource for the treatment of unmanageable CDAD with toxic megacolon or colon perforations.
Finally, the perspective for a *C. difficile* vaccine look very promising at the present time.

Pathogenesis

O236 Surfactant protein A promotes the interaction of *Pseudomonas aeruginosa* with the airway epithelial cells and enhances the inflammatory response

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Background: The association of surfactant protein A with *P. aeruginosa* enhances uptake by alveolar macrophages and modulates inflammatory responses including cytokine production and the oxidant burst. However, the role of this association in the interaction with the airway epithelial cells is poorly investigated.
Objective: The aim of this study was to characterise the role of surfactant protein A in the interaction of *P. aeruginosa* with the airway epithelial cells and its biological significance in the pulmonary pathogenesis of this microorganism.
Methods: Surfactant protein A was purified by affinity chromatography from human bronchoalveolar lavage. Binding of purified surfactant protein A to of 22 genetic unrelated *P. aeruginosa* isolates (11 from chronic infections and 11 from acute infections) was analysed by ELISA and Western blot analysis using specific monoclonal anti-surfactant protein A antibody.
To investigate the role of surfactant protein A in the interaction of *P. aeruginosa* with the airway epithelial cells, standard adhesion and invasion assays were performed using 16HBE14- bronchoepithelial cells and A549 pneumocyte type II cells incubated with *P. aeruginosa* pre-opsonised with surfactant protein A either in the presence of calcium or EDTA.
The production of IL-8, TNF-alfa and IL-6 by airway epithelial cells was determined by ELISA according to the manufacturer's instructions.
Results: Binding of purified surfactant protein A varied widely among *P. aeruginosa* clinical isolates. However, the isolates from acute infections bound surfactant protein A more efficiently than those from chronic infections. Surfactant protein A opsonisation of *P. aeruginosa* in the presence of calcium enhanced dramatically bacterial attachment and internalisation by both bronchoepithelial cells and pneumocytes type II. This interaction facilitated the airway epithelial cells mediated inflammatory response increasing the synthesis of IL-8, TNF-alfa and IL-6.
Conclusions: Surfactant protein A promotes the interaction of *P. aeruginosa* with the airway epithelial cells and enhances the production of IL-8, TNF-alfa and IL-6 by these cells. This mechanism is more effective with the isolates from acute infections than from chronic infections.

O237 Fine tuning of *P. aeruginosa* physiology during chronic cystic fibrosis lung disease

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To provide a detailed survey of adaptation of *P. aeruginosa* during chronic infection of the cystic fibrosis (CF) lung, we performed a comparative proteome and transcriptome analysis of isogenic non-mutator and mutator isolates from three selected CF patients. Recently, we showed that during CF lung persistence PA mutators converge to a virulence-attenuated phenotype. In this study, we demonstrate that the adaptation process of PA predominantly comprises metabolic pathways. In end-stage mutator strains, several transcripts of genes or proteins involved in metabolism of fatty acids, nucleotides, amino acids and the generation of energy were increased. Of particular interest is the increased expression level of genes involved in (i) the anaerobic arginine-deiminase pathway, (ii) the anaerobic respiration (iii) the tricarboxylic acid cycle (TCA) and glyoxylate shunt and (iv) the uptake of dicarboxylates. These changes in transcriptome and proteome indicate an adaptive shift towards constitutive expression of genes of metabolic pathways obviously required for growth under micro-aerobic and nutritional conditions of suppurative CF lung tissue. Strikingly, these data provide us with new potential targets for antimicrobial agents to combat chronic CF lung disease.

O238 Effect of antibiotics, alone and in association, on Panton-Valentine leukocidin production by *Staphylococcus aureus*

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Objectives: To examine the capacity of *Staphylococcus aureus* to release Panton-Valentine leukocidin (PVL) in the presence of subinhibitory concentrations of the main anti-staphylococcal drugs.
Methods: *S. aureus* reference and clinical strains were grown in presence of subinhibitory concentrations of ten antibiotics (oxacillin, vancomycin, ofloxacin, co-trimoxazole, pristinamycin, clindamycin, fusidic acid, linezolid, tetracycline and rifampicin) and of four antibiotic associations (oxacillin + clindamycin; oxacillin + linezolid; oxacillin + fusidic acid and oxacillin + rifampicin). The PVL concentration was measured in the supernatant with an ELISA method after 18 h of culture. Pellets of cultures grown with $\frac{1}{4}$ MIC of antibiotics were used for quantitative RT-PCR with specific lukSF-PV and gyrA primers.
Results: The effect of subinhibitory concentrations depended on the antibiotics: oxacillin enhanced PVL level by up to 3-fold, while clindamycin, linezolid, fusidic acid and rifampicin were inhibitory, and vancomycin, pristinamycin, tetracycline, ofloxacin and co-trimoxazole had roughly no effect. We then examined whether the inhibitoriest molecules for PVL could abolish the PVL increase induced by oxacillin. Subinhibitory concentrations of clindamycin and rifampicin dramatically inhibited PVL induction by oxacillin. Linezolid had a slighter dose-dependent inhibitory effect on PVL release. By contrast, fusidic acid was not able to inhibit the PVL induction by oxacillin, except at 50%of the MIC. RT-PCR results confirmed PVL induction by oxacillin and lukSF-PV inhibition of transcription by clindamycin and rifampicin. RT-PCR results of cultures grown with antibiotic associations showed lukSF-PV inhibition of transcription for oxacillin + clindamycin and oxacillin + rifampicin and PVL induction for oxacillin + fusidic acid.
Conclusion: These data support that for staphylococcal infections related to PVL production, antibiotic can either up-regulate or down-regulate PVL release. Against PVL-producing MSSA, β-lactams should be associated with either clindamycin or rifampicin while in PVL-producing MRSA infections, linezolid may be of more benefit than is vancomycin.

O239 Toll-like receptor agonists stimulate phagocytosis of *Escherichia coli* by murine microglial cells

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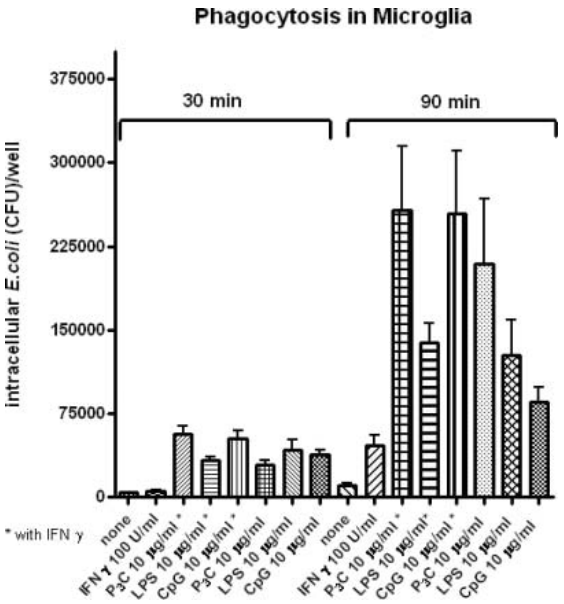
Objectives: Bacterial phagocytosis by microglia during infections contributes to the resistance of the brain. Microglia cells express toll-like receptors (TLR) which can be stimulated by pathogen-associated molecular patterns (PAMPs). *Escherichia coli* is one of the leading Gram-negative bacteria that cause sepsis or neonatal meningitis. We hypothesised that PAMPs may stimulate microglia thereby increasing their ability to phagocytose bacteria.

Methods: Primary cultures of mouse microglia were exposed to the TLR agonists: tripalmitoyl-S-glyceryl-cysteine (Pam3Cys; TLR 2), endotoxin (LPS; TLR 4) and oligonucleotides containing unmethylated cytosin-guanosin motifs (CpG; TLR 9) alone and in combination with interferon-gamma (IFN-g) for 24h. After stimulation, cultures were challenged with *E. coli* DH5alpha at a ratio of 100 bacteria per cell. Phagocytosis was left to proceed for 30 and 90 min at 37ºC. Then, after washing, the microglial cultures were incubated in medium containing gentamicin (200 mg/l) for 1 h to kill extracellular bacteria. Thereafter, cells were washed and lysed with distilled water. For phagocytosis inhibition studies, cytochalasin D (CD) was used at 10 microM. Viable intracellular bacteria were enumerated by quantitative plating of serial 10-fold dilutions. To monitor intracellular survival, microglia cells were allowed to ingest bacteria for 1.5 h. Then, incubation in medium with gentamicin and CD was performed for 1h. Thereafter, intracellular bacteria were determined at various time points by quantitative plating after cell lysis. Kruskall-Wallis test (followed by Dunn's multiple comparisons test) was performed to analyse differences in phagocytosed bacteria between groups ($n \geqslant 10$); $p < 0.05$ was considered statistically significant.
Results: Unstimulated and IFNg-stimulated microglia ingested bacteria at a low rate. LPS, Pam3Cys and CpG strongly increased the number of phagocytosed bacteria ($p < 0.01$ at 30 and 90 min). Co-stimulation by a TLR agonist and IFNg did not significantly increase the number of phagocytosed bacteria compared to stimulation by a TLR agonist alone. CD inhibited phagocytosis >99% in all groups. Intracellular survival assays showed that the number of viable intracellular bacteria reached a plateau at 3h and then started to decrease in all groups.
Conclusion: After stimulation with bacterial TLR agonists, phagocytosis of bacteria by microglial cells is increased. This increase is not dependent on the presence of IFNg.



O240 The pathophysiological importance of apoptosis for the septic patient

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Objectives: The early apoptosis of the monocytes is related to good prognosis in septic shock (Giamarellos-Bourboulis et al. Crit Care 2006). The precise mechanism of the latter process has not yet been investigated. The purpose of this study is to clarify whether the apoptotic procedure is stimulated by the septic patient's serum.
Methods: Serum from 48 patients was isolated within 24 hours from the diagnosis of sepsis. The patients had severe sepsis or septic shock according to the ACCP/SCCM CRITERIA 1992. Serum sampled from healthy donors was used as the control group. Peripheral blood mononuclear cells (PBMC's) were isolated after gradient centrifugation. 5×10^6/ml PBMC's were incubated with septic patient's serum or serum of healthy controls for 24 hours in 5% CO2 at 37ºC. PBMC's were washed and stained with Annexin-V-FITC-anti-CD4-PE-PI and annexin-V-FITC-anti-CD14-PE-PI and analysed by flow cytometry.
Results: Median (IQR) expression of ANNEXIN(+)/CD14(+)/PI(−) on PBMC's incubated with serum of patients and with serum of healthy volunteers was 6.01 (15.28)% and 24.12 (37.56)% respectively (p: 0.004). Respective values for the expression of ANNEXIN(+)/CD4(+)/PI(−) on PBMC's were 7.93 (18.52)% and 3.65 (4.22)% ($p < 0.0001$). After incubation with serum of non-survivors, median (IQR) of the rate of apoptosis of PBMC's was 16.80 (25.18)%; after stimulation with serum of survivors it was 5.04 (8.47)% (p: 0.015).
Conclusion: Serum of patients with severe sepsis or septic shock induces the apoptosis of the lymphocytes and inhibits the apoptosis of the monocytes. This could explain why the induction of CD4-lymphopenia is connected with poor survival and how circulating factors in serum contribute to the perpetuation of the septic procedure through the non-apoptotic monocyte.

O241 Role of the AcrAB efflux pump in *Klebsiella pneumoniae* respiratory infections

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Background: AcrAB efflux pump activity is critical to mediate antimicrobial resistance in *K. pneumoniae*. However, the role of this efflux pump in the virulence of this opportunistic pathogen has been poorly investigated.
Objective: The aim of this study was to investigate the role of the AcrAB efflux pump of *K. pneumoniae* in the respiratory infections caused by this microorganism.
Methods: We constructed by insertion-duplication mutagenesis a specific AcrB mutant, designed as 52DB, from the *K. pneumoniae* virulent strain 52145. Characterisation of the mutant was performed by Southern blot analysis, RT-PCR analysis of AcrB expression, and determination of the MIC of several antimicrobial agents.
To investigate the virulence of the AcrB-deficient mutant, standard survival assays were performed incubating the bacterial cells with human bronchoalveolar lavage, polymyxin B or human beta-defensin. Virulence was also tested in a murine model of pneumonia.
Results: Southern blot and RT-PCR analysis confirmed the interruption of the AcrB gene and the abolishment of the expression of AcrB in 52DB. The mutant was more susceptible to cefoxitin, erythromycin, and nalidixic acid than the parent strain. MICs (mg/l) of antibiotics for strain 52145 and its 52DB mutant were respectively; 8 and 1 (cefoxitin), 64 and 1 (erythromycin), and 4 and 0.5 (nalidixic acid).
The mutant 52DB exhibited a significant reduction of more than three-folds in its capacity to survive in the presence of bronchoalveolar lavage, polymixin or human beta-defensin compared with the parent strain. Furthermore, the mutant was less virulent than the parent strain in a murine model of pneumonia.
Conclusions: AcrAB efflux pump contributes to both antimicrobial resistance and virulence of *K. pneumoniae*.

O242 The two predominant HA-MRSA clones in France exhibit specific adhesive and virulence properties in vitro and in vivo in a mice sepsis model in comparison with MSSA isolates

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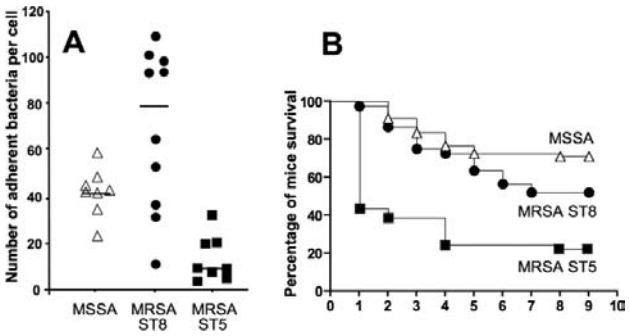
Objectives: To determine if the two predominant pandemic hospital-acquired (HA) meticillin-resistant *S. aureus* (MRSA) clones in France have specific adhesive properties in vitro and virulence properties in a mouse sepsis model in vivo.

Methods: 26 *S. aureus* isolates responsible for bloodstream infections in France in 2003–2007 were included: 10 HA-MRSA isolates belonging to the predominant "Lyon clone" in France (sequence type [ST] 8, SCCmec type IV), 8 HA-MRSA isolates belonging to a minor but emergent MRSA clone in France (ST5, SCCmec type IV), and 10 MSSA isolates with various genetic background. The in vitro binding to human airway epithelial cells (HAECs) and the virulence in a mouse sepsis model were determined. C57Bl/6 and Balb/C mice were i.v. infected with inocula of 10^7 to 10^8 (five to ten female per isolate). agr dysfunction (that may enhance adhesion) and virulence factors such as the capsular type (CP) and the toxin gene profile were screened in all isolates to interpret the results.
Results: In comparison to the MSSA group, a higher adhesion (mean of 69.1 ± 10.9 versus 42.2 ± 3.7 adherent bacteria per cell, $P < 0.001$; Figure, panel A) and a higher virulence in the mouse sepsis model ($P = 0.072$ with C57BL/6 and $P = 0.01$ with Balb/c) was observed for the MRSA ST8 group (Figure, panel B). The MRSA ST5 group was significantly less adhesive to HAECs (mean of 12.1 ± 3.3, $P < 0.001$; Figure, panel A), but induced a high mortality (79% at day nine; Figure, panel B) in the sepsis model in comparison with MSSA ($P < 0.001$). We observed heterogeneity in the results obtained with the MRSA ST8 group both in the adhesion and in the sepsis model, with three MRSA ST8 isolates not being lethal at all. The higher adhesion of MRSA ST8 isolates did not correlate with agr dysfunction. Virulence of MRSA ST8 and ST5 isolates may be due, at least in part, to the combination of expression of CP5 and production of superantigenic toxins. Indeed, all MRSA isolates: (i) expressed CP5, contrary to four MSSA, only; (ii) harbored genes encoding SEA (MRSA ST8) or TSST-1 (MRSA ST5) whereas none of the MSSA isolates were positive for these genes.



Conclusion: The two pandemic MRSA clones in France exhibit specific adhesive and virulence properties that may explain their success. CP5 and production of superantigenic toxins may be key virulence factors for these pandemic MRSA clones.

O243 The ferric yersiniabactin uptake receptor FyuA is required for efficient biofilm formation by urinary tract infectious *Escherichia coli* in human urine

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Objectives: Urinary tract infection (UTI) is the most common infection in patients with indwelling urinary catheters and bacterial biofilm formation is a major problem in this type of infections. Bacterial biofilms are highly resistant towards fierce flows and high concentrations of antibiotics. Understanding the mechanisms behind biofilm formation is necessary in order to combat these infections.
Methods: Microarray analysis was performed on samples from *E. coli* UTI strain VR50 grown in MOPS/shake flask, urine/shake flask and urine/biofilm. Biofilm formation of knockout mutants and wild-type strains was investigated in microtitre plates and flow-cell chambers.
Results: The ferric yersiniabactin uptake receptor (FyuA) which is encoded on the high pathogenicity island (HPI) was found to be highly important for biofilm formation by UTI *E. coli* in human urine. Global gene expression profiling of UTI strain VR50 during biofilm formation in urine showed that all genes located on the HPI were significantly up-regulated; interestingly, this was the only iron acquisition system displaying up-regulation in biofilm urine growth compared with planktonic urine that was not up-regulated in planktonic urine compared with minimal medium.
The fyuA gene was among the highest up-regulated of all genes, 63-fold. Furthermore, an fyuA mutant showed significant reduction in biofilm formation compared with its parent – both on polystyrene (microtitre plates) and on glass (flow chambers). In urine flow-cell chambers the mutant showed 92% less biofilm compared with the wild-type (Fig. 1). However, when complemented in trans or supplied with extra iron the mutant regained its biofilm-forming faculty. Also, introduction of an fyuA-encoding plasmid into three UPEC strains increased biofilm formation significantly.

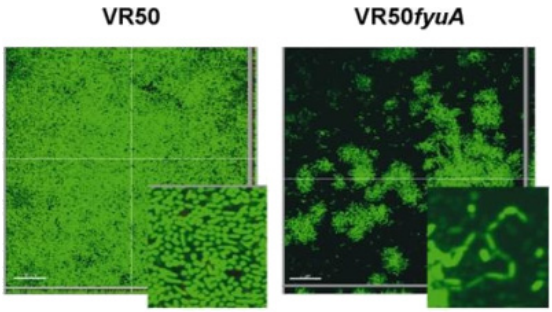

Figure 1. Biofilm formation of VR50 and the *fyuA* mutant in flow chambers in human urine. The mutant showed 92% less biofilm compared with the wild-type. Scale bars, 30 μm.

Conclusion: Free iron is strictly limited in the human urinary tract and there is fierce competition between the host and infectious bacteria for this essential metal. UTI *E. coli* has highly efficient mechanisms of iron acquisition, one of which is yersiniabactin encoded on the HPI. Here we demonstrate a direct link between FyuA and biofilm formation in iron-poor environments such as human urine. We also show that the availability of iron greatly influences the UTI strains' ability to form biofilm.

O244 SgrA, a serine-glutamate repeat containing MSCRAMM of *Enterococcus faecium* CC17 binds fibrinogen

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Objectives: The incidence of infections caused by *Enterococcus faecium* has dramatically increased in hospitals world wide. Nosocomial outbreak associated and most clinical isolates cluster together by multilocus sequence typing in a hospital-adapted genogroup, designated as clonal complex-17 (CC17). Recently, five genes encoding LPXTG surface proteins were found to be enriched in CC17 *E. faecium* isolates. One of these five, sgrA, formerly known as orf2351, is present in all of the CC17 *E. faecium* isolates and in only 26% of the non-CC17 *E. faecium* isolates. The objective was to functionally characterise SgrA, which is a potential target for immunotherapy to prevent and treat *E. faecium* infections.
Methods: Sequence encoding sgrA was cloned, expressed as a 6xHis fusion protein (rSgrA) in *Escherichia coli* and purified using affinity chromatography. Binding of rSgrA to extracellular matrix molecules as fibronectin, fibrinogen, laminin, vitronectin and BSA (negative control) was assessed by ELISA and ligand-affinity Western blotting. Reverse transcriptase (RT) PCR was used to detect mRNA transcripts of sgrA. Transmission immuno electron microscopy (TEM) was used to determine association of the SgrA surface protein with the cell wall.
Results: SgrA has similarities with the Clumping factor B protein of *Staphylococcus aureus* and contains a serine-glutamate repeat region. In an ELISA, the purified rSgrA protein showed concentration dependent binding to immobilised fibrinogen in a saturable manner and to a lesser extend to vitronectin and not to fibronectin or BSA. By use of

ligand-affinity blotting, rSgrA showed to bind to the alpha, beta and gamma subunits of fibrinogen. Using RT-PCR, mRNA transcripts of sgrA were detected in all phases of growth at 37ºC and TEM demonstrated association SgrA with the cell wall.
Conclusion: We showed that sgrA is constitutively expressed at mRNA level at 37ºC, encodes a LPXTG surface protein, and mediates adherence to fibrinogen. This makes SgrA the second member of the MSCRAMM (microbial surface component recognising adhesive macromolecules) family identified in *E. faecium*. The ability to adhere to fibrinogen may play a role in the pathogenesis of CC17 *E. faecium* in hospital-related infections and may have contributed to successful adaptation in the hospital setting.

O245 Gliotoxin is an important virulence factor in cerebral aspergillosis

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Objective: The high lethality of cerebral aspergillosis urgently asks for a deeper insight into the pathogenic mechanisms of this disease. Therefore we studied whether mycotoxins, especially gliotoxin, contribute to neural damage and to the inefficient immune response in cerebral aspergillosis. Furthermore we tested the putative capacity of antioxidant components to interfere with the harmful activity of gliotoxin.
Methods: The secretion of gliotoxin after fungal growth in cerebrospinal fluid (CSF) was measured using HPLC and tandem mass spectrometry. An effect of gliotoxin on the viability and proliferation of astrocytes, neurons and microglia was tested by MTS assays. Phagocytic activity of cells in the presence of gliotoxin was quantified using fluorescent latex beads and subsequent microscopic analysis; oxidative burst was studied by FACS.
Results: Pathogenic *Aspergillus* species like *A. fumigatus* secrete significant levels of gliotoxin when cultivated in CSF. The corresponding concentration of gliotoxin was sufficient to affect the viability of astrocytes, neurons and microglial cells, with neurons and microglia being the most sensitive cell types. Subtoxic concentrations of gliotoxin diminished the capacity of microglia to phagocyte pathogens. Furthermore gliotoxin exaggerated the oxidative burst in infiltrating granulocytes induced by stimuli such as PMA.
Therapeutic approaches might aim to neutralize the inhibitory or even toxic effect of gliotoxin and thus to reconstitute the potency of cerebral immunity. We used glutathione for this purpose, a tripeptide made up from cysteine, glutamate and glycin which act as a reducing compound. In first promising experiments with glutathione we were able to protect brain cells from gliotoxin concentrations which otherwise were shown to kill the cells. Furthermore glutathione reconstituted the phagocytic activity of immune cells in the presence of gliotoxin.
Conclusions: These data indicate an essential contribution of gliotoxin to the pathogenesis of cerebral aspergillosis. As a therapeutic approach neuroprotective substances such as glutathione might be used to neutralize the toxic or immune-inhibitory activity of gliotoxin.

Public health and community-acquired infections

O246 Current status of diphtheria and related infections in Europe

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Objectives: To describe the current status of diphtheria and related infections caused by *Corynebacterium diphtheriae* and *C. ulcerans* in Europe, as well as the changing epidemiology with respect to C. ulcerans infection. Also in liaison with the ECDC and WHO EURO to compare the different surveillance systems across the European region, leading to the development of a European surveillance database.
Methods: A survey was undertaken of the number of microbiologically confirmed cases due to *C. diphtheriae* and C. ulcerans, and the current surveillance systems of the 25 Diphtheria Surveillance Network (DIPNET) member countries. DIPNET was officially recognised as a Dedicated Surveillance Network by the European Commission in 2006, and originates from the European Laboratory Working Group on Diphtheria, formed in 1993 because of the re-emergence of diphtheria to epidemic levels in the Russian Federation and Newly Independent States during the 1990s. One of DIPNET's projects is the development of a database in order to link surveillance, microbiological and molecular data, creating an integrated tool for both microbiologists and epidemiologists.
Results: The number of toxigenic strains reported in the last seven years (2000–2006) varied widely from none (in 12 countries), to 487 (in Latvia). Some countries, mainly those with strong microbiological support reported mild cases of diphtheria caused by toxigenic *C. diphtheriae* and C. ulcerans. Interestingly, the isolation of toxigenic C. ulcerans is increasing, relative to toxigenic *C. diphtheriae* in some countries. All member countries provided information about their current surveillance systems for diphtheria, and their potential to collect case-based data as part of a standardised European dataset.
Conclusion: Whilst diphtheria is generally well controlled by vaccination in most European countries, there is a continued threat of re-emergence. The absence of reported cases in some countries may reflect the lack of appropriate epidemiological and microbiological investigation. The increasing prevalence, in some countries, of C. ulcerans infection, which can present with symptoms of classical diphtheria, also highlights the need for increased understanding of this pathogen, associated with contact with domestic animals. Development of the integrated surveillance database should provide a useful tool for monitoring changes in the epidemiology and increasing our understanding of both *C. diphtheriae* and C. ulcerans infections.

O247 Recent temporal trends in morbidity and mortality due to pneumonia in the Netherlands

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Objective: Community acquired pneumiae (CAP) remains a major cause of morbidity and mortality in the Netherlands, however little is known about recent temporal trends in the general population, which is essential for an adequate policy on infectious diseases. We conducted a population-based retrospective study to examine and compare these recent trends in the Netherlands between 1997 and 2006
Methods: We analysed three national databases to assess incidence of general practitioner consultations (1) and hospitalisation (2) and mortality rates due to pneumonia (3) (1:Netherlands Investigation Network of General Practice (LinH), 2:Dutch Monitoring Network (LMR), 3:Statistics Netherlands (CBS)). We assessed incidences and mortality rates for respiratory years (May 1st year N until April 30th year N+1), adjusted for demographic changes in age and gender. For the GP visits we excluded repeated visits for pneumonia by the same patient within 90 days after the initial consult. Hospital admissions were included when pneumonia was the main diagnosis of a patient. For mortality we included cases with pneumonia being the primary or secondary cause of death
Results (preliminary): Between 1997/1998 and 2005/2006, the adjusted mortality rate of pneumonia decreased with about 1% per year. The adjusted hospital admission rates increased with approximately 7% per year in the period between 1999/2000 and 2005/2006.
The incidence of patients visiting their GP with a (suspected) pneumonia did increase with about 8% per year between 2001/2002 and 2005/2006.
Conclusion: Though the Dutch population is aging, the mortality rate of pneumonia is slowly but surely decreasing. On the other hand, the morbidity has increased considerably in the recent years, which proves that pneumonia remains a great clinical and public health concern.

O248 Body mass index at age 50 to 64 and risk of subsequent hospitalisation with pneumonia

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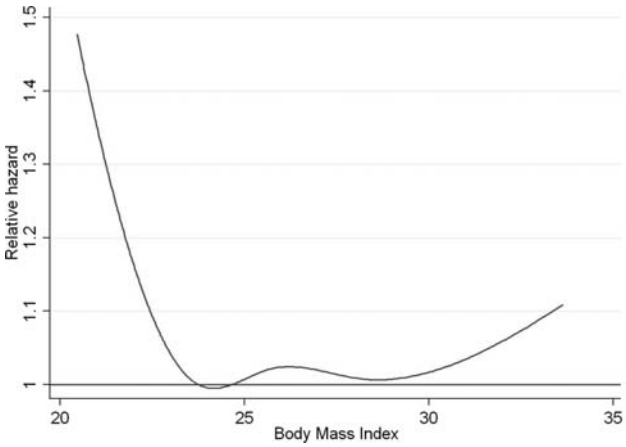
Objectives: The incidence of hospitalised pneumonia in Denmark has increased considerably during the last decade. Little information is available on the impact of body mass on the risk of pneumonia. The aim of this study was thus to examine the association between BMI and risk of hospitalised pneumonia.
Methods: We conducted this large prospective cohort study using data from the Danish cohort "Diet, Cancer and Health" including individuals 50–64 years of age recruited during 1993–97 with detailed information concerning diet and other lifestyle factors and anthropometric measurements. We linked data from the cohort study to the Danish National Patients Registry and retrieved information on all incident hospitalisations with pneumonia before 2006. We excluded individuals with a prior hospitalisation for pneumonia and those with incomplete information on anthropometric measurements or potential confounders (n = 1,102), leaving 55,951 for the analysis. We categorised BMI into five groups (<22.5, 22.5–24.9, 25.0–29.9, 30.0–34.9, 35+) and estimated the cumulative risk of hospitalisation for pneumonia within the first 5-years of observation. We used Cox's regression to compute hazard ratios as measure of relative risk (RR) for hospitalisation for pneumonia (reference: BMI = 22.5–24.9). We adjusted for comorbidity, smoking, and alcohol intake. Since changes in body composition differ in men and women we stratified the analysis according to gender.
Results: A total of 2,664 subjects had a first episode of hospitalised pneumonia during a median follow-up of 8.9 years. The 5-year risks of hospitalisation with pneumonia were 7% for BMI < 22.5, 5% for BMI = 22.5–24.9, 5% for BMI = 25.0–29.9, 6% for BMI = 30.0–34.9, and 7% for BMI = 35+. The association between BMI and adjusted relative hazard of pneumonia (Reference is BMI = 23.8)is shown in fig. 1. Compared with participants with a BMI of 22.5–24.9 the adjusted RRs were for men: 1.5 (95% CI:1.2–1.8) for BMI < 22.5, 1.1 (95% CI:1.0–1.3) for BMI = 25.0–29.9, 1.2 (95% CI:1.0–1.4) for BMI = 30.0–34.9, and 1.8 (95% CI:1.4–2.4) for BMI of 35+. For women the adjusted RRs were 1.3 (95% CI:1.1–1.5) for BMI < 22.5, 0.9 (95% CI:0.8–1.0) for BMI = 25.0–29.9, 0.9 (95% CI:0.7–1.0) for BMI = 30.0–34.9, and 0.9 (95% CI:0.6–1.2) for BMI of 35+.



Conclusion: We found a clearly increased risk of pneumonia among individuals with a BMI below 22.5 and for men also among those with a BMI of more than 35.

O249 Guidelines observance by general practitioners for respiratory tract infections: a quantitative study using the "Small Samples Approach" for in-depth, case-based analysis in French-speaking Belgium

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Background and Objectives: Belgium is a "high antibiotics prescribing" country in Europe for outpatients (Clin Infect Dis. 2007, 44:1091–5; 44:1259). This has triggered (i) the development of national guidelines for ambulatory practice using Evidence-Based Medicine data (supported by the official Belgian Antibiotic Policy Coordination Committee), and (ii) the sending to each general practitioner (GP) of an individual feedback comparing her/his personal prescribing habits with an "average GP" in her/his region. We wanted to assess how these guidelines and feedbacks are perceived by GPs, and to determine how they are followed for respiratory tract infections (RTI).
Methods: SSA (in-depth analysis of actors' behaviour aiming to identify the rationale of a decision when faced with actual data) was used retrospectively on a cohort of patient contacts (n = 150) for antibiotic prescription for RTI. GPs (n = 38) were randomly selected and approached for data collection from medical records and direct interview (n = 30), to document medical history, reasons for encounter, symptoms, clinical examination, patient's demand, imaging or laboratory tests, diagnostic, prescribed antibiotic, and compliance with guidelines. Data were anonymously analysed in a double-blinded fashion by two independent researchers (both GPs) for assessment of guideline observance (antibiotic need and choice).
Results: Level of enrolment was 79%. Observance of guidelines (with CI 95%) as assessed by the GPs themselves was 41% (33–49) [non-observance: 26% (18–34); guidelines not know: 32% (24–40)]. Inappropriateness of antibiotic prescription, as assessed by the independent researchers, was 56% (48–64). Reasons for non-observance of guidelines (as expressed by the GP's; by order of frequency) were that guidelines are (i) too restrictive; (ii) unusable in everyday practice, (iii) not credible, (iv) only money-saving oriented, or (v) not known. Patients' expectations were judged as a major factor in the prescribing decision, overtaking the opinion of official scientific authorities.
Conclusions: Official guidelines and recommendations have only a limited impact on actual prescribing behaviour. Efforts to curb overprescription of antibiotics in RTI for community patients must aim at (i) decreasing patients' demands, and (ii) making guidelines more usable in everyday practice, independent of financial considerations, based on more credible sources, and with goals that the practitioner consider as being reachable.

O250 Do Bugs Need Drugs? Pharmacist train-the-trainer programme in Alberta, Canada

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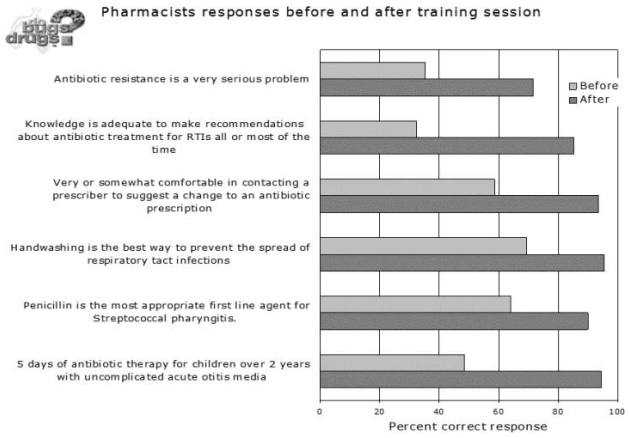
Objective: To increase the capacity to deliver education about appropriate use of antibiotics in communities in Alberta, Canada (population 3.3 million).
Methods: Previous studies showed that parents whose children received Do Bugs Need Drugs? (DBND) instruction in school or daycare were better informed about appropriate antibiotic use than controls. A train-the-trainer programme was designed to increase capacity to deliver DBND key messages: 1) handwashing prevents infections, 2) bacteria and viruses are different, and 3) use antibiotics wisely. The DBND programme is funded by Alberta Health and Wellness.
Based on interest and enthusiasm expressed by pharmacists, DBND training workshops were provided for pharmacists in five communities March-June 2006 and were monitored by before and after surveys. Pharmacist trainers received an antimicrobial handbook, educational tools and honoraria for teaching others, including nursing and early childhood education (ECE) students and daycare managers. Subsequently, nursing students used DBND kits to teach 7–8 year olds

in grade two classrooms. ECE students and daycare managers were given a kit to teach children and staff in daycares. Signs and booklets were provided for the school, daycare and parents.
Results: After the training session, pharmacists knew significantly more about antibiotic use, were more confident about recommending treatment options and were more comfortable in contacting a prescriber to change an antibiotic prescription (Fig).



Of the 176 pharmacists attending training workshops, 50 became DBND trainers. By 13-Nov-07, 50 lectures had been provided by DBND trainers at 6 nursing schools and 19 ECE programmes, targeting over 600 nursing and 250 ECE students or managers. Over 450 classrooms and 13 childcare agencies have participated in this programme, impacting over 12,500 children, their teachers and families.
Feedback from pharmacist trainers, nursing instructors and students, children and teachers has been positive. The programme has opened new lines of communication with school boards and public health departments.
Conclusions: This multi-step approach has improved pharmacists' knowledge about antibiotic resistance and confidence in communicating with others. Involvement of nursing and ECE students has increased the capacity to provide community education about appropriate antibiotic use in Alberta with the aim of reducing rates of antibiotic resistance.

O251 Q fever outbreak in southeastern Netherlands

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Objectives: Q fever is a worldwide zoonosis caused by Coxiella burnetii. Goats, sheep and cattle have been described as the most common animal reservoir and birth products from infected animals are an important source of environmental contamination. Transmission to humans occurs by the aerosol route or by ingestion of non pasteurised milk products. In the late spring of 2007 there was an outbreak of Q fever in the South East of the Netherlands.
Results: From January until November there were 150 confirmed (and 23 probable) cases of Q fever in the South East of the Netherlands whereas normally 5–20 cases would be expected annually in the whole country. The area has a population of 90.000 and is primarily rural.
Patients reported a sudden onset of high fever, sweats, cough, headaches and muscle pain. There were 3 to every 2 females, their median age was 51 years and 71% had a history of smoking. Forty-nine percent of the patients were admitted to the hospital all with pneumonia except two patients who had hepatitis and one patient who had endocarditis. Most patients were treated with moxifloxacin 400 mg once daily for 7 days and recovered within 24–48 hours. However, 15% of the patients still had complaints, mainly fatigue and may develop chronic Q fever. Of the 18 pregnant women who were screened 2 (11%) had an active infection and were treated with co-trimoxazole for the rest of their pregnancy.
There was a long dry and hot spell during the spring of this year and Coxiella burnetii is highly resistant to chemicals and heat and can survive for long periods in the environment which may have contributed to this outbreak. The source of the outbreak is still under investigation but transmission was almost certainly by way of aerosols.
Conclusion: There was a large outbreak of Q fever in the South East of the Netherlands. Most patients suffered from a pneumonia implicating that the transmission is probably causes by the aerosol route and climate change may have contributed to this exceptionally large outbreak of Q fever in the Netherlands.

O252 Co-infection with zoonotic *Campylobacter* and *Salmonella*

K.O. Gradel, B. Kristensen, T. Ejlertsen, H.C. Schønheyder, H. Nielsen (Aalborg, Aarhus, DK)

Objectives: We hypothesised that patients co-infected with thermophilic *Campylobacter* (TC) and non-typhoid *Salmonella* (NTS) had different characteristics than patients with TC or NTS mono-infections.
Methods: Population-based registry study comprising all TC/NTS co-infected patients (0–7 days between TC and NTS detection) and all first-time mono-infected TC or NTS patients in two Danish counties from 1991 through 2003. Data on recorded comorbidity were retrieved from the Danish Hospital Discharge Registry and data on one-year mortality from the Danish Civil Registration System.
Results: A number of 114 patients were co-infected and 13,335 had mono-infections (6,768 TC [50.8%] and 6,567 NTS [49.2%]). The age distribution (median [1st-3rd quartile]: 26.2 [19.2–44.4] years for co-infected vs. 29.7 [17.6–48.2] years for mono-infected), comorbidity (15 co-infected (13.2%) vs. 1,611 mono-infected [12.1%]; OR [95% CI] = 1.1 [0.6–1.9]), and seasonal distribution (peak date and peak-to-trough ratio [95% CI]: 22 August and 2.6 [1.4–4.9] for co-infected vs. 28 August and 4.4 [4.1–4.8] for mono-infected) did not differ between the two patient groups. All co-infected and 13,100 mono-infected patients (98.2%) were alive after one year (p = 0.15). Co-infected patients had a higher ratio of exotic NTS serotypes (in contrast to *Salmonella* Enteritidis and *S.* Typhimurium) than mono-infected NTS patients (53.5% vs. 21.8%; OR [95% CI] = 4.1 [2.8–6.1]), whilst there were no differences between S. Enteritidis and S. Typhimurium (OR [95% CI] = 1.1 [0.6–2.0]).
Conclusion: Co-infected patients were not frailer than mono-infected patients. The differences in serotype distribution amongst NTS infected patients indicate that infection sources and behavioural factors (e.g., travel) are more important determinants of co-infection vs. mono-infection than baseline patient characteristics.

O253 Waterborne gastro-enteritis at a scout camp caused by Norovirus types 1 and 2

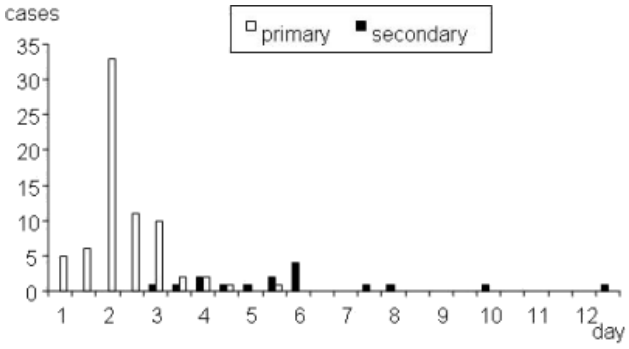
H.L.G. ter Waarbeek, N.H.T.M. Dukers-Muijrers, H. Vennema, E.I.G.B. de Brauwer, R.C.H. Boesten, C.J.P.A. Hoebe (Geleen, Bilthoven, Heerlen, NL)

A gastro-enteritis outbreak in July 2007 at a scouting camp (77 children/29 leaders), 40% hospital admission, was investigated.
A retrospective study was performed. Epidemiological investigation included a standardised questionnaire about sex, age, risks, symptoms and family members. A primary case was defined as diarrhoea/vomiting (in any 24-h period). Stool and water was collected for microbiological analysis. Another questionnaire study was done, to identify household secondary cases. Attack rates and risk factors were determined.
Questionnaires were returned by 65 children/19 leaders (response rate 82.4%, median age:13 (IQR 11–17.7, range 7–47), female: 50%). Primary attack rate was 67% (71 cases), with 87% vomiting, 73% diarrhoea, 41% fever.
Water was obtained from a farmer's well. Participants who drank water more often fell ill (RR:3.9, 95%CI:1.5–10.5) with clear dose-response effect. 97% of the cases drank water. Using the primitive toilet was also associated with illness (RR: 4.9 (2.0–11.9)), whereas food was not. Stool samples (68 cases/11 non-cases) were negative for bacteria and parasites. 95.8% of the cases returned stool; PCR showed norovirus in 75% (51):

51% type 1 only, 25% type 2 only, 24% both 1–2, genotype II.7 Leeds, I.2 Southampton. Looking only at cases with positive stool revealed a stronger risk for water (RR=17.0, p = 0.02) than toilet use (RR=2.8, p = 0.4). Of all cases 77.5% were early cases (day 1–2): more often <16 yrs (RR 1.5), vomiting (RR 4.1), fever (RR 2.1) and often type 1. Of 47 primary cases 32 households (79 members at risk) completed a second questionnaire (response rate 63%). Secondary AR was 20.3% and did not differ for the primary cases' virus type: AR type 1=22.9%, type 2=23.1%, type 1–2=18.2%. Only a small water sample was available. Analysis showed coliform bacteria, *Escherichia coli* and *Enterococcus*. Norovirus was negative.

This unique outbreak shows a point source infection with two water-transmitted noroviruses: type 1 and 2, proved by epidemiological and microbiological findings, compatible incubation time and symptoms, also high attack rate, clear secondary attack rate, faecal water contamination and detection of mostly type 1 but also type 1–2. Not enough water was at hand to detect norovirus.

Waterborne norovirus outbreaks regularly occur, but probably often stay undetected. Monitoring water for viruses should be encouraged. At this camp using bottled water and adequate toilets could have prevented this epidemic.



O254 VZIG administration in pregnant women exposed to chickenpox

J. Troughton, G. Crealey, V. Crawford, C. McCaughey, D. Wyatt, H. O'Neill, P. Coyle (Belfast, UK)

Objectives: Varicella infection during pregnancy poses a serious risk for both foetus and mother. The current UK practice regarding exposure in pregnancy is immunity testing and varicella immunoglobulin (VZIG) administration to non-immune women. This may not provide best patient care, since a vaccine is now available. Additionally verbal followed by serological screening of primigravidas giving a negative history at antenatal booking with post-natal vaccination of those non-immune may be more cost-effective than current policy.

The study aims to retrospectively compare the cost of the current policy with a cost estimate for antenatal screening with post-partum vaccination in N. Ireland.

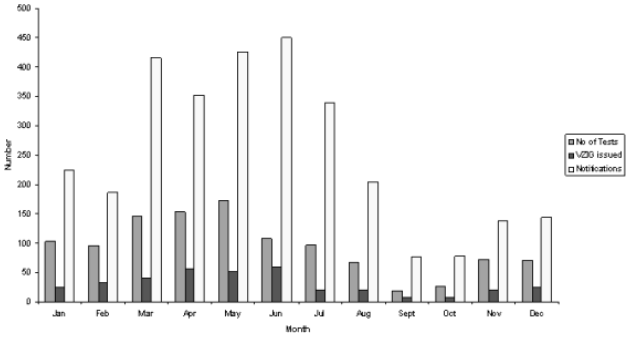
Method: The cost of VZIG issued in 2006 was calculated from the number of vials issued to pregnant women, at £280 per vial.

A cost estimate of antenatal screening of primigravidas, with post-partum vaccination, was calculated for two models: 1) verbal screening, with serological testing of those with no history of varicella infection; 2) serological screening of all primigravidas. The calculation included: number of first pregnancies in N. Ireland; cost per varicella immunity test; estimated number of non-immune primigravidas; and cost of a varicella vaccine course.

Results: The varicella epidemic curve spans the entire 12 month period (see graph). The cost of VZIG issued to pregnant women in 2006 was £100,800; 43% of births were to primigravidas therefore the estimated cost of VZIG issued to multigravidas was £58,100. The cost of verbal screening with serological screening of those with no history followed by post-partum vaccination is estimated at £23,750 p.a, saving £34,350 over current policy.

The estimated cost of serological screening of all primigravidas with post-partum vaccination is £43,000, saving £15,100.

Conclusion: This retrospective study suggests that in N. Ireland either of the proposed antenatal screening strategies with post-natal vaccination would be less costly than current practice, and may improve patient care.



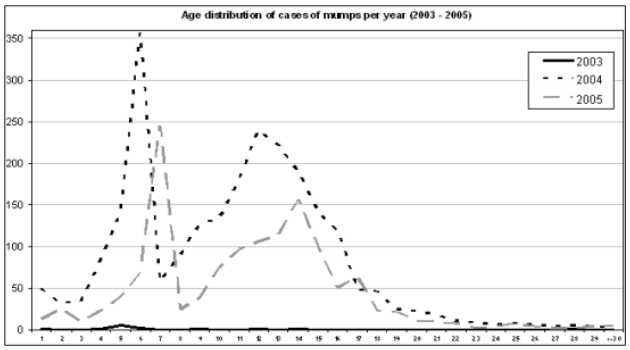
Graph 1. Vericella Notifications, Tests and Immunoglobulin in 2006.

O255 Sustained mumps transmission in a highly vaccinated population

M. Wohoush, M. Obeidi, M. Hindiyeh, F. Riccardo, G. Sabatinelli (Jerusalem, IL; Amman, JO)

Objectives: To describe a parotitis outbreak in a highly vaccinated population of Palestine refugees in West Bank (WB).

Methods: The United Nations Relief and Works Agency (UNRWA) surveillance system targets various diseases including mumps. In December 2003, following an increase in the reported incidence of mumps among WB Palestinian refugees, an outbreak line listing was established, serum IgM and IgG test was adopted for confirmation and RT-PCR for differential diagnosis and parotitis virus isolation. The immunisation status of patients was assessed from immunisation cards. Univariate analysis was performed and attack rates calculated.



Results: On average 3 cases meeting the WHO clinical case definition for mumps are reported each month from WB (670,613 refugees of whom 176,726 living in 19 refugee camps). When 5 mumps cases were reported from a single camp in northern WB in December 2003 the system was alerted. In 2004 and 2005, mumps cases increased by more than 4 folds the baseline. The epidemic started in north-eastern WB (Nablus) spread westwards and extended to central WB (Jerusalem). Only one case was reported in 2005 in southern WB (Hebron). A total of 3928 cases were detected (males 56%, mean age 11.7±7 years, 70.8% in the 6–15 years age group). Attack rates in the camps varied between 0.03% and 4.3% (overall 1.2%). Complications were reported in 1% of cases and all outcomes were favourable. UNRWA introduced MMR vaccination (single dose) in 1988. 67.5% of all patients and 80% of those born after 1988 had been immunised at least one month before the onset of symptoms. 6 year-olds in 2004 and 7 year-olds in 2005 were the mostly affected age groups suggesting a cohort effect for vaccinated in 1999 (Fig.) moreover almost 30% of all vaccinated patients had been

immunised between 1999 and 2000. In order to control the outbreak an MMR vaccination campaign was conducted in May 2005 targeting 58,561 students from UNRWA schools and vocational training centres (coverage 96%). In 2006 and 2007 cases dropped below the outbreak threshold line but settled at higher than pre-outbreak levels.
Conclusions: High immunisation coverage did not prevent the parotitis outbreak although complications seemed to occur more rarely than expected. Although preliminary data suggests a cohort effect pointing to a problem occurred during a specific vaccination year, further investigations are planned to explain this event and avoid future epidemics.

Antibiotic stewardship in the ICU: today and tomorrow (Symposium arranged with ESGAP)

S280 Future antibiotics for intensive care patients?

U. Theuretzbacher (Vienna, AT)

Although there are good treatment options available for most common infections, antibacterial resistance has risen dramatically in ICUs and the need for new compounds without cross-resistance to existing drugs is apparent. Meticillin-resistant *Staphylococcus aureus*, pan-resistant *Pseudomonas* and *Acinetobacter* have been recognised as major resistance threats in many ICUs. Some patients are left without a therapeutic option because of resistance against all available antibiotics. No doubt, resistance is outrunning antibacterial research and development. Since the 1980s, the focus of most pharmaceutical companies shifted from antibacterial to more lucrative disease areas that promise to meet the financial targets of the big companies. To reduce the risk and to speed up development time, many companies are presently limiting their antibacterial programmes to building on existing classes of antibiotics – developing analogues that lead to second and third generations of the original compound. Thus, cross-resistance to the newer agents can develop in short order because of the abundant pool of existing resistance genes.
In general, development efforts are mainly focused on anti-staphylococcal drugs that target a sufficiently large market. These new drugs are analogues of known antibacterial groups. In any case, the medical need for new anti-staphylococcal drugs will be met with several new antibiotics expected to be launched within the next two years. Clinical development activities against multi-resistant Gram-negative pathogens including *Pseudomonas* and *Acinetobacter* are virtually non-existent. Looking towards the coming years, no new antibiotics are anticipated and no new scaffolds are close to preclinical stages of development.
Recently, there have been signs of regained interest in the field of resistant Gram-negative infections from a few companies. These companies and also academic research sites are exploiting unconventional high-risk approaches and pursuing new targets. It is difficult to assess the potential of such new approaches and it remains to be seen if they will yield useful antibiotics for ICUs in the future. In any case, it will take at least 10 years before we can see the fruits of current research efforts in the Gram-negative field. Therefore, prudent use of existing antibiotics, improved dosage regimes, surveillance efforts, and stringent adherence to infection control guidelines are essential to preserve the power of existing antibiotics.

S281 Consumption, resistance and policies in European ICUs

H. Hanberger (Linköping, SE)

Critically ill-patients admitted to ICUs are highly susceptible to infections because of predisposing illnesses and the use of invasive procedures, and are therefore exposed to high antibiotic pressure. The high and increasing prevalence of antibiotic resistant bacteria among patients admitted to European hospitals in addition to high antibiotic consumption and shortcomings in infection control measures increase the risk of significant outbreaks caused by antibiotic-resistant pathogens among the critically ill. In countries with previously low resistance levels (Scandinavia, Finland and the Netherlands), the frequency of multi-drug resistance (MDR), particularly MRSA, may increase rapidly if surveillance and measures to combat outbreaks fails. The need for an early warning system of emerging and/or increasing antibiotic resistance for local, national and international use is apparent. A programme for continuous yearly registration of antibiotic use, resistance and clinical practices with automatic feedback through a website was developed for use in ICUs. It was launched as "CARE-ICU" in 2005. Antibiotics to which >90% of isolates of a species were susceptible were defined as treatment alternatives (TA90) which is a novel index of susceptibility to measure the magnitude of MDR. Thirty four ICUs in 8 countries participated in the large pilot during 2005. The median yearly admissions and admission days were 551 and 2595 respectively. There were four important main findings in this first report from CARE-ICU. First, antibiotic consumption varied widely from 348 to 4992 DDD1000 with a relatively high median consumption of 1254 DDD1000. Second, levels of antibiotic resistance was very high in many settings but varied also greatly between species, ICUs and countries and the finding that more than half of all participating ICUs had no or only one treatment alternative (TA90) for *P. aeruginosa* with at least 90% susceptibility to aminoglycoside, ceftazidime, ciprofloxacin and carbapenem was alarming. Third, there was a lack of rooms for isolation precautions and cohort care for patients colonised or infected with alert organism. Four, antibiotic guidelines including routines for discontinuation of antibiotics, was available in some of the high antibiotic consuming ICUs but with low compliance which shows the need for more effective tools for change of prescription behaviour and a sustainable surveillance of compliance to guidelines in general.

Why should adults care about paediatric vaccination?

S283 Role of pneumococcal conjugate vaccines in protecting adults from disease and antibiotic resistance

R. Dagan (Beer-Sheva, IL)

Despite the extensive global contribution of paediatric vaccines to health in all ages in the last decade, the adult infectious disease expert community continues to show a lack of interest in paediatric vaccines. Yet, each of the licensed paediatric vaccines has had a tremendous effect on morbidity of adults. Two main mechanisms are responsible for the effect of childhood vaccination on adults: 1) A vaccinated child grows to be a vaccinated adult. In some instances the adult may be protected and in other he/she might show a modified disease; and 2) vaccinated children often show reduction in carriage and then spread of pathogens to adults, protecting them from disease (herd immunity).
Most of the times these 2 effects are beneficial to adults, but in some instances, such as pertussis or varicella vaccines, these effects could increase disease in adults if vaccination programmes are not implemented properly.
The complex relationship between paediatric vaccines and adult disease will be demonstrated through the examples of pertussis, varicella, pneumococcal and Hepatitis A vaccines.
Time has come to change the term "paediatric vaccines" for a more appropriate term such as "vaccines for life".

S284 Can paediatric influenza vaccination protect adult populations?

C. Weil-Olivier (Colombes, FR)

Children, especially infants, toddlers and school-age ones, have a high attack rate during yearly influenza seasonal epidemics. Epidemiological data confirm the anteriority of children' epidemics peak from the adults'

one by two weeks. A longer duration of viral shedding and higher viral titres in the naso-pharynx compared with adults, associated with promiscuity (day care centres, school) explain the children' major vector role in this respiratory transmission. Any intervention in childhood limiting Influenza virus circulation in the community should provide indirect protection ("herd immunity") to adults.

Large immunisation programmes in children have been investigated in different settings measuring the protection of contacts (households) or the community at large. So far, routine immunisation of school-age children appear to be effective in preventing the disease burden in the community and adults contacts in several studies. Nevertheless, methodological discrepancies between them render imprecise the estimation of this strategy on effectiveness: which preventable fraction is achievable in adults? The impact in the community depends on the coverage rate achieved, the vaccine efficacy and the contact patterns. Some simulation models taking in account different efficacy figures of Influenza vaccines (most assume a protective efficacy of 70% and a 80% efficacy for infectiousness) and different coverage levels suggest that a coverage rate of 20% of schoolchildren would result in as much mortality reduction in elderly as vaccinating 90% of persons aged 64 and over.

Nevertheless, if the universal influenza immunisation strategy in school-children has demonstrated some benefits in term of reduction of the disease burden – either in observational studies or in mathematical models – in adults or elderly, some points must be raised:

- Routine vaccination of school aged children has not been shown to decrease by indirect effect the burden of disease in infants and younger children who are highly infectious (thus transmitting the virus) or more at risk of hospitalisation for severe Influenza disease. So far the American recommendation of immunisation first before 23 months of age, then, since 2006 up to 59 months of age is mainly based on a reduction of morbidity, hospitalisation, mortality in the targeted group.
- An other source of concern is the low coverage rates achieved post-recommendation in the US, especially for the full primo-vaccination demanding two doses below 9 years of age.
- It has not been demonstrated yet that routine vaccination of children below 2 years of age has an indirect impact in the population.
- In Europe, the injectable trivalent inactivated influenza vaccine TIV, sole available raises the issues of acceptability by the families, and on a national public health point of view the feasibility of such a programme, the huge increase of resources and the adequate information strategies.
- TIV efficacy may vary depending of the child's age, the matching of the strains – wild/vaccine – although mathematical models assume its upper range (70%) and never more conservative hypothesis (the real life).
- Clearly the availability of an intra-nasal live attenuated influenza vaccine in Europe could be an opportunity to enlarge the strategies due to its higher protective efficacy and easiness of administration.

S285 Pertussis in children, adolescents and adults: mutual protection by mutual vaccination

N. Guiso (Paris, FR)

Pertussis is a human respiratory disease due to a bacterium, Bordetella pertussis. Since the generalised use, for newborns and children only, of the first pertussis vaccines, called Pertussis whole-cell (Pw) vaccines, because composed of inactivated bacterial suspensions, Pertussis is still endemic. Why? The use of Pw vaccines led to a dramatic decrease in the incidence of the disease in children but conducted to demonstrate and confirm that Pertussis is not a paediatric disease. Pertussis can infect children, as well as adolescents or adults. The disease is dramatic for newborns but also for persons at risk such as elderly and pregnant women. One of the characteristics of Pertussis is to induce a short immunity, as well as Pw vaccines. A human can be infected two or three times during its life. Twenty years after the introduction of Pw vaccines for children, adults are no more immune and can contaminate newborns too young to be vaccinated. For this reason, the introduction of vaccine boosters was needed. This was possible after the development of Pertussis acellular (Pa) vaccines, composed of purified inactivated bacterial proteins. Since 1995, these vaccines are used for newborns and children but also for adolescents and adults in many developed countries in Australia, North America and Europe. However, vaccine coverage of adolescents and adults is still low and mortality of newborns less than 2 months of age is now again observed in developed countries. Adequate pertussis surveillance and reporting system are important to implement vaccine strategy. They must be reinforced to evaluate the consequences of the changes of (i) pertussis vaccine used (Pa instead of Pw), (ii) vaccine strategy (addition of adolescents and adults boosters), (iii) characteristics of the disease necessitating the development of new specific, sensitive and standardised biological diagnoses, (iv) bacteria circulating since herd immunity will be modified by universal immunisation and might have an impact on the properties of the agent of the disease.

S286 Rotavirus – does it affect adults and will infant vaccination protect adults?

T. Vesikari (Tampere, FI)

Worldwide, rotaviruses are responsible for half a million deaths from severe acute childhood gastroenteritis annually. In developed countries deaths are rare, but hospitalisation because of rotavirus-associated dehydration is common. With dehydration corrected, the disease in well-nourished children is self-limiting. Most hospitalisations occur in children 6 to 24 months of age, when susceptibility to life threatening dehydration is highest. However, both rotavirus infections and rotavirus gastroenteritis continue to occur in older children as well as adults.

In adults rotavirus infections occasionally may lead to symptomatic disease. There is evidence of endemic circulation of rotavirus in adults that is independent from the winter epidemic cycle in children. In addition, adults exposed to children with rotavirus gastroenteritis are frequently infected and often come down with symptoms. Rotaviruses may also cause outbreaks in adults in closed communities and, particularly, in the elderly.

Two live oral rotavirus vaccines have been licensed recently. These are: Rotarix^{TM} (GSK), a human G1P[8] vaccine given in two doses, and RotaTeq^{TM} (Merck & Co., Inc.), a human-bovine reassortant vaccine given in three doses. Both induce protection against severe rotavirus gastroenteritis caused by multiple serotypes of rotavirus, and neither protects against rotavirus infection. Both vaccines are being shed and might be rarely transmitted, but they do not remain in circulation. Vaccine induced protection will be sustained for a few years – enough to pass the most susceptible age for dehydration. However, both asymptomatic rotavirus infections and mild breakthrough cases of rotavirus gastroenteritis will occur in vaccinated children.

Universal mass vaccination against rotavirus has been introduced in a number of countries, including USA, Brazil, Venezuela, Mexico, and, in Europe, Austria and Belgium. While both licensed rotavirus vaccines effectively protect the immunised individual, the long term consequences of mass vaccinations are uncertain and purely speculative. There is no evidence of herd immunity. As a result of vaccination, exposure of adults to wild type rotavirus gastroenteritis in children might result in waning immunity and actual increase in rotavirus gastroenteritis in adult populations. The prospect of immunising target populations of adults with rotavirus vaccines remains a distant possibility to be explored in the future.

The role of modelling in understanding the dynamics and prevention of antimicrobial resistance

S287 Transmission dynamics of infectious diseases – the inclusion of evolutionary concepts with our thinking on transmission and control

R. Anderson (London, UK)

Conventional approaches to the study of the transmission dynamics and control of infectious diseases rest on the principles of population ecology and to a degree on traditional statistical methods. With the rise and rise of ever cheaper tools to examine the structure, and changes their in over time and space, of both pathogen and host genomes, the need arises to modify population and transmission dynamic frameworks to encompass natural selection acting on both host and pathogen, and more generally the concepts of evolutionary change. The talk will discuss recent work to examine evolutionary concepts based on (a) stimulated phylogenetic trees for pathogens within a transmission framework and (b) the evolution of drug resistance in pathogen populations including both viruses and bacteria, and the parasitic tropical diseases.

S288 Catastrophic failure of MRSA control in the hospitals: myth or reality?

B.S. Cooper (London, UK)

Successful control of an infectious disease has a precise mathematical definition: if the mean number of secondary cases caused by a typical infectious case is maintained below one, an epidemic is said to be under control. When this condition is met, though transmission may still occur, and infection rates may temporarily increase as well as decrease, the long-term reduction in incidence and ultimately the termination of the chain of transmission is guaranteed (provided immigration of the infectious agent into the population can be prevented).
While some interventions, such as hand hygiene, may be expected to be equally effective regardless of the number of cases, resource-limited control measures, such as nurse cohorting and side-room isolation, can become less effective as the number of cases increases. For example, in a low-level MRSA setting isolation facilities or staffing levels may be sufficient to allow all detected cases to be effectively and promptly isolated. In contrast, in high-level MRSA settings resource limitations (e.g. limited availability of isolation beds and insufficient staffing levels) can delay the time to effective isolation or make some interventions, such as nurse cohorting, entirely unfeasible. Under such circumstances an intervention that results in epidemic control in a setting with a low MRSA prevalence can fail in a high prevalence setting. Thus, two stable outcomes are possible even with identical control measures are in place. If interventions are put in place early enough they can successfully maintain infection at a low level preventing high-level MRSA becoming established. If put in place too late, or compromised by immigration of MRSA from other settings, identical interventions can have a much smaller effect, only slightly suppressing MRSA levels, and failing to reduce prevalence to a low level. Transitions between these two outcomes are known mathematically as catastrophes, and can also have catastrophic consequences for patient welfare. Simulation models have shown that both stochastic fluctuations, and immigration of MRSA cases from other settings can, in principle, lead to such catastrophic shifts from successful control to control failure. In this talk, the evidence that such catastrophic transitions also occur in practice will be reviewed, making use of new statistical developments for analysing hospital infection data and highly detailed MRSA transmission data.

New aspects of *Enterococcus faecalis* and *E. faecium* infections, including VRE

S291 New insights into the pathogenesis and treatment of enterococcal endocarditis

B.E. Murray (Houston, US)

Enterococci were first identified in the late 1890s from the blood of a patient with infective endocarditis (IE) and, since then, enterococci (primarily *E. faecalis*) have been recognised as causing 5–15% of cases of IE, 3rd behind staphylococci and streptococci. They have also been reported as the 2nd most common cause of nosocomial IE. Because cell-wall active agents are not bactericidal against many enterococci, the recommended therapy for enterococcal IE has, for many decades, been a synergistic combination including 4–6 weeks of a cell-wall active agent plus an aminoglycoside (AG). Recent studies have suggested that shorter AG regimens and, for strains with high-level resistance to all AG, the combination of ampicillin with ceftriaxone may also have adequate efficacy for *E. faecalis* IE. Therapy for endocarditis caused by vancomycin resistant *E. faecium* remains more problematic.
Relatively little is known about the pathogenesis of enterococcal IE. Our recent studies have identified pili in *E. faecalis*, encoded by the ubiquitous ebp (for endocarditis and biofilm related pili) locus and made up of 3 subunits, each of which have the characteristics of cell wall anchored MSCRAMMs. We showed the importance of these pili in initiating biofilm, in experimental IE (EIE) and in serum-induced adherence to fibrinogen, suggesting these pilus structures are involved in initiating *E. faecalis* IE. The ebp locus is regulated in part by the Fsr system, a homologue of the Agr system of staphylococci. We have also identified a ubiquitous collagen adhesin, Ace (also an MSCRAMM), whose expression is induced by serum and collagen, and found that this adhesin is also important in *E. faecalis* EIE. Similarly, we have shown that its homologue (Acm, an adhesin to collagen of *E. faecium*), is important in the pathogenesis of *E. faecium* EIE and adherence to heart valves, the first factor shown to be important for virulence in that species. Preliminary data suggest that immunisation against recombinant Ace or Acm is protective against EIE by the producing species. Based on genome searchs, both *E. faecalis* and *E. faecium* have a number of other MSCRAMM genes encoding adhesins to ECM proteins, and future studies may reveal additional contributions to IE. Another factor shown to be importance in *E. faecalis* IE is gelatinase, a protease produced by only ~60% of *E. faecalis* despite the fact that the encoding gene gelE is found in over 90% of stains; deletion of part of the regulatory locus, fsr, explains the lack of gelatinase production by many strains. Aggregation substance, encoded by pheromone responsive plasmids found in some *E. faecalis*, and a cytolysin/hemolysin may influence the size of EIE vegetations and mortality produced by *E. faecalis*, respectively. Additional studies are needed to define the role of other factors in enterococcal EIE and the ability of passive or active immunisation to prevent this disease.

S292 Conserved, co-evolved genes of invasive *Enterococcus faecium* and *Enterococcus faecalis* and insights into special lineages

R.J.L. Willems (Utrecht, NL)

Enterococcus faecalis and *Enterococcus faecium* have both evolved in so-called high-risk enterococcal clonal complexes (HiRECC), defined as specific clades of isolates with multiple antibiotic resistance traits, epidemiological associated with either colonisation or infection of hospitalised patients. One of these HiRECC is *E. faecium* CC17, which represents a distinct pandemic clonal complex of extremely successful hospital acquired isolates. Also in *E. faecalis* specific genetic subpopulations exist, like CC2, CC9, and CC40, which are enriched among nosocomial isolates and as such can be classified as HiRECC. Thus *E. faecalis* and *E. faecium* isolates belonging to these HiRECC share niches with the potential of virulence gene exchange. The extent

of genetic exchange, especially of virulence genes between these two species is unknown but can be disclosed by assessing the presence of orthologs in *E. faecalis* and *E. faecium* clinical isolates. Comparative genomic hybridisation and whole genome sequencing, implemented to identify genes specific for CC17, distinguished more than 100 genes with most notably CC17 specific IS elements, resistance genes, genes putatively encoding cell surface proteins and metabolic pathways. Of a subset of these *E. faecium* CC17 genes orthologous genes were identified among *E. faecalis* bloodstream isolates. Eight of these orthologs were highly similar to genes previously found on EfaB5, an *E. faecalis* Tn916-like element containing several genes related to virulence in other species. The sequence identity of EfaB5 orthologous genes between *E. faecalis* and *E. faecium* was >85%, which is considerably higher than the sequence identity of the core genomes of the two species, based on an in silico comparison and reflected by the relative high average of 16S rRNA sequence divergence (4.1%) and the low average nucleotide identity of housekeeping genes (74.2%). The *E. faecium* EfaB5-like orthologs, though highly similar to the *E. faecalis* EfaB5-like element, are not similarly organised in one cluster but separated by stretches of other genes and the order partially inverted. The presence of highly similar genes in HiRECC *E. faecium* CC17 and *E. faecalis* CC2, 9, and 40, suggests genetic exchange between the most prevalent clinical enterococcal subpopulations. A common EfaB5-like element, in addition to *E. faecium* and *E. faecalis* specific virulence determinants, might have contributed to pandemic spread and nosocomial enterococcal infections.

S293 Novel immunotherapy strategies to prevent enterococcal infections/colonisations

J. Huebner (Freiburg, DE)

Enterococci are among the three most common nosocomial pathogens and due to their multiple antibiotic resistances cause substantial morbidity and mortality, especially among intensive care patients and the immunocompromised. While several new antibiotics have been introduced in the last decade, resistance against these new drugs is developing and spreading rapidly. Alternative treatment and prevention strategies are desperately needed to counter the rise of multiply resistant clones in hospitals and nursing homes worldwide.

There is only a basic understanding of the mechanisms that are responsible for the transition of a normally benign commensal into a dangerous pathogen. A better understanding of the virulence factors that enable enterococci to access the bloodstream and cause systemic infections will help to target new therapeutic and prophylactic approaches.

Compared to other Gram-positive pathogens, relatively little is know about surface antigens in enterococci that may be used as vaccine targets. Several proteins such as aggregation substance, ACE (adhesin to collagen), EfaA, an ABC transporter complex, and Esp have been studied, although only few data exist on the protective efficacy of these antigens in appropriate animal models. Newly identified pili in *E. faecalis* are associated with adhesion, biofilm formation and pathogenicity, and since these antigens are highly immunogenic antibodies against these structures may be promising candidates for immunotherapy.

Several carbohydrate antigens have been identified in *E. faecalis*, while so far no information exists for *E. faecium*. Similar to other Gram-positive bacteria, enterococci possess membrane-associated lipoteichoic acid (LTA) and peptidoglycan-associated wall teichoic acids (WTA). While LTA has been shown to be the target of protective antibodies for *E. faecalis* and *E. faecium* infections, no information exists for WTA. Additional capsular polysaccharide antigens consisting of a diheteroglycan have been identified in some *E. faecalis* strains and these antigens also elicit opsonic antibodies making them likely candidates for vaccine approaches.

Taken together, there are several promising vaccine candidates among surface carbohydrate antigens, as well as among the protein antigens studied so far. Conjugation of a protein target to a carbohydrate component may enhance immunogenicity and broaden the coverage of such a vaccine. Knowledge of the clinical application, mode of vaccination (passive vs. active immunotherapy), timing of application and the identification of a patient population that will profit most needs to be acquired in order to develop a successful immunotherapy strategy.

S294 The emergence of VRE (multi-resistant enterococci) and its clinical impact in hospitals in Europe

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The epidemiology of vancomycin-resistant enterococci (VRE) in Europe has recently changed. The initial scenario consisted of polyclonal enterococcal population with a high diversity of strains and transposable elements encoding vancomycin resistance in the community and with scarce presence in the nosocomial setting. In the last few years six European countries have reported prevalence rates in hospitals >20% and nosocomial VRE outbreaks are frequently described. Clonal and plasmid outbreaks have been documented in European hospitals but complex epidemiological situations involving strains (*Enterococcus faecalis* and/or *Enterococcus faecium*) and different mobile genetic elements are frequently reported in institutions with high VRE rates. Population genetics studies have shown that most VRE isolates from hospitalised patients belong to worldwide disseminated hospital high-risk clonal complexes (HHRCCs) of *E. faecalis* (CC2, CC9, CC21, CC87) and *E. faecium* (CC17) often resistant to multiple antibiotics, while VRE from non-hospitalised individuals or animals belong to other CCs. In addition, specific HHRCCs or clones within these HHRCCs become relevant in the persistence and dissemination of VRE. Horizontal transfer is also important in the recent spread of VRE in European hospitals although plasmid outbreaks have been described since early 90's. The most frequent type of acquired glycopeptide resistance found is VanA and the genes coding for resistance are part of Tn1546, usually located in conjugative plasmids. Variants of Tn1546 containing mutations, deletions and different insertion sequences have been described and an apparent high diversity and host specificity of these genetic elements has been suggested. However, the detection of different Tn1546 variants in common plasmids platforms suggests frequent genetic exchange events within particular wide disseminated plasmids. A change in the epidemiology of VRE in European hospitals seems to have occurred with the emergence and dissemination of specific plasmids and HHRCCs resistant to multiple antibiotics and possibly more transmissible and virulent.than others Situations of endemicity or polyclonal outbreaks are difficult to handle since limite therapeutic options and require genotyping-targeted infection containment. Detailed characterisation of epidemic VRE strains and mobile elements will help to understand the reasons involved in the evolutionary outcomes of HHRCC in different countries.

MDR *Acinetobacter* from molecular biology to hospital epidemiology

O295 In vivo transposition of insertion sequence ISAba1 at the origin of genome plasticity and acquired antibiotic resistance in *Acinetobacter baumannii*

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Background: Insertion sequence ISAba1 belonging to the IS4 family has been identified repeatedly in *Acinetobacter baumannii*, being often associated with antibiotic resistance genes. In particular, it has been shown to provide promoter sequences enhancing expression of the natural cephalosporinase AmpC and oxacillinase OXA-51 of *A. baumannii*. In addition, two copies of ISAba1 bracketing blaOXA-23 may form composite transposon Tn2006 at the origin of acquisition of this carbapenem-hydrolysing oxacillinase gene. The transposase of ISAba1 is formed by two open reading frames Orf1 and Orf2 likely giving rise to a functional transposase when a frameshift occurs. Our study was aimed to evaluate the transposition ability of ISAba1.

Methods: We evaluated experimentally the ability of ISAba1 to transpose in *Escherichia coli*. In a first step, the β-lactamase gene

blaTEM-1 was inserted into ISAba1 to provide an ampicillin resistance marker allowing its tracing. This ISAba1::blaTEM-1 structure was cloned into pTOPO vector and then transformed into recA(−) *E. coli* RZ201 harboring conjugative plasmid pOX38-Gen used as a target for transposition events. Transposition events onto pOX38-Gen were selected by conjugating this plasmid into azide-resistant *E. coli* J53. The same technique was used to evaluate the transposition ability of Tn2006. Also the role of the frameshift in the transposase expression was evaluated by eliminating the frameshift in the transposase encoding gene by site-directed mutagenesis.

Results: Transposition of ISAba1 was obtained at a frequency of 1×10^5 ($\pm0.7\times10^5$) per *E. coli* donor. Sequencing of the target sites of transposition revealed i) a systematic 9-bp duplication upon transposition, and an adenine-rich hotspot of target transposition. Transposition frequency of the mutated ISAba1 with a transposase gene made of a unique frame was 2×10^3 ($\pm0.2\times10^3$). The mobility of Tn2006 was demonstrated and its frequency of transposition was estimated to be 1.6×10^8 ($\pm2.5\times10^2$).

Conclusion: This study iss the very first demonstration of the functionality of the widespread ISAba1 as a mobile element. The frameshift-mediated down-regulation of transposition has been assessed, as well as the functionality of the ISAba1-made composite transposon. This in-vivo model will allow further experiments able to evaluate the possible selective factors such as antibiotics for ISAba1-transposition enhance

O296 The OXA-51-like enzymes of *Acinetobacter baumannii*: markers of success?

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Objectives: *Acinetobacter baumannii* has become a prevalent nosocomial pathogen due to the spread of major epidemic lineages. The diversity of the intrinsic blaOXA-51-like genes may provide insights into the evolution of this species. This study aimed to examine the relationship between the sequences of the OXA-51-like enzyme family and the properties of a collection of *A. baumannii* isolates.

Methods: Sixty *A. baumannii* isolates had their blaOXA-51-like gene amplified by PCR using external primers, and the products sequenced and identified using BLAST and MultAlin software. The external primers allowed the simultaneous identification of ISAba1 upstream of the blaOXA-51-like genes. MICs for imipenem and meropenem were determined according to British Society for Antimicrobial Chemotherapy (BSAC) guidelines. Isolates were assigned to sequence groups (SGs) based on PCR amplification of fragments of their blaOXA-51-like, ompA and csuE genes. All publicly available OXA-51-like amino-acid sequences were retrieved and used to construct a linkage map showing the relationships of the enzymes to one another.

Results: The linkage map revealed that some of the enzymes form closely related clusters while others are less closely related. The largest number of isolates, including European clone II, contained enzymes in the OXA-66 cluster, and were assigned to SG1. The second largest group of isolates, including European clone I, contained enzymes in the OXA-69 cluster, and were assigned to SG2. The third largest isolate group, including European clone III, were assigned to SG3 and contained an OXA-71 enzyme. Eight isolates contained enzymes not found in a major cluster, or could not be assigned to a SG. ISAba1 was found upstream of the blaOXA-51-like gene in ten isolates, but was only associated with enzymes on branch tips in the OXA-66 and OXA-69 clusters. MICs for imipenem and meropenem ranged from 0.06 and 0.12 mg/L respectively up to >128 mg/L for both antibiotics. Isolates with ISAba1 upstream of the blaOXA-51-like gene tended to have MICs towards the higher end of this range, from 0.5 and 2 mg/L up to 8 and 32 mg/L.

Conclusion: SG1 and SG2 represent the most prevalent epidemic lineages of *A. baumannii* and encode specific sub-sets of OXA-51-like enzymes. SG3 is not as prevalent, but is also associated with a specific OXA-51-like enzyme. A minority of isolates cannot be grouped using this typing scheme. Evolution of the OXA-51-like enzymes appears to be occurring in real time.

O297 Outbreak of multidrug-resistant *Acinetobacter baumannii*-producing OXA-23 carbapenemase at one Belgian acute care hospital

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Objectives: *Acinetobacter baumannii* (Ab) is a well recognised nosocomial pathogen, especially for critically-ill patients. The emergence of multi-drug resistant (MDR) and carbapenem-resistant Ab isolates constitutes a major therapeutic challenge. Outbreaks caused by MDR Ab are mostly reported from tropical or sub-tropical areas and from Mediterranean countries. Here we report the characterisation of OXA-23 producing Ab strains isolated in an acute Belgian hospital.

Methods: All MDR Ab isolates recovered from patients and from the environment were phenotypically and genetically (PCR-sequencing and PFGE) characterised. Resistance patterns were analysed by disc diffusion and MICs determination. PCR-sequencing was performed for blaIMP, blaVIM, blaOXA-23-like, blaOXA-26-like, blaOXA-58 genes, blaOXA-51-like genes, blaTEM gene, blaOXA-20, and the chromosomal class C β-lactamase blaAmpC gene. The presence of insertion sequence (IS) elements upstream the detected β-lactamases genes was also analysed.

Results: In 09/2007, a MDR- and carbapenem-resistant Ab was recovered from one Belgian resident transferred from Morocco with a diagnosis of pneumonia. Subsequently, over a 2 month period, similar MDR-Ab isolates were found in 6 patients (4 with pneumonia, 2 with asymptomatic throat colonisation). Five of the patients were hospitalised in the pneumology ward and 2 in the intensive care unit where the outbreak secondarily spread.

All MDR-Ab clinical isolates were positive for blaOXA-23 with an ISAba4 sequence being present immediately upstream this gene. Further, 14 environmental samples obtained during the outbreak also yielded the same MDR OXA-23-producing Ab. All clinical and environmental isolates were confirmed by PFGE to be clonally related. After the implementation of reinforced infection control measures no additional MDR Ab were isolated neither from patient nor from the environment.

Conclusion: Our data highlight the importance of inter-country transfer in the spread of MDR carbapenem-resistant OXA-23 producing Ab and the propensity of this opportunistic pathogen to cause major nosocomial outbreaks. Systematic screening, and implementation of additional precautions are mandatory for any patients transferred from high-risk areas.

O298 Outbreak of carbapenem-resistant *Acinetobacter baumannii* carrying the carbapenemase OXA-23 in a German university medical centre

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Objective: Investigation and control of an outbreak of carbapenem-resistant *Acinetobacter baumannii*.

Methods: An outbreak investigation with a descriptive analysis, a case-control study, environmental sampling and molecular typing of *Acinetobacter baumannii* isolates using randomly amplified polymorphic DNA (RAPD) with M13 primer and pulsed-field gel electrophoresis (PFGE) was carried out. Detection of OXA-type carbapenemases was performed by multiplex PCR. For the case-control study cases defined as patients with the outbreak strain and controls defined as patients with a minimum duration of intensive care unit (ICU) stay of 5 days were selected from the mainly affected ICU during the outbreak period. Odds ratios (OR) were calculated in a univariate analysis and a multiple logistic regression analysis was performed to identify independent risk factors for acquisition of the outbreak strain.

Results: 35 patients acquired carbapenem-resistant *A. baumannii* with a similar antibiogram in five ICUs and two wards of two different

tertiary care hospitals within a time period of ten months in 2006. All but two isolates were resistant to all penicillins, cephalosporins, ciprofloxacin, gentamicin, tobramycin, imipenem, and meropenem, and remained susceptible only to colistin and tigecycline, i.e. isolates were pandrug-resistant. All isolates belonged to one major strain type with RAPD pattern a and pulsotype A with subtypes A1–A4. All but two isolates belonging to subtypes A2 and A3 carried the OXA-23-like gene in addition to the intrinsic OXA-51-like gene. The most likely mode of transmission was cross-transmission from colonised or infected patients and the environment via the hands of personnel. The multiple logistic regression analysis showed that severity of illness (mean daily SAPS II score > median) and intensity of care (mean daily TISS-28 score > median) were independent risk factors for acquisition of the outbreak strain (OR 6.67; CI95 1.55–36.56 for both variables).
Enhanced infection control measures with enforcement of standard precautions, education of personnel, cohorting of patients with the outbreak strain in separate ward areas and screening of ICU patients for *A. baumannii* on admission and once weekly were successful in controlling the outbreak.
Conclusion: This is the first report of an outbreak of *Acinetobacter baumannii* carrying the carbapenemase OXA-23 in Germany.

O299 Switching populations: establishment of OXA-40-producing *Acinetobacter baumannii* within a hospital setting

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Objectives: Endemicity of an OXA-40-producing *A. baumannii* clone has been observed at numerous hospitals within the Iberian Peninsula associated with mortality events in Portugal. In this study, the epidemiological evolution, within a university hospital, of 3 *A. baumannii* imipenem-resistant clones is described throughout a period of six consecutive years.
Methods: From 2001 to 2006, 252 imipenem-resistant *Acinetobacter baumannii* (IRAb) were collected from several specimens and different wards, where *A. baumannii* was associated with nosocomial infections and colonisations.
Isolates were identified by API32GN and by 16S rRNA sequencing. PFGE (ApaI restriction enzyme) was performed in representative isolates. Oxacilinase genes were sought by PCR.
Results: Clonal dissemination of 3 pulsetypes (A,B,C), widespread throughout the hospital, contributed to the observed *A. baumannii* imipenem resistance, which has persisted since 2001 despite several elimination attempts, including the use of polymyxin. Pulsotype B was predominant from 2001 to 2002, after which clone A (identical to the previously described Iberian OXA-40-producing clone) emerged as the dominant type. Pulsotype C (2 isolates) seemed to represent a sporadic event within the prevalence of clones A and B. Absence of imipenem-hydrolysing enzymes was observed for both B and C.
Resistance to β-lactams (including carbapenems) and variable susceptibility to aztreonam, amikacin and tobramycin was a common in clone A. B and C clones showed lower resistance profiles (e.g. susceptibility to ceftazidime, netilmicin and minocycline; variable susceptibility to meropenem, cefepime, and aztreonam). Curiously, excluding one isolate, all the remaining IRAb isolates (n = 27), recovered in 2006, produced an OXA-carbapenemase (OXA-40 and, for the first time in this hospital, OXA-23). blaOXA-40 producers were characterised as pulsotype A while clones B and C were not detected.
Conclusion: The strong selective forces within hospital settings have been historically implicated in the establishment of resistance phenotypes. The evolution of bacterial resistance together with aggressive therapeutic strategies envisions the maintenance of only the best adapted, able to cope with various stressful agents. Our data highlights an apparent succession in IRAb populations, from more susceptible bacteria to the establishment of an OXA-40 producing clone expressing a wider resistance profile to several antibiotics (including tigecycline).

O300 First detection of carbapenem-resistant *Acinetobacter baumannii* producing the OXA-24 carbapenemase in Italy

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Background: *Acinetobacter baumannii* is an important opportunistic pathogen responsible of serious nosocomial infections. Multi-drug resistance is common, and carbapenems often remain among the few therapeutic options. Production of acquired OXA-type carbapenemases (Ambler class D) of the OXA-23, OXA-24/40 and OXA-58 lineages appears to be a major mechanism of acquired carbapenem resistance in *A. baumannii*. Here we report the first detection of a carbapenem-resistant *A. baumannii* producing the OXA-24 carbapenemase in Italy.
Methods: Bacterial identification was carried out with phenotypic and molecular approach. Susceptibility testing were carried out by broth microdilution and Etest. Genotyping was carried out by RAPD and typing of the blaOXA-51-like, csuE and ompA genes. OXA-type and metallo-carbapenemase determinants were investigated by PCR and sequencing.
Results: Two carbapenem-resistant *A. baumannii* were isolated in 2000, at Varese University Hospital (northern Italy), from the BAL of two ICU-patients. The isolates differed from other carbapenem-resistant *A. baumannii* from the same hospital for a very high level of carbapenem resistance (imipenem and meropenem MICs, >32 mg/L). They were also resistant to expanded-spectrum cephalosporins, aminoglycosides (amikacin and gentamicin), ciprofloxacin, but susceptible to colistin. Characterisation of carbapenemase genes revealed the presence of a blaOXA-24 allele in both isolates. Genotyping showed that the two isolates were identical to each other and belonged to European clone II. They were also clonally related to other lower-level carbapenem-resistant isolates from the same hospital, which were found to produce OXA-58. The blaOXA-24 determinant was surrounded by a genetic context similar to a previously described plasmid-mediated blaOXA-24 determinant.
Conclusions: To our best knowledge this is the first report of *A. baumannii* producing OXA-24 in Italy. The isolates, noticed for their exceedingly high level of carbapenem resistance, did not spread in the hospital. Interestingly, they were clonally related to the European clone II, which exhibits a remarkable propensity to evolve carbapenem resistance by different mechanisms.

O301 Dissemination of OXA-23-producing *Acinetobacter baumannii* Iberian clone in hospitals and ambulatory

F. Grosso, S. Quinteira, L. Cavaleiro, L. Peixe on behalf of the Portuguese Resistance Study Group

Objectives: Class D carbapenemases carrying *Acinetobacter* spp. have been confined, in Portugal, to OXA-40 producers. Recently, we identified the first OXA-23-producing *A. baumannii* in a clinical isolate. The aim of this work was to characterise the recently isolated strains of OXA-23-producing *A. baumannii*, some of them associated to outbreaks in Portuguese hospitals, and to investigate the clonal relatdness between them and the endemic OXA-40-producing clone
Methods: Nineteen imipenem-resistant isolates of *A. baumannii* revovered from four Portuguese hospitals and an ambulatory patient between 2006 and 2007 were studied. Isolates were identified by API32GN and by sequencing the 16S rRNA gene. Susceptibility to antibiotics was tested by disk diffusion method and Vitek 2 system. MICs for tigecycline and colistin were determined by the agar dilution method. Oxacillinase genes (OXA-23, 40, 51, and 58) were sought by a multiplex PCR. ISAba1 insertion sequence upstream of blaOXA-23 gene was also searched by PCR.
PFGE (ApaI) was performed for comparison of these isolates with the endemic clone.
Results: *A. baumannii* isolates were resistant to all β-lactamic antibiotics tested, tigecycline and colistin. The isolates involved in hospital outbreaks were mostly susceptible to aminoglicosides, being one susceptible only to amikacin and another one resistant to all antibiotics. PCR assays detected the presence of blaOXA-23. ISAba1 was detected

upstream of the OXA-23 gene for all isolates except one. PFGE showed that the isolates' profile was related with the imipenem-resistant *A. baumannii* clones disseminated throughout the country, including the endemic OXA-40 producing *A. baumannii* clone.
Conclusions: Emergence of OXA-23 producing *A. baumannii* is occurring in several Portuguese hospitals associated to outbreaks and sporadic cases. Of interest is the relatdness with the endemic OXA-40 clone, although with an enlarged resistance profile, which supports the plasmidic acquisition of these two oxacillinases by a well adapted *A. baumannii* clone. It is of note the recovery of an OXA-23 producer from an ambulatory patient which can further promote community dissemination.

O302 Emergence during therapy of efflux-mediated tigecycline resistance in *Acinetobacter baumannii* belonging to a UK epidemic clone

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Objectives: Up-regulation of the RND-type efflux system AdeABC has been implicated in reduced susceptibility to tigecycline in *A. baumannii*. AdeABC expression is controlled by the two-component regulatory system AdeRS. We investigated the role of this pump in the emergence of tigecycline resistance using a pre- and post-treatment pair of clinical isolates.
Methods: Isolates were identified by API20NE profile, and susceptibilities were first determined by BSAC disc methodology, then by agar dilution and Etest on IsoSensitest agar. PFGE was used to determine relatedness. Expression of the efflux pump, AdeABC, and its regulatory proteins, AdeRS, was examined by real-time reverse transcriptase (RT)-PCR using primers specific for adeB and adeR, respectively, and quantified relative to the RNA polymerase beta subunit gene, rpoB, which was used as a reference.
Results: The two *A. baumannii* isolates were recovered from abdominal drain fluid of a 38-yr old woman who had undergone a cholecystectomy. Inter alia she had received a 14-day course of tigecycline, and a 32-fold difference in susceptibility was observed between pre- and post-treatment isolates (MICs, 0.5 mg/L and 16 mg/L, respectively). The patient has since made a full recovery. The isolates had identical PFGE profiles and belonged to a prevalent UK strain, OXA-23 clone 1, with OXA-23 carbapenemase and initial susceptibility only to tigecycline and polymyxin. Real-time RT-PCR identified a 24-fold increase in adeB gene expression in the post-treatment isolate. No concomitant difference in adeR expression was observed.
Conclusions: OXA-23 clone 1 is a widespread multi-resistant lineage and we report here the emergence of resistance to tigecycline during therapy in a representative. This resistance was associated with increased expression of the AdeABC efflux system, but not with altered expression of its known regulatory genes adeRS. This suggests that there may be 'cross-talk' between adeABC and other trans-acting regulatory factors.

O303 The recent increase in *Acinetobacter baumannii* resistance to carbapenems in the Czech Republic is associated with the spread of genotypically highly related strains of European clone II

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Objective: In 2003, a significant increase of *Acinetobacter* resistance to carbapenems has been noted in the Czech Republic. The aim of this study was to assess the prevalence and epidemiology of this resistance among hospital strains of *Acinetobacter* spp.
Methods: Isolates were collected prospectively via a network of diagnostic laboratories between January 2005 and April 2006. The laboratories were asked to send clinical isolates of *Acinetobacter* spp. from intensive care units (ICUs), with no more than one isolate per patient and 10 isolates per ICU. The isolates were identified to species by AFLP and assessed for relatedness using AFLP and PFGE. Susceptibility to 12 antimicrobial agents primarily effective against *A. baumannii* was tested by disk diffusion, and MICs were determined for carbapenems.
Results: A total of 150 *Acinetobacter* isolates were obtained from 56 ICUs of 20 hospitals in 15 cities. They were identified as *A. baumannii* (n = 108), genomic sp. 3 (n = 30), genomic sp. 13 TU (n = 8) or other species (n = 4). Using AFLP cluster analysis, *A. baumannii* isolates were allocated to EU clone II (n = 66), EU clone I (n = 5), 6 clusters with 2–5 isolates (n = 15) or 22 unique genotypes. Nearly all clone II isolates yielded identical or highly similar PFGE and AFLP patterns. A total of 24 (16%) isolates were resistant to at least one carbapenem. Seventy isolates were susceptible to all antimicrobials while 7 isolates showed resistance to 1–3 agents and 73 isolates were resistant to >3 agents. Resistance to >3 agents and/or carbapenem MICs >1 mg/l were found only in the EU clones and four unique strains while resistance to one or two carbapenems (MIC >8 mg/l) was confined to EU clone II. The 66 EU clone II isolates originated from 37 ICUs in 12 cities and showed the following susceptibility rates: piperacillin (0%), ceftazidime (5%), ampicillin-sulbactam (23%), imipenem (71%), meropenem (67%), gentamicin (15%), tobramycin (97%), amikacin (61%), netilmicin (52%), ofloxacin (0%), doxycycline (0%), co-trimoxazole (12%).
Conclusion: The increase in *Acinetobacter* resistance to carbapenems in the Czech Republic is associated with the spread of multidrug resistant *A. baumannii* strains belonging to EU clone II. The high genotypic similarity of the isolates suggests that they represent a recent subgroup within this clone.
Supported by grant NR8554–3 of the Ministry of Health of the Czech Republic.

O304 Nosocomial multidrug-resistant *Acinetobacter baumannii* bloodstream infections: epidemiology, clinical features, treatment, and outcome, Bangkok, Thailand

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Objective: To determine the epidemiology, clinical features, antibiotic susceptibility, treatment, and outcome of nosocomial *Acinetobacter baumannii* bacteraemia.
Design: A retrospective study was conducted at KCMH in all hospitalised patients with microbiologically and clinically documented *A. baumannii* bacteraemia from January 2005 to February 2007.
Results: A total of 83 patients with nosocomial *A. baumannii* bacteraemia were identified. There were 51 males and 32 females with the mean age of 62 years (range: 19 to 99 years). Most patients had received antibiotics within three months before the diagnosis of *A. baumannii* bacteraemia. Third-generation cephalosporin was the most commonly prescribed antibiotic (78.9%). Ventilator-associated pneumonia (VAP) was the most common primary site of infection, followed by catheter-related blood stream infection (CRBSI), intraabdominal infection, and skin and soft tissue infection (45.8%, 20.5%, 7.2% and 2.4%, respectively). There were 16 patients (19.3%) with unknown source of infection. The mean duration of intubation in the patients with VAP and that of central venous catheter insertion in those with CRBSI before the diagnosis of *A. baumannii* infection were 9.46 days and 12.07 days, respectively. Most *A. baumannii* isolates were susceptible to cefoperazone-sulbactam (48.2%), followed by netilmicin (44.6%) and amikacin (31.3%). Of all isolates, the frequency of carbapenem-, multidrug-, and pan-drug-resistant *A. baumannii* was 69%, 45.8%, and 27.7%, respectively. The overall inhospital mortality rate was 69.9%, with high mortality rate in the patients in medical intensive care units (ICUs), compared to those in non-medical ICUs [92.6% and 58.9%, odds ratio (OR): 8.712, 95% confidence interval (CI): 1.876–40.457, p = 0.002]. The patients infected by multidrug- and pandrug-resistant strains had much higher mortality rate than those infected by non-multi- and pandrug-resistant strains (80.3% and 40.9%, OR: 5.898, 95% CI: 2.046–17.002, p = 0.001). Pneumonia had higher mortality rate than other source of infections (81.6% and 60%, OR: 2.952, 95% CI: 1.071–8.136, p = 0.03)

Conclusions: Multidrug- and pandrug-resistant *A. baumannii* bacteraemia are a common cause of nosocomial infections in KCMH. The high inhospital mortality rate is observed in the patients in medical ICUs. This situation emphasizes the importance to maintain rigorous measures of infection control as well as appropriate antibiotic prescription in our institute.

Mycobacterial infections

O305 Identification of *Mycobacterium tuberculosis*-specific CD4 and CD8 IFN-gamma and/or IL-2 secreting cells by a novel method contributes to defining the different stages of tuberculosis

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Objectives: A more detailed characterisation of the immune response to *Mycobacterium tuberculosis* (Mtb) antigens would provide new insights into the complex host-parasite interactions that take place in the course of tuberculosis (TB) and offer new tools for a more accurate diagnosis of the different stages that characterise TB as a disease.
Methods: Here we measured CD4 and CD8 T-cell responses to Bacillus Calmette-Guérin (BCG), purified protein derivative (PPD), early secretory antigenic target-6 (ESAT-6) protein and culture filtrate protein-10 kDa (CFP-10), measured as interferon (IFN)-gamma and interleukin (IL)-2 release, using the flow cytometric cells-secreting cytokine detection technique on peripheral blood mononuclear cells (PBMC) obtained from active TB patients, latently Mtb infected individuals, patients with pulmonary diseases different from TB and healthy donors. IL-10 and IL-17 were also measured to test their possible role as indicators of disease activity.
Results: We confirmed that the enumeration of IFN-gamma releasing cells upon Mtb-specific stimulation is sufficient to identify TB patients and that CD8 T cells contribute to the specificity of the response. IL-2 secreting cells were more frequently observed in latent TB infected individuals than in active TB patients, suggesting that measurement of cells secreting this cytokine could be a marker of disease stages. No discriminating role was associated to IL-10 and IL-17 release in TB patients.
Conclusions: Together, data indicate that the flow cytometric cytokine-secreting cell detection technique is a powerful tool for TB diagnosis which allows the analysis of the immune response to Mtb-related antigens in the different stages of TB.

O306 RD-1 antigen based in vitro diagnosis of TB infection: IP-10/CXCL10 is a novel potent biomarker with comparable performance to the Quantiferon In Tube test

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Objectives: A major breakthrough in clinical management of tuberculosis infection has been the development of in vitro diagnostic tests measuring IFN-g in response to stimulation with RD-1 antigens (IGRA tests). The tests perform with excellent specificity, but there is a concern that the sensitivity is not optimal, especially in patients with immune-suppression and in children. We have identified the chemokine IP-10/CXCL10 as a novel potential diagnostic biomarker for TB. The aim of the study was to establish a diagnostic test algorithm based on IP-10, and compare it with the QFT-IT results.
Materials and Methods: We measured IP-10 levels in plasma supernatants from Quantiferon In Tube (QFT-IT) stimulated whole blood from 80 patients with culture and/or PCR proven active TB, 86 unexposed Danish high school students, and 38 unexposed high school teachers. Based on ROC curve analysis we established two IP-10 cut-off points and constructed a diagnostic test algorithm inspired by the QFT-IT test; with positive, negative, and indeterminate test outcome.
Results: TB patients produced significantly higher levels of IP-10 and IFN-g compared with controls: for IFN-g median 215 pg/ml (IQR 22–651 pg/ml) vs. 0 pg/ml (IQR 0–0 pg/ml), for IP-10 median 2158 pg/ml (IQR 582–5882 pg/ml) vs. 37 pg/ml (IQR 13–99 pg/ml. IP-10 and IFN-g responses were highly correlated (0.81, $p < 0.0001$). Based on ROC curve analysis we selected a sensitive IP-10 cut-off (237 pg/ml), and a specific cut-off (673 pg/ml). A cut off for indeterminate test was established by comparing the mitogen IP-10/IFN-g ratio. QFT-IT and the specific IP-10 test showed comparable performance with equal sensitivity/specificity and 90% agreement (kappa 0.79), table 1. The sensitive IP-10 test had a higher sensitivity 71/80 (90%), but also a high degree of positive responders among the controls 14/124 (11%), primarily among the older teachers (10/14)

Table 1. Concordance between Quantiferon in Tube test (QFT-IT) and the Specific IP-10 test.

	Quantiferon In Tube test			
Specific IP-10-test	Neg.	Pos.	Indet.	Total
Neg.	128	7	1	136
Pos.	7	54	1	62
Indet.	0	4	2	6
Total	135	65	4	204

Agreement 90%, *kappa* = 0.79.
IFN-g and IP-10 levels were measured in plasma supernatants of 80 patients with culture and/or PCR proven active TB, 86 unexposed Danish high school students, and 38 unexposed high school teachers. IFN-g levels were analysed according to the QFT-IT test algorithm (cut off for positive test 17.5 pg/ml and 25 pg/ml for indeterminate test). IP-10 levels were analysed according to an IP-10 inspired algorithm (cut off for positive test 637 pg/ml and 201 pg/ml for indeterminate test). Cut offs were calculated using ROC curve analysis.

Discussion: IP-10 is expressed in response to the RD1 antigens in consistent and significant higher levels than IFN-g. IP-10 has properties that enable the development of diagnostic test algorithm with positive, negative and indeterminate outcomes, performing with excellent concordance and comparable or even superior discriminatory power compared with the QFT-IT. As IP-10 is expressed in high amounts, it holds promise for the development of a new generation of more sensitive TB tests with simpler and potentially field-friendly read out formats, such as the lateral flow dipstick.

O307 Biodiversity of *M. tuberculosis* complex strains circulating in Greater London area: implications for prospective epidemiology and phylogenesis

V. Nikolayevskyy, P. Velji, T. Brown, F. Drobniewski (London, UK)

Objectives: i) To characterise *M. tuberculosis* complex (MTBC) isolates circulating in the Greater London area with respect to drug resistance, geographic origin and phylogeny; ii) To evaluate the discriminatory power of various genotyping methods and select highly discriminant reproducible loci for prospective molecular epidemiological studies and phylogenetic analysis.
Methods: A total of 2261 individual MTBC isolates (one isolate per patient) representing 95.7% of the total number of bacteriologically confirmed TB cases reported in the Greater London area over the 12 month period (1/04/2005 – 31/03/2006), were genotyped using 22 loci VNTR typing (MIRU2, 4, 10, 16, 20, 23, 24, 26, 27, 31, 39, 40; ETR-A, -B, -C; VNTR 2163A, 2163B, 2347, 3232, 1982, 3336, 4052) and spoligotyping, followed by detection of deletions in RD9 and TbD1 regions in a selection of isolates. Diagnostic identification and drug resistance testing was performed on liquid media followed by a range of phenotypical and molecular tests. Limited demographic data, including gender, date of birth, and country of birth was available for these patients.
Results: Most (84.7%) of *M. tuberculosis* (strictly sensum) isolates were fully drug sensitive and 83.7% strains were isolated from patients

born outside the UK (primarily in the Indian Subcontinent and Africa). Analysis of discriminatory power of different genotyping methods and their combinations have demonstrated that inclusion of additional VNTR loci allows to improve discrimination significantly and avoid formation of false clusters which is critical for prospective molecular epidemiological studies (Table 1).

Table 1.

Genotyping method	No. of distinct profiles (variety of types)	No. of clusters	Size of clusters (isolates)	Clustering rate (%)	No. of unique isolates
Spoligotyping	656	198	2–221	79.7%	458
MIRU15	1271	235	2–53	54.2%	1036
MIRU15 + Spoligotyping	1619	196	2–48	37.1%	1423
MIRU15 + VNTR7	1888	158	2–35	22.2%	1730
All three methods	1897	153	2–35	21.6%	1744

The CAS family was the most prevalent in the study population (24.7%) followed by the T, EAI, and LAM families comprising 16.4%; 15.9%, and 15.5% of total isolates respectively; CAS and EAI strains were dominant in patients born in the Indian subcontinent, East Africa and South-East Asia, and LAM and T families were more common in patients born in Europe, UK and other parts of the world.
Analysis of allelic diversity within certain loci in combination with results of spoligotyping and deletion mapping allowed the selection of loci of greater phylogenetical relevance (MIRU4, 24, 26, ETR-A, -B, -C) and propose VNTR codes (combinations of allelic variants) which can be potentially used for identification of families, major lineages and species within MTBC.

O308 Multiple-mutations in the katG gene of *Mycobacterium tuberculosis* isolates correlate with high-level of resistance to isoniazid in patients with active pulmonary tuberculosis in Belarus

S. Zakerbostanabad, A. Bahrmand, E. Graviss, L. Titov (Tehran, Houston, Minsk, IR)

The aim of this study was to investigate the significance of multiple-mutations in the katG gene, predominant nucleotide changes and its correlation with high level of resistance to isoniazid in *Mycobacterium tuberculosis* isolates that were randomly collected from sputa of 42 patients with primary and secondary active pulmonary tuberculosis from different geographic regions of Belarus. Drug susceptibility testing was determined using the CDC standard conventional proportional method. DNA extraction, katG gene amplification, and DNA sequencing analysis were performed. Thirty four (80%) isolates were found to have multiple-mutations (composed of 2–5 mutations) in the katG gene. Increased number of predominant mutations and nucleotide changes were demonstrated in codons 315 (AGC → ACC), 316 (GGC → AGC), 309 (GGT → GTT) with a higher frequency among patients bearing secondary tuberculosis infection with elevated levels of resistance to isoniazid (MIC μg/ml ⩾ 5–10). Furthermore it was demonstrated that the combination of mutations with their predominant nucleotide changes were also observed in codons 315, 316, and 309 indicating higher frequencies of mutations among patients with secondary infection respectively. In this study 62% (n = 21) of multi-mutated isolates found to have combination of mutations with predominant nucleotide changes in codons 315 (AGC → ACC), 316 (GGC → GTT), 309 (GGT → GGT), and also demonstrated to be more frequent in isolates of patients with secondary infections, bearing higher level of resistance to isoniazid (⩾ 5–10 μg/ml).

O309 Outcomes of patients with XDR-TB in Iran

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Introduction: To date there has been little information reported about the outcome of treatment in XDR-TB cases around the world. Here, we will report the outcome of the first cohort of XDR-TB patients in Iran.
Material and Method: All patients with documented MDR-TB based on drug susceptibility tests (DST) are treated with the standard second-line regimen, consisting of cycloserine, prothionamide, amikacin, and ofloxacin since 2002 in Iran. After performance of second line drug susceptibility in 2006, all XDR-TB cases were identified and treatment outcome was extracted from their medical records.
Results: Between 2002–2006, 105 patients with MDR-TB were identified at Masih Daneshvari Hospital in Tehran, Iran. Of those patients, seven (6.6%) were diagnosed with XDR-TB. Four (57.1%) patients were male and three (42.9%) were female. All seven patients were HIV-negative. Mean age was 52.85± 21.61.4 patients were Iranian, however 2 were from republic of Azerbaijan and one from Afghanistan. One of the seven patients had primary XDR-TB, with no history of previous treatment and no known close contact. Of the remaining six patients, all had a history of anti-TB treatment.
Cure, failure and death each occurred in 2 cases (28.6%).Treatment outcome was unknown in one patient from republic of Azerbaijan.
Conclusion: Our study shows a poor prognosis in patients with XDR-TB, since only two patients out the seven were successfully treated without relapse.

Table 1. Drug susceptibility tests and outcomes of patients with XDR-TB in Iran

	Age	Sex	Nation	OFX	CIP	CS	AM	KM	ETO	PAS	CM	PZA	EMB	Outcome
1	22	M	Azerbaijan	R	R	S	R	R	S	R	R	R	R	Cure
2	63	F	Iran	R	R	S	R	R	R	R	R	R	R	Cure
3	63	M	Iran	R	R	R	R	R	R	R	R	R	R	Relapse
4	55	F	Afghanistan	R	R	S	R	R	R	S	R	R	R	Failure
5	64	M	Iran	R	R	S	R	R	S	S	R	S	S	Death
6*	79	F	Iran	R	R	R	R	S	R	S	R	R	R	Death
7	24	M	Azerbaijan	R	R	R	R	R	R	R	R	R	R	Unknown

*Primary XDR-TB. Summary of abbreviations: S = Sensitive, R = Resistant, OFX = Ofloxacin, CIP = Ciprofloxacin, CS = Cycloserine, AM = Amikacin, KM = Kanamycin, ETO = Ethionamide, PAS = Para-aminosalicylic Acid, CM = Capreomycin, PZA = Pyrazinamide, EMB = Ethambutol.

O310 A comparison between QuantiFERON and tuberculin skin test during screening for tuberculosis infection in a contact investigation among students

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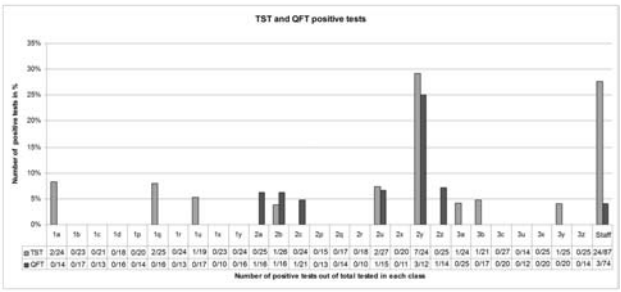
Introduction: Two cases of tuberculosis (TB) were discovered at a high school in Copenhagen. A contact investigation was performed with Mantoux skin test (TST) and QuantiFERON® TB In Tube test (QFT) and all staff and students were asked to participate in the study.
Objective: To compare QFT with TST in a contact investigation.
Methods: Students and staff were screened with TST, QFT and risk factors were registered in a questionnaire.
Results: A total of 724 had a TST applied of which 689 (94%) were read. Twenty-seven (4%) were TST positive. A total of 490 (62%) had a QFT done and 11 (2.2%) were positive and 4 (0.8%) inconclusive. We found that 17/603 (2.8%) of the students and 24/87 (28%) of the staff were TST positive whereas, 8/419 (1.9%) of the students and 3/71 (4.2%) of

the staff were QFT positive. Agreement between the TST and the QFT was moderate 97% (Kappa 0.407, CI (0.124–0.689)) among students and poor among staff, 68 (Kappa 0.036, CI (–0.102–0.175)). The majority of positive QFT and TST persons were found in the index class and the neighbouring classes. Logistic regression analysis showed a significant association between positive TST results and BCG vaccination and we concluded that prior BCG vaccination could explain the high prevalence of TST positive staff. Only 5/10 (50%) of the QFT positive individuals were also TST positive indicating a suboptimal sensitivity of TST among persons at risk.

Conclusion: The two tests found an equally amount of M tuberculosis infected amongst the young non BCG vaccinated group taking into account that not everyone volunteered for both tests. 50% (4/8) of the QFT positives students were not detected with the TST. Among the staff, the high frequency of TST positives was explained by former BCG vaccination.

The choice of test preferred for a TB contact investigation should be based on the profile of the screening population. BCG status, age, country of origin, the use of both tests at the same time or in two steps should be evaluated according to the risk in the population of developing TB.



O311 In only half of the tuberculosis cases diagnosed in Zambia, *Mycobacterium tuberculosis* is the causative agent

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Diagnosis of tuberculosis (TB) in resource-poor countries is almost exclusively based on microscopy of Ziehl-Neelsen stained (ZN) sputum smears; in some settings supplemented by chest X-ray.

Objectives: The aim of this study was to determine the accuracy of this diagnosis of TB in Zambia in the era of increasing HIV prevalence. By applying MGIT liquid culture technique, this study distinguished between (i) TB cases confirmed by positive *Mycobacterium tuberculosis* cultures, (ii) mycobacteriosis caused by non-tuberculous mycobacteria (NTM) and, (iii) tuberculosis-like disease caused by organisms other than mycobacteria.

Methods: Adult patients were included who had been diagnosed with TB on basis of respiratory complaints lasting two or more weeks, failure to improve on two courses of empiric antibiotics, a positive ZN sputum smear and/or findings on the chest X-ray consistent with TB. Sputa of the presumptively diagnosed TB cases were subjected to MGIT liquid culture. *Mycobacterium* isolates were identified using a nucleic acid amplification method and 16S DNA sequencing.

Results: In only 47% of the 187 presumptively diagnosed TB cases was *M. tuberculosis* cultured. In 19% exclusively NTM were found. In another 12% of the presumptive cases, a combination of *M. tuberculosis* and NTM was isolated. In the remaining 29% of cases in which TB was diagnosed, no mycobacteria were cultivable. HIV positivity was significantly correlated with the isolation of NTM from sputum and inversely associated with the isolation of exclusively *M. tuberculosis* ($p < 0.05$).

Conclusions: Basing the diagnosis of tuberculosis on symptoms, sputum smear and/or chest X-ray may lead to significant numbers of false-positive cases of tuberculosis in Zambia, due to the increased prevalence of HIV.

O312 A rapid, fully-automated PCR system for sample processing, diagnosis, and rifampin resistance detection of *Mycobacterium tuberculosis*

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Background: Current methods for the detection and determination of drug resistance of *Mycobacterium tuberculosis* (MTB) are insensitive and slow. PCR is a more accurate and rapid technique, but requires substantial labour and technical competence. PCR is also affected by inhibitors, sample cross-contamination, and limited ability to concentrate samples. All of these limitations are overcome by the GeneXpert® system. This automated system uses a low-cost plastic cartridge to concentrate all the bacilli present in a sputum sample, remove inhibitors, lyse cells, and transfer the DNA into an integrated PCR tube. This DNA is amplified in a nested, real-time PCR using an optimised, six-colour Molecular Beacon assay to detect more than 95% of all rifampin-resistant TB strains. An internal sample processing and reagent control is also included. Sample processing and nested PCR occur within the same cartridge and no external manipulation is required after the sample is initially loaded.

Objectives: To comprehensively evaluate the GeneXpert MTB assay using sputum and sputum pellet samples and simultaneously detect rifampin resistance-associated mutations in the rpoB core region. Key evaluation criteria were analytical performance, stability of reagents, and sample inactivation.

Methods: Proficiency panels, clinical and spiked samples are mixed with a sample treatment buffer (STB), incubated and added to the cartridges containing reagent beads and buffer. Wild type and mutant MTB strains were used to determine the system's analytical performance. Inactivation studies determined the amount of viable TB cells before and after treatment with STB. Stability was assessed by testing cartridges incubated from 4 ºC to 45 ºC.

Results: The STB kills >6 logs of MTB organisms in sputum, disinfecting the sputum and reducing the risk from infectious aerosols. The GeneXpert detected as few as 50 bacilli/mL of sputum in 90 min, making it as rapid as microscopy, but much more sensitive. Eleven out of 12 of the QCMD 2002 TB panel samples were correctly identified. At two alpha test sites this system diagnosed and provided semi-quantitative information about TB infections and accurately detected rifampin resistance. To date reagent stability is >3 months at 45ºC.

Xpert™ MTB sensitivity & specificity (per patient analysis)

	Sensitivity:		Specificity:
	smear-pos, cul-pos	smear-neg, cul-pos	smear-neg, cul-neg
Peru	100% (99/99)	71.4% (10/14)	95.6% (130/136)
Latvia	100% (14/14)	66.6% (10/15)	97.6% (40/41)
Total	100% (113/113)	68.9% (20/29)	96.0% (170/177)

Xpert™ MTB – Rif resistance detection (per patient analysis)

	Sensitivity in Rif resistant cases	Specificity in Rif sensitive cases
Peru	100% (13/13)	100% (88/88)
Latvia	100% (6/6)	100% (7/7)
Total	100% (19/19)	100% (95/95)

Conclusion: The combination of sample processing with real-time PCR into a single automated step makes this assay simple to perform, in near-patient and laboratory settings, with minimal effort in <2 h.

O313 Tuberculosis and HIV in Mexico City: a single-centre cohort

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Objective: To determine the frequency, clinical characteristics, anti-tuberculosis drug resistance pattern, and mortality in HIV and TB co-infected patients.
Methods: A cohort of all HIV infected patients was followed at a tertiary care referral centre in Mexico City. Specific endpoints were defined and analysed in relation to tuberculosis infection, and other co-morbidities.
Results: We found 92 cases of active TB (5.5%) among 1651 HIV patients. The rate of co-infection was 3% in 1998, 2% in 2003, and 1% in 2007. In 32 (34%) of the 92 cases, TB and HIV were diagnosed simultaneously ($\pm$ 30 days). Their median age was 36.9 years and 91.3% were male. We observed a statistical difference in the median CD4 cell count between co-infected [74 X 106 cells/L (IQR 25–169)] and non-co-infected [197 X 106 cells/L (IQR 66–346)] patients ($p < 0.007$). Forty-eight (52%) patients had extra-pulmonary TB of whom 11 (23%) had disseminated disease. Chest x-rays in the 44 (48%) patients with pulmonary involvement showed: bilateral diffuse infiltrates in 70%, localised infiltrates in 9%, cavitations in 14%, and no infiltrates in 7%. Tuberculosis was diagnosed with culture in 64 patients (69%): *Mycobacterium tuberculosis* was isolated in 47 cases and *Mycobacterium bovis* in 17. Acid-fast staining contributed to diagnosis in 39% of the cases, and histopathology in 15%. Of the 86 patients (92%) that started treatment for TB, 7 (8.4%) experienced liver toxicity, 51 (56%) achieved cure, 5 (5%) relapsed, and 17% (16) were lost to follow-up. Primary resistance to anti-TB drugs was 9.3%, whereas secondary resistance was 5.4%. Only two cases of MDR-TB were detected, both in patients with relapses and poor compliance to anti-TB treatment. Mortality was observed in 18.5% (17 cases) at their last visit. Tuberculosis was the cause of death in 11% (10). The median time to death attributed to TB was 12 days (IQR 3–428).
Conclusions: The frequency of co-infection in this population was lower than expected for a medium income country; primary resistance to anti-TB drugs was similar to that observed in Mexico; mortality attributed to TB was low but it happened shortly after diagnosis. Of note, extra-pulmonary TB was common in this cohort, and *Mycobacterium bovis* was a frequent pathogen.

O314 Epidemiological study of an outbreak of non-tuberculous Mycobacteria subcutaneous infections after mesotherapy

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Objectives: Mesotherapy is an increasing used technique involving subcutaneous injections of minute quantity of various medical drugs for cosmetic or rheumatism purposes. This practice was already reported to be related to infection risk.
In January 2007, a general practitioner notified to the health authorities and the regional centre for infection control a cluster of subcutaneous infections due to non-tuberculosis Mycobacteria (NTM) following mesotherapy. An epidemiological investigation was performed to describe the outbreak, to identify the source and the mechanism of contamination and to determine risk factors.
Methods: The case definition was based on typical clinical subcutaneous lesion associated with positive specimens for NTM. An assessment practice study was performed to determine potential risk factors to be tested in a comparative epidemiological study. Data were collected including schedules of outpatient visits, localisation of injections, and type of injected products. Tap water of the medical examination room was sampled for search of Mycobacteria. *Mycobacterium chelonae* strains were compared using Pulsed Field Gel Electrophoresis (PFGE).
Results: Overall, 16 cases were identified among 105 outpatients (attack rate: 15.2%), including 11 positive for *Mycobacterium chelonae* and 2 for M. frederiksbergense. *M. chelonae* was found in the tap water. Assessment practice study identified inappropriate cleaning and rinsing of the multiple injection device (automatic repetitive machine) using tap water which was likely to be the source of NTM contamination. Indeed, PFGE *M. chelonae* strains patterns were similar between patients and tap water samples. The multivariate logistic regression analysis showed that to be at the second rank during a mesotherapy session (Odds ratio = 4.15 [1.2–13.9]) and to have more days of mesotherapy cares delivered after room closure (Odds ratio = 1.03 [1.01–1.05]) were independent risk factors of NTM infection.
Conclusion: This outbreak investigation highlights that failure in disinfection of injecting material could generate severe infections with highly resistant bacteria related to non regular medical cares. Efforts should focus on control of hygiene practices in non hospital settings based on appropriate guideline recommendations.

Antifungal therapy: optimisation for life-threatening *Candida* infections (Symposium organised by Astellas)

S315 Epidemiology of *Candida* infections: European focus

A.M. Tortorano (Milan, IT)

Invasive candidiasis and candidaemia are the most common systemic fungal infections observed in hospitals. The increased prevalence of risk factors for these infections over the past two decades have dramatically increased their incidence during that period. These infections are frequently severe, with a crude mortality rate of 38%.

Category (*n*)	*C. albicans*	*C. glabrata*	*C. parapsilosis*	*C. tropicalis*
Underlying condition				
Surgery (933)	58.0	16.3	12.6	6.1
Intensive care (839)	60.5	11.9	12.9	6.1
Solid tumour (471)	58.0	15.9	10.6	8.3
Haematological malignancy (257)	34.6*	9.7	14.8	17.9*
Foetal immaturity (125)	60.8	4.8*	28.8*	2.4
HIV infection (63)	65.1	9.5	6.3	6.3
Age group				
<1 y (158)	59.6	3.1*	27.9*	3.1
1–19 y (144)	47.9	3.6*	32.9*	5.7
20–69 y (1189)	57.1	14.0	11.2	8.3
$\geqslant$70 y (590)	60.0	19.3*	6.9*	7.1
Total population	56.4	13.6	13.3	7.2

*$p \leqslant 0.01$ for comparison of percentage for each underlying condition/age group vs. percentage in total population.
Table reproduced from Tortorano AM, et al. *Eur J Clin Microbiol Infect Dis* 2004; 23: 317–322.

The nature of systemic *Candida* infections appears to be changing. Until recently, the majority of infections were caused by *Candida albicans*, but this species is becoming less common as non-*albicans Candida* species begin to proliferate, particularly in certain patient types. In a recent survey of the epidemiological and mycological profile of *Candida* species in Europe, while *Candida albicans* was still the most common cause of infection overall, 43.6% of infections were caused by non-*albicans Candida* species [1]. Furthermore, in surgical patients and those with solid tumours, and in patients aged $\geqslant$70 years, the prevalence of *C. glabrata* approached 20% (see Table) [1].
The significance of these changes in *Candida* epidemiology may be profound, since non-*albicans Candida* species seem to be associated

with an increased risk of mortality compared with *C. albicans* (*C. krusei*: 55.3%; *C. glabrata*: 45.0%; *C. tropicalis*: 41.4%; *C. albicans*: 38.5%). However, these differences may be explained by the increased prevalence of these species in patients with more severe underlying illness [2]. While isolates of *C. albicans* are generally susceptible to most antifungal agents, non-*albicans Candida* species, particularly *C. glabrata* and *C. krusei*, are often non-susceptible to older agents, such as fluconazole [3]. This has important implications for the selection of appropriate antifungal therapy in patients with systemic *Candida* infections.

Reference(s)

[1] Tortorano AM, et al. Eur J Clin Microbiol Infect Dis 2004; 23:317–322.
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S316 Antifungals: in vitro activity

M.C. Arendrup (Copenhagen, DK)

In vitro activity is an important measure of the potency of antifungal agents as well as a useful guide to their relative clinical activity and to the extent of resistance development. Over recent decades, an increasing proportion of candidaemia cases involve species not fully susceptible to fluconazole (mainly *C. glabrata* and *C. krusei*) [1,2]. Furthermore, although *Candida* isolates with acquired azole resistance are still observed only sporadically, recent studies have documented the increased emergence of *C. glabrata* isolates against which voriconazole has elevated MICs.2 Conversely, amphotericin B and the echinocandins (anidulafungin, caspofungin and micafungin) are potent and cidal in vitro against isolates of the major *Candida* spp., including *C. glabrata*, although slightly higher MICs have been observed for the echinocandins against the less virulent species *C. parapsilosis* (see Table) [3].

Table

Organism	MIC_{90} (μg/ml)			
	Number of isolates	Anidulafungin	Caspofungin	Micafungin
C. albicans	2,869	0.06	0.06	0.03
C. parapsilosis	759	2	1	2
C. glabrata	747	0.12	0.06	0.015
C. tropicalis	625	0.06	0.06	0.06
C. krusei	136	0.06	0.25	0.12
G. guilliermondii	61	2	1	1
C. lusitaniae	58	0.5	0.5	0.25
C. kefyr	37	0.12	0.015	0.06
C. famata	24	2	1	1
Candida spp.	30	1	0.25	0.5
Total	5,346	2	0.25	1

While in vitro activity alone is a useful measure of potency, susceptibility data can be applied further to the development of susceptibility breakpoints. Studies examining the correlation between PK/PD, MICs and clinical success rates have resulted in the establishment of breakpoints for fluconazole against *Candida* spp. of $\leqslant$2 microg/ml by EUCAST and $\leqslant$8 microg/ml by CLSI. CLSI have suggested further breakpoints of $\leqslant$1 microg/ml and $\leqslant$2 microg/ml for voriconazole and the echinocandins, respectively. However, in a recent large population-based survey, including 410 micafungin-treated patients, a lower susceptibility breakpoint for micafungin of $\leqslant$1 microg/ml was suggested [4]. As clinical experience is still very limited regarding isolates with MICs above the normal range predicted by the species identification, further studies are warranted.

The results of in vitro activity studies have a substantial impact on treatment choices for systemic *Candida* infections in an era when 1) non-*albicans Candida* species are prevalent, 2) susceptibility testing methods applicable for routine testing are available, but 3) species identification is often not rapidly available. The echinocandins are likely to play an important role in the treatment of systemic *Candida* infections and with their increased use, continued close monitoring of susceptibility and epidemiology is necessary.

Reference(s)

[1] Messer SA, et al. J Clin Microbiol 2006; 44:1782–87.
[2] Arendrup MC, et al. Clin Microbiol Infect; in press.
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S317 Antifungals for life-threatening candidiasis

O. Cornely (Cologne, DE)

Invasive candidiasis and candidaemia are rarely seen in normal, healthy hosts, since *Candida* spp. are opportunistic pathogens. Risk factors that increase the likelihood of development of a systemic *Candida* infection include malignant diseases, diabetes, deeply invasive medical procedures (e.g. intravenous catheters, central lines, major abdominal surgery) and prematurity.

Treatment success of systemic *Candida* infections may also depend on patient conditions and other factors, such as age, gender, type of infection, neutropenic status and severity of illness. For example, removal of catheters is regarded as standard of care in patients with systemic *Candida* infections, since *Candida* biofilms that may form on these invasive devices can act as a reservoir of continued infection. In a multivariate logistic regression analysis of Phase III trials conducted with the echinocandin agent micafungin in patients with systemic *Candida* infections, a number of factors were associated with a reduction in the likelihood of treatment success. These included higher APACHE II score, non-removal of catheters, disseminated infection, age $\geqslant$70 years and steroid treatment.

Micafungin has demonstrated efficacy in adults with invasive candidiasis and candidaemia and is also the echinocandin with the most extensive experience in children and neonates with these infections. Furthermore, micafungin has been shown to be effective in both neutropenic and non-neutropenic patients and in both deep invasive *Candida* infections and candidaemia. Micafungin also has broad-spectrum efficacy across all major *Candida* species. These data suggest that micafungin is a valuable option for the treatment of serious invasive *Candida* infections.

S318 Optimising antifungal therapy – key points

D. Denning (Manchester, UK)

Effective treatment of invasive candidiasis and candidaemia is currently limited by the lack of accurate, reliable diagnostic tools, although steps towards this ideal are being taken presently. Furthermore, resistance in certain *Candida* species, such as *C. glabrata* and *C. krusei*, is a significant and growing problem with some antifungal treatments, particularly fluconazole and other agents of the azole class.

Micafungin, a new echinocandin agent, offers much promise for the treatment of invasive candidiasis and candidaemia, having demonstrated high treatment success rates in adults, children and neonates. Micafungin has been approved for the treatment of systemic *Candida* infections in Japan for a number of years, accounting for 157,069 patient-months' exposure, during which time it has been shown both to be effective and to have excellent tolerability. Globally, micafungin has been administered to around 365,000 patients, including 3,300 patients in clinical studies. Pooled analyses of safety data from all clinical studies, which were conducted in a wide range of indications including systemic *Candida* infections, prophylaxis against fungal infections in haematopoietic stem cell transplant recipients and treatment of

oesophageal candidiasis, demonstrate the excellent tolerability associated with micafungin treatment in a broad range of patient types (see Table).

Common treatment-related adverse events (≥2%) by age group

	Age group, patients, n (%)			
	Overall	Adults, non-elderly (16–64 years)	Adults, elderly (≥65 years)	Paediatrics (<16 years)
AE	N = 3028	n = 2345	n = 387	n = 296
Hypokalaemia	63 (2.1)	45 (1.9)	9 (2.3)	9 (3.0)
Phlebitis	75 (2.5)	65 (2.8)	7 (1.8)	3 (1.0)
Diarrhoea	61 (2.0)	52 (2.2)	6 (1.6)	3 (1.0)
Vomiting	75 (2.5)	62 (2.6)	9 (2.3)	4 (1.4)
Nausea	84 (2.8)	73 (3.1)	8 (2.1)	3 (1.0)
Pyrexia	63 (2.1)	51 (2.2)	8 (2.1)	4 (1.4)
AST increased	71 (2.3)	63 (2.7)	2 (0.5)	6 (2.0)
ALT increased	61 (2.0)	49 (2.1)	3 (0.8)	9 (3.0)
Alk Phos increased	81 (2.7)	65 (2.8)	10 (2.6)	6 (2.0)

Alk Phos: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase.

Challenges from the rise of resistant pathogens: Part 2 (Symposium organised by Janssen Cilag)

S319 Changing landscape of resistance in Europe: focus on Gram-negative bacteria

D.M. Livermore (London, UK)

The 1970s and 1980s saw development of many cephalosporins and fluoroquinolones, and it briefly appeared that these had beaten Gram-negative pathogens. But, as so often occurs in the battle between man and microbe, victory proved illusory, and resistance is now widespread. This has serious clinical implications, because inadequate treatment, owing to resistance, correlates with increased mortality among seriously ill patients.

Recent critical shifts in Europe and elsewhere include a huge increase of quinolone resistance in Enterobacteriaceae, particularly *Escherichia coli*, where rates among bacteraemia isolates mostly now exceed 25%. Cephalosporin resistance has increased too, largely via the spread of plasmids, encoding extended-spectrum β-lactamases (ESBLs). Until 2000, most ESBLs in Europe were mutants of the old TEM or SHV penicillinases and largely occurred in nosocomial *Klebsiella* spp. Subsequently "CTX-M" ESBLs have spread extensively, becoming frequent in *E. coli* as well as *Klebsiella* spp. Producers cause infections in "complicated community" patients as well as those in hospitals. The predominant CTX-M types and the clonality of their producers vary among countries, but their "takeover" is remarkably consistent. Many producers are resistant to all antibiotics except carbapenems, fosfomycin, and nitrofurantoin.

This accumulation of cephalosporin and quinolone resistance is driving use of carbapenems. These largely evade ESBLs and AmpC enzymes, although resistance can arise if a strain with one of these enzymes is unusually impermeable. At present, true acquired carbapenemases remain rare in most species, but there are disturbing pointers to their future spread. First, *Acinetobacter baumannii* clones with OXA carbapenemases are already prevalent in intensive care units (ICUs) worldwide. Secondly, *Klebsiella* clones with KPC carbapenemases are spreading in the United States, with a few reports from Europe. Thirdly, there is a growing scatter of Enterobacteriaceae and non-fermenters with IMP and VIM metallo-β-lactamases, particularly in Greece and Italy. Perhaps most disturbing of all is that there is no new, consistently active family of anti-Gram-negative agents in reserve behind the carbapenems, nor any likely to become available in the next decade.

S320 Nosocomial pneumonia in the critical care setting

J. Rello (Tarragona, ES)

Hospital-acquired pneumonia (HAP) is the most common healthcare-acquired infection contributing to death and has been estimated to increase the length of hospitalisation by 7 to 9 days. Ventilator-associated pneumonia (VAP) is also associated with increased mortality, increased length of stay, and need for continued mechanical ventilation, and therefore, presents its own unique management challenges. Key pathogens associated with nosocomial pneumonias include meticillin-resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*. Effective management involves accurate diagnosis, timely selection of initial appropriate antibiotics at effective doses and administration routes, and implementation of optimal prevention strategies. Due to variability of offending organisms within different treatment settings, initial therapy for HAP and VAP should be individualised. Selection of broad-spectrum agents should be based on many variables, including patient risk factors (eg, recent antibiotic exposure, comorbidities, duration of hospitalisation), suspected pathogens, pharmacokinetic considerations, and resistance dynamics. Following 48 to 72 hours of therapy, the patient should be assessed for de-escalation strategies based on clinical response and laboratory tests. Dosing flexibility in patients with VAP or severe sepsis is crucial to ensuring optimal resolution. Recent clinical trials suggest that doripenem might provide additional value for therapy of HAP, including VAP. A multidisciplinary approach with implementation of pan-European guidelines for HAP based on care bundles should be viewed as an opportunity to optimise management of HAP in Europe.

S321 New options for the management of complicated intra-abdominal infections

J. Solomkin (Cincinnati, US)

Complicated intra-abdominal infections (CIAIs) are a therapeutic challenge and consume significant hospital resources. The infecting organism can often be suspected based on the likely source of the infection (eg, community acquired or healthcare associated) and the location of insult or intestinal perforation(s). Therapy is complicated by the infection's polymicrobial nature. The continued rise of resistant organisms in the community (eg, *Escherichia coli*) and in the hospital setting (eg, *Pseudomonas aeruginosa*) has made antibiotic selection even more challenging. Effective management strategies for the treatment of CIAI include, but are not limited to: rapid and accurate diagnosis, anti-sepsis therapy, and the timely administration of empiric antimicrobial therapy. Clinical features associated with poor outcomes include: acute physiologic severity and an inability to obtain adequate control of the source of infection. Patients with severe CIAIs may benefit from broad-spectrum and more active agent(s). These agents include carbapenems, β-lactam/β-lactamase inhibitor combinations, select cephalosporins, or other combined classes of agents. Recent clinical trials suggest that doripenem may be an effective initial therapy for CIAIs due to its enhanced microbiologic potency and resistance profile for *P. aeruginosa*, as well as its dosing optimisation flexibility.

S322 Optimising outcomes: getting it right from the start

Y. Carmeli (Tel Aviv, IL)

Resistance to antimicrobial drugs is a growing health and economic concern. Rates of resistance to community- and hospital-acquired Gram-negative and Gram-positive pathogens have risen significantly over the past several years. Infections caused by these drug-resistant pathogens are associated with greater morbidity and mortality, prolonged

hospitalisations, and increased costs, compared with infections from sensitive strains. The early identification of patients at higher risk for polymicrobial-resistant infections is a key factor in guiding the selection of empiric antibiotic therapy. Selection of agent(s) from various classes of antibiotics should be individualised based on the patient's clinical status and renal function and by the agent's microbiologic activity (coverage), resistance characteristics, and therapeutic dynamics. The prompt selection of appropriate broad-spectrum empiric antibiotic therapy is essential to provide adequate coverage for the pathogens of concern and Getting it Right from the Start! The use of pharmacokinetic (PK) and pharmacodynamic (PD) simulations can help guide the decision-making process for the selection of antibiotic(s), dosage(s), and duration of infusion(s). For example, extending infusion times may lead to better outcomes for the treatment of infections caused by organisms with high minimum inhibitory concentration (MIC) values by extending the time above the MIC without requiring an increase in dosage. The use of PK/PD data for augmenting antibiotic exposure with carbapenems, including doripenem, will be presented.

Neonatal immunisation – Needs and reality

K330 Neonatal immunisation – needs and reality

C-A. Siegrist (Geneva, CH)

Millions of infants die annually of acute respiratory and diarrhoeal infections that could have been prevented, had these infants been immunised earlier. Unfortunately, the immune immaturity that is responsible for their increased vulnerability to infections also limits vaccine responses. This major challenge is being addressed, and information is rapidly accumulating on the mechanisms that limit vaccine responses in early life and potential circumventing strategies.

The recognition of limited B cell responses to bacterial polysaccharides led to the development of conjugate vaccine which had a major impact in preventing infant disease by encapsulated bacteria. However, even potent conjugate or protein vaccines require multiple vaccine doses to overcome immune immaturity when given early in life, an issue which limits their introduction in many countries of the world. The limitations of infant B cell responses result not only from the interference by antibodies of maternal origin but also from the weaker induction of antibody-secreting plasma cells within Germinal Centres, which has emerged as a generic determinant of the magnitude of early life antibody responses and calls for the development of specific adjuvant or formulations. Importantly, infant antibody responses are also of much shorter duration than those elicited later in life, which may result into secondary vaccine failure. In infant mice, this rapid decline of vaccine-induced antibodies results from the failure of bone marrow niches to efficiently support plasma cell survival through the production of sufficient level of APRIL, a limiting factor that is unlikely to be easily circumvented and calls for the administration of early booster doses.

In contrast to the limitations of antibody-secreting-cells, the induction of memory B cells may be readily elicited already in the neonatal period. This understanding led to clinical studies in which human neonates received a first dose of pertussis vaccine in their first week of life. Markedly enhanced responses to subsequent vaccine doses were observed in certain studies, whereas others were confronted to the issue of vaccine interference. Numerous questions therefore remain open, including the relative magnitude and persistence of vaccine memory elicited early or later in life.

Aiming at "zero". Impact of "bundles" and other new initiatives to reduce nosocomial infections

K331 Aiming at "zero": impact of "bundles" and other new initiatives to reduce nosocomial infections

D.A. Goldmann (Cambridge, US)

For decades, the infection prevention and control community has regarded nosocomial infections as a "right of passage", especially for critically ill patients with invasive devices. Now there is cause for optimism as an increasing number of hospitals and intensive care units report dramatic reductions in the rates of catheter-associated bloodstream infections, ventilator-associated pneumonias, and other serious infections. At last, evidence regarding effective infection control processes of care is being translated into practice. Progress has been facilitated by: 1) application of principles of reliability science (in which failures to adhere to best practice are regarded as "defects" in care); 2) development of concise "bundles" of evidence-based practices (culled from exhaustive and somewhat cumbersome guidelines); 3) application of an "execution framework" (including clear aims supported by leadership, multi-disciplinary teams employing quality improvement methods at the bedside in clinical microsystems, clear measures of outcomes and success, and a parsimonious set of "drivers" required to achieve these outcomes); and 4) advances in technology. These principles can be illustrated by examining methods used to reduce major nosocomial infection problems, such as MRSA, catheter-associated bloodstream infection, and ventilator-associated pneumonia. In deploying these strategies, it is important not to underestimate the complexity of working on multiple problems at the same time, as well as the impact on cost/resources and the potential for unintended consequences.

Molecular basis and clinical impact of mutators

S335 Mutators: mechanisms and roles in bacterial infections

A. Oliver (Palma, ES)

Laboratory and theoretical approaches have shown that under particular circumstances such as exposure to new environments or stressful conditions, cells with increased (up to 1000-fold) spontaneous mutation rates (hypermutable or mutator) are frequently selected in bacterial populations, speeding up bacterial evolution (adaptation to the environment). The first surveys in natural bacterial populations, conducted in *E. coli* and *Salmonella* spp., revealed a higher than expected prevalence of mutators (around 1%), supporting the hypothesis that hypermutation could act as a mechanism for acceleration of bacterial evolution in nature. Nevertheless, the first evidence for a specific role of hypermutation in human infections was obtained from the study of *P. aeruginosa* chronic lung infection in cystic fibrosis (CF) patients, in which the prevalence of hypermutable strains was by far the highest ever found in nature. Subsequent studies have demonstrated that hypermutation is indeed a common feature of *P. aeruginosa* chronic respiratory infections occurring in patients with chronic underlying respiratory diseases such as CF, bronchiectasis, or COPD with a prevalence ranging from 30 to 60% of the patients, whereas the prevalence of mutator strains is very low (<1%) in acute infections. A high prevalence of mutators in the CF setting was also found later for other pathogens such as *S. aureus* and *H. influenzae*. Results from recent studies, involving mouse models of chronic infection and long-term longitudinal studies of CF isolates, have shown that hypermutation indeed plays a relevant role in two major aspects of *P. aeruginosa* chronic respiratory infections: bacterial adaptation for long-term persistence of infections and antimicrobial resistance development. The molecular basis of hypermutation are defects in genes involved in DNA repair or error avoidance system which have been deeply studied in *E. coli*. Among them, the gene coding for

the Epsilon subunit of the DNA polymerase III (dnaQ or mutD), the genes involved in the Mismatch Repair (MMR) System, mutS, mutL, mutH, and UvrD (mutU) or the genes belonging to the GO system for DNA oxidative lesion repair, mutT, mutM and mutY. In CF *P. aeruginosa* strains, as well as in other natural bacterial populations, the most frequent mechanism leading to hypermutation is the inactivation of the MMR System, that along with mutation rates, increases also by 1000-fold the rates of recombination between divergent DNA sequences.

S336 The role of mutators in emergence of antibiotic resistance in the clinical setting

I. Chopra (Leeds, UK)

Mutation plays an important role in the evolution of antibiotic resistance mechanisms in bacteria either by refining existing horizontally acquired genetic determinants (e.g. those encoding β-lactamases), or by giving rise to variant drug targets with decreased affinity for antibiotics through point mutations (e.g. in the case of resistance to fluroquinolones. rifampicin, mupirocin and fusidic acid). Therefore bacteria exhibiting elevated mutation frequencies, known as mutators or hypermutators, may have a selective advantage when exposed to antibiotics in the clinical setting.

Naturally occurring hypermutators have been described in a number of pathogenic Gram-negative species. They appear to be particularly prominent in isolates of *Pseudomonas aeruginosa* from cystic fibrosis patients and the possible molecular basis for their selection in this environment will be discussed. Although *S. aureus* hypermutators, deficient in the methyl-directed mismatch repair (MMR) system have been created in the laboratory (e.g. mutS and mutL mutants), the occurrence of staphylococcal hypermutators in the clinical setting appears to be variable, with some groups reporting their presence and others not. Furthermore, a *S. aureus* mutL mutant has recently been reported to exhibit decreased fitness in a model of chronic bone infection and there is no evidence that intermediate susceptibility to vancomycin in clinical isolates of *S. aureus* (VISA) evolves in hypermutable hosts, despite the fact that resistance to vancomycin in these strains probably requires multiple mutations in chromosomal genes. Therefore hypermutation may not play a major role in the development of antibiotic resistance in *S. aureus*. Possible reasons for this situation will be discussed.

Innovative treatment of experimentally infected animals

O337 New osteotropic prodrugs prevent bone infection in a rat model of osteomyelitis

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Objectives: Osteomyelitis is a difficult to treat bone infection mainly caused by *Staphylococcus aureus*. Its treatment often requires a combination of surgical intervention and prolonged antibiotic therapy. Recently, new prodrugs were synthesised by attaching fluoroquinolone (FQ) or rifamycin (RIF) antibiotics to a bisphosphonate (BP) moiety, to target bone, including prodrugs of moxifloxacin (MOX), gatifloxacin (GAT), rifabutin (RFB) and rifalazil (RFZ). Here we tested the in vivo efficacy of these prodrugs as prophylactic treatments in a rat model of osteomyelitis.

Methods: *S. aureus* ATCC 13709 (MSSA) was used for all in vivo studies. Minimum inhibitory concentrations (MICs) were determined by CLSI broth microdilution. MICs for the parent drugs GAT, MOX, RFB and RFZ were 0.12, 0.06, 0.016, and 0.001 mg/L, respectively. Osteomyelitis was established in anaesthetised CD rats by injecting 0.05 mL of 5% sodium morrhuate followed by 0.05 mL of 10^5 cells of *S. aureus* into the medullar cavity of the tibia. Groups of rats (n = 5/group) received no treatment, or single doses of parent drugs (MOX, GAT, RFB and RFZ) or prodrugs (intravenously (IV)) administered before infection. Treatments were initiated: (1) −24h for MOX (IV) and TT99000520 (MOX-BP) at 10 mg/kg parent equivalent (eq.), or −28 days at 20 mg/kg eq., (2) −48h for GAT (IV) and TT99000559 (GAT-BP) at 10, 1, 0.1 mg/kg eq. (3) −5 days for RFB (subcutaneous (SC)) and TT99000647 (RFB-BP) at 20 mg/kg eq., and (4) −3 days for RFZ (SC) and TT99000665 (RFZ-BP) at 10 mg/kg eq. Tibiae were harvested 24h after the infection and ground to determine the bacterial titer in CFU/g of bone.

Results: 1) Prophylactic treatment with TT99000520 resulted in greater efficacy (−2.8±0.2 Log CFU/g) than MOX. Also, TT99000520 remained statistically ($p = 0.004$) more active even when injected 28 days before the infection. 2) Prophylaxis with GAT was completely ineffective, while TT99000559 at 10, 1, and 0.1 mg/kg eq. resulted in a dose dependent efficacy (2.8±0.9, 5.0±1.5 and 5.8±0.8 Log CFU/g, respectively). 3) Prophylaxis with TT99000647 reduced the bacterial load by 3.5±1.5 Log CFU/g as compared to RFB. 4) 40 and 100% of rats treated with RFZ and TT99000665 had sterilised tibia, while untreated rats had 6.1±0.9 Log CFU/g.

Conclusion: Bisphosphonated prodrugs tested under this format were highly efficacious, demonstrating the potential of a single dose of these prodrugs to prevent osteomyelitis.

O338 Sequential combination of rifampicin and ceftriaxone for bacterial meningitis treatment reduced the release of bacterial compounds by *Streptococcus pneumoniae* in vitro and attenuated inflammation and neuronal damage in vivo

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Objectives: In bacterial meningitis, severe systemic and local inflammatory processes cause long-term impairment and death. Current standard therapy relies on members of the group of β-lactam antibiotics such as ceftriaxone. By inhibition of cell wall synthesis these antibiotics induce bacteriolysis, which leads to a sudden release of high amounts of proinflammatory and toxic bacterial products including bacterial DNA and haemolysins (e.g. pneumolysin produced by *S. pneumoniae*). Bactericidal protein-synthesis inhibiting antibiotics like rifampicin prevent bacteriolysis and the resulting burst of inflammation. With respect to a certain risk of secondary resistance development during rifampicin monotherapy, combination of rifampicin with other antibiotics is mandatory. Therefore, we evaluated a sequential combination regimen with a short-time rifampicin pretreatment followed by the addition of ceftriaxone.

Methods: In vitro we analysed the release of pneumolysin and bacterial DNA from pneumococcal broth culture by quantitative immunoblotting and real-time-PCR. In vivo, we evaluated in a rabbit model of pneumococcal meningitis the influence of rifampicin pretreatment on cerebrospinal fluid (CSF) inflammation and on hippocampal neuronal damage.

Results: In pneumococcal broth culture, rifampicin pretreatment for only 30 minutes significantly reduced the ceftriaxone-induced release of pneumolysin and bacterial DNA. Likewise, in vivo, rifampicin pretreatment for 1 hour followed by the addition of ceftriaxone significantly reduced markers of neuroinflammation in the CSF, i.e. prostaglandin E2 and total protein 2 hours after initiation of antibiotic therapy. Reduced inflammation resulted in a significantly reduced density of apoptotic neurons in the hippocampal dentate gyrus compared to animals treated with ceftriaxone alone. The combined antibiotic treatment resulted in a slight decrease in the speed of bacterial killing, but was nevertheless rapidly bactericidal.

Conclusion: A pretreatment of only 1 hour with the bactericidal broad-spectrum antibiotic rifampicin prior to therapy with a β-lactam antibiotic reduced the release of proinflammatory bacterial products in vitro and attenuated inflammation and neuronal damage in vivo. This concept holds promise to reduce inflammation-associated damage in severe bacterial infections and should therefore be evaluated for meningitis therapy in clinical trials.

O339 Augmented effect by early antibiotic treatment of lung infected mice using sequentially adapted mucoid *Pseudomonas aeruginosa*

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Objective: During chronic pulmonary infection of cystic fibrosis (CF) patients there is a continuous adaptation of *Pseudomonas aeruginosa*. One important trait is overproduction of alginate, which results in appearance of mucoid phenotypes and contributes to protection against antibiotic treatment and the host immune response.
We have recently introduced a novel chronic *P. aeruginosa* lung infection model in mice using three pairs of sequentially isolated (1988, 1997 and 2003) isotypic, non-mucoid and mucoid strains from one patient. The model provides a CF-like time perspective of the chronic lung infection and previous bacteriological results indicate that the mucoid phenotype becomes more virulent with time.
Methods: Seaweed alginate embedded mucoid isolates were installed in the lungs of female BALB/c mice (n = 8/gr). For every isolate there were three groups: 1) Treatment initiated 1 hour after infection, 2) Treatment initiated 24 hours after infection and 3) Untreated control group. Mice were treated s.c. with 120 mg/kg tobramycin q.d. On day three mice were euthanised for histopathology. The inflamed part of the lung was removed, fixed in formalin and slides were prepared for evaluation of pathological changes.
Results: Early treatment significantly reduced quantitative bacteriology compared to delayed treatment for isolates 1997 and 2003 ($p < 0.0004$). Early treatment and delayed treatment differed significantly when compared to the untreated group for all the isolates ($p < 0.02$).
A shift from the acute, severe inflammatory response dominated by PMNs to the chronic inflammatory response also involving mononuclear cells (MNs), was seen when comparing the mucoid isolate from 2003 with the isolates from 1988 and 1997 ($p < 0.0001$).
When comparing the degree of inflammation, treatment initiated after 1 hour resulted in less inflammation compared to the untreated group in all the isolates ($p < 0.01$). The importance of early treatment compared to delayed treatment was seen with the two late isolates from 1997 and 2003, where a significant reduction was observed ($p < 0.0015$). Only the isolates from 1988 and 2003 showed a reduction in degree of inflammation between treatment initiated after 24 hours and the untreated group ($p < 0.02$).
Conclusion: The present histopatological study emphasizes the importance of early treatment, which should be aimed at the mucoid phenotype in patients with CF. Such strategy reduce the inflammation and thereby decrease lung tissue damage.

O340 Thioridazine and other helper compounds as enhancers of the killing activity of macrophages infected with *Mycobacterium tuberculosis*: correlation with the curing of infected mice

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Objectives: The emergence of Multi-Drug Resistant and Extensively-Drug Resistant *Mycobacterium tuberculosis* (MDRTB/XDRTB) represents a major threat to public health worldwide, as many of these resistant strains are untreatable with available drugs. In order to control these infections, there is the need for new and non-toxic antimicrobial compounds. Phenothiazines have been shown to have direct and indirect activities against a large gamut of bacteria and may be considered for the therapy of these infections. We have demonstrated with previous studies that Thioridazine (TZ) enhances the killing of MDRTB phagocytosed by human monocyte-derived macrophages. However, the mechanism by which this agent enhances the killing of intracellular bacteria is not fully understood. The killing activity of some phagocytic cells, such as the neutrophils have been demonstrated to be correlated with the K+ availability which is dependent upon transport processes affected by agents that inhibit Ca2+-activated K+ pumps. In order to clarify the mechanism by which TZ enhances the killing activity of infected macrophages, we have studied the activity of TZ, twenty-two TZ derivatives, ouabain, reserpine and verapamil (other known inhibitors of K+ and Ca2+ transport) against intracellular bacteria.
Methods: Ex vivo studies were conducted using human monocyte-derived macrophages infected with MDRTB and subsequently treated with TZ, its derivatives and other inhibitors of K+ and Ca2+ transport. Animal studies were conducted using BALB/c mice infected with *M. tuberculosis* and daily treated with different doses of TZ. The bacterial load in the lungs was monthly assessed by CFU counting.
Results: Each of these compounds enhanced the killing of intracellular MDRTB ex vivo and TZ showed a significant effect in the mouse curing of the *M. tuberculosis* infection.
Conclusion: Because each of these compounds also inhibit Ca2+ and K+ transport processes, enhanced killing is postulated to be due to the inhibition of Ca2+ and K+ transport, processes which when inhibited promote the activation of hydrolases and subsequent killing of intracellular bacteria. A model that provides the sequence of events that lead to killing of intracellular bacteria by non-killing human macrophages will be presented in detail and related to the curing of BALB/c mice infected with *M. tuberculosis* by administration of TZ at dose and interval equivalent to that employed for the therapy of psychosis.

O341 Daptomycin therapy is associated with less demonecrosis compared with vancomycin therapy in a murine soft tissue infection model caused by community-associated isolates of *Staphylococcus aureus*

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Objectives: We have previously reported that exposure of clinical isolates of community-associated isolates of *Staphylococcus aureus* (CA-MRSA) to daptomycin (DAP) compared with vancomycin (VAN) results in a dampened macrophage inflammatory response with reduced production of tumour necrosis factor (TNF) and nitric oxide (NO). We hypothesised that daptomycin therapy of soft tissue infections caused by CA-MRSA would be associated with a blunted host inflammatory response and result in less severe soft tissue lesions and less dermonecrosis.
Methods: We injected groups of 10 Charles River hairless (immunocompetent) mice in the right upper thigh muscle with 5×10^7–1×10^9 cfu of three different clinical isolates of CA-MRSA. Two USA300 isolates were employed: 6U24, a previously-studied clinical isolate from a paediatric patient in Memphis with osteomyelitis and bacteraemia; and LAC, a well-characterised California isolate. One USA 400 isolate was used – the well-characterised Minnesota strain, MW2. After inoculation, we initiated antibiotic treatment (either DAP or VAN) at either 18 hours or 40 hours after bacterial inoculation. All animals were observed daily and lesion size and character were recorded.
Results: Mice injected with the higher inoculum (10^9 cfu) of either of the two USA300 isolates (6U24 or LAC) – but not the USA400 isolate, MW2 – consistently developed large soft tissue lesions with dermonecrosis. Lesions in the mice treated with DAP compared with VAN developed markedly less dermonecrosis and were also somewhat smaller (approximately 20% reduction in size).
Conclusions: We found that in this mouse model, DAP is more effective than VAN treatment of severe soft tissue infections caused by USA300 strains of CA-MRSA.

Resistance and fitness

O342 Evolutionary trajectories among ESBL enzymes belonging to the CTX-M-1 cluster

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Objectives: To define the different mutational pathways driving the diversification of blaCTX-M-1-like genes, and to establish a hypothetical evolutionary scenario among CTX-M-1-cluster enzymes.
Methods: All known CTX-M-1-like sequences were aligned using clustalW. MODELTEST programme was used to establish the best model of DNA substitution and parameters for phylogenetic reconstruction. Maximum likelihood trees were estimated with PhymL. The obtained consensus tree was used to reconstruct the most likely ancestral sequence by Mesquite software. Changes in positions 77, 114, 140, 167, 240 and 288 were considered as they are frequently found among CTX-M-1-like enzymes. As distinct phenotypic profiles might be observed depending on the allelic background (epistasis), 30 combinations of mutations on these positions were constructed by site-directed mutagenesis using blaCTX-M-3 as template DNA. blaCTX-M mutants were cloned in pBGS18 plasmid and transformed into *E. coli* MI1443 (delta-ampC). Cefotaxime (CTX), ceftazidime (CAZ), and cefepime (FEP) MICs were determined by E-test.
Results: Phylogenetic reconstruction analysis predicted that CTX-M-3 might be the ancestor of all CTX-M-1-like enzymes, except of CTX-M-10, -34, -37 and -53. The branch-site model indicated a strong positive selection in CTX-M-3 sub-branch, with five positions (A77V, N114D, P167S, D240G and D288N) being implicated. Four of these mutations (A77V, N114D, P167S, and D288N) were required in successive steps to obtain CTX-M-58, which showed the highest CAZ MIC (256 μg/mL). Distinct evolutionary pathways to obtain CTX-M-58 are possible. Moreover, A140S change (also present in CTX-M-58) was highly beneficial to the CAZ/CTX resistance phenotype when introduced in the last step, although its presence in intermediate mutants yielded a neutral or even deleterious effect (sign epistasis). In evolutionary routes involving D240G change, lower increases in MIC values than those observed among P167S route were observed, being the presence of both changes in the same background selectively inaccessible. This result would suggest that the fitness landscape in CTX-M-1 cluster is two-peaked.
Conclusions: All aminoacid changes associated with positive selection by bioinformatics tools contributed to favourable resistance phenotypes in specific contexts. Different evolutionary paths leading to more efficient enzymes have been identified, indicating a rugged fitness landscape for CTX-M-1-like enzymes.

O343 Relative fitness of different plasmids carrying blaCTX-M-15 gene

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Objective: The presence of plasmids harbouring resistance genes has been associated with relative fitness. In this study we analyse, in competition experiments, if there are different fitness among isogenic strains harbouring related plasmids carrying the blaCTX-M-15 gene.
Methods: Three related IncFII plasmids carrying blaCTX-M-15 gene (RFLP patterns denominated A, M and J) identified in our hospital were selected. Plasmids belonging to RFLP patterns A and M showed only one replicon (RepFII) and similar size (85Kb), while the plasmid with RFLP pattern J showed two replicons (RepFII+RepFIA) and its size was 100 Kb. Only plasmid belonging to pattern A is epidemic in our environment (highly related to pC15–1a). The plasmids were transformed in two *Escherichia coli* isogenic strains, REL606(ara–) and REL607(ara+). Competition experiments were performed in Davis minimal (DM) broth supplemented with glucose during seven days. Each day 50 mcl from coculture were inoculated into 20mL of fresh DM and another sample was spread onto MacConkey plates with arabinose to estimate the relative fitness (W) of each plasmid alone and between them (in this case 1 mg/L cefotaxime was added to plates). The values expressed the mean of six independent experiments. The magnitude of the fitness difference corresponds to how much more slowly one strain grew relative to the other during the course of the experiments.
Results: Strains harbouring the epidemic plasmid (pattern A) yielded a growth rate 9% slower than its parental strain ($W=0.91\pm0.09$). On the contrary, strains harbouring the non-epidemic plasmids (pattern M and J) showed higher cost than epidemic plasmid, growing 16% and 17% slower than its parental strain ($W=0.84\pm0.08$ and $W=0.82\pm0.02$) respectively. However, in competition assay between isogenic strains harbouring epidemic (pattern A) and non-epidemic plasmid (pattern M), the relative fitness was next to 1. The competition assay between strains harbouring plasmids belonging to pattern A and J showed a slight advantage ($\leqslant 10\%$) for the plasmid carrying two replicons ($W=1.09\pm0.10$), although this advantage was detected after five days of competition.
Conclusion: The epidemic plasmid show higher fitness, which might facilitate its dissemination. Although other posibilities can not be excluded, the presence of plasmids with multireplicons could give even more advantage in stable environments.

O344 Increased fluoroquinolone resistance co-selected with improved bacterial fitness

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Objectives: We asked how fluoroquinolone-resistant clinical isolates of *E. coli* maintain fitness despite accumulating several mutations affecting DNA gyrase, topoisomerase IV and drug efflux. We investigated the possibility that different resistance-associated mutations might mutually compensate fitness costs. We also asked whether bacterial fitness itself might be a selected parameter driving the evolution of fluoroquinolone resistance.
Methods: To test this experimentally we constructed a set of 29 isogenic strains carrying combinations of up to 5 resistance mutations found commonly in clinical isolates, and measured their associated levels of resistance and fitness. Fitness was measured in pairwise growth competition in vitro.
Results: The mean MIC for ciprofloxacin increased as a simple function of the number of resistance mutations in the isogenic strains. As expected, mean relative fitness decreased as a function of addition of the first three resistance mutations (1; 0.95; 0.89; 0.8). However, the downward trend was reversed by the addition of 4th and 5th mutations (0.8; 0.87; 0.9). We identified 6 strains in which an additional resistance mutation increased both resistance and fitness. These strains were deconstructed and reconstructed to confirm the relationship between the resistance mutations present and the resulting fitness/resistance phenotype. We also evolved strains for increased fitness in the absence of drug and observed the selection of variants with decreased susceptibility to fluoroquinolones.
Conclusions: Bacteria can use different strategies to minimize the fitness costs of the genetic alterations selected for drug resistance. Two of these are well documented: the selection of low-cost mutations, and the selection of additional fitness compensating mutations. Here we identify a third strategy that reduces the fitness costs of resistance to fluoroquinolones: particular resistance mutations, when in combination, increase both resistance level and bacterial fitness. This relationship between drug-resistance mutations and improved bacterial fitness could be one force driving the evolution of fluoroquinolone resistance. An important implication is that strains carrying low level resistance mutations could evolve by Darwinian selection to higher levels of resistance, in the absence of direct selection by the drug.

O345 Fitness cost of linezolid resistance in *Enterococcus faecalis*

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Objectives: Resistance to linezolid (LZ) in clinical isolates of *Enterococcus faecalis* is associated to the substitution G2576U in domain V of 23S rRNA. All previously described LZ resistant (R) clinical isolates of *E. faecalis* harboured at least 2 mutated copies of the four 23S rRNA genes (rrl). The associated fitness cost of LZ resistance in *E. faecalis* was investigated according to the number of mutated copies of the rrl gene.
Methods: Four LZ-R strains obtained in the digestive tract of gnotobiotic mice colonised by *E. faecalis* JH2–2 and treated orally by LZ, were studied (Bourgeois-Nicolaos et al., J. Inf. Dis., 2007). These LZ-R strains, 2576–1, 2576–2, 2576–3 and 2576–4 had the mutation G2576U in 1, 2, 3 and 4 copies of 23s rRNA. Growth rates of LZ-R mutants were determined in brain-heart infusion broth and compared with JH2–2 growth. The fitness of these mutants was estimated by pairwise competition experiments into batch cultures and into chemostats. Competition index (CI) was calculated as $(X-Y)/(X+Y)$ where X was the number of resistant colonies and Y was the number of susceptible colonies. CI values approaching +1 indicated dominance by resistant strains, whereas CI values approaching −1 indicated dominance by susceptible strains.
Results: 2576–1 LZ-R mutant was associated with a generation time (GT) longer (38.9 min) than the one of the parental strain JH2–2 (31.8 min); 2576–2 mutant's GT (32.4 min) was similar to the GT of JH2–2. On the contrary, the mutants 2576–3 (29.6 min) and 2576–4 (29.2 min), were associated with a GT, significantly shorter ($p < 0.05$). In competition experiments into batch cultures, CIs after 3 days were negative for the 4 mutants. In the chemostats experiments, we have observed after 6 days a negative CI for the 2576–1 mutant ($CI = -0.64$) and a positive CI for 2576–2 mutant ($CI = 0.06$) and 2576–4 mutant ($CI = 0.82$).
Conclusions: The impact of the acquisition of the mutation G2576U depends on the number of mutated copies of the 23S rRNA and on, the environment in which the cost of the resistance is testing. The presence of the mutation G2576U in 4 copies seems to confer an advantage for *E. faecalis*. On the contrary, the presence of only one mutated copy leads to a disadvantage for growth and fitness, which could explain the scarcity of this type of mutant in the clinical isolates selected under treatment.

O346 A quantitative-PCR based method to estimate the fitness cost of antibiotic resistance in competition experiments

M. Rodriguez-Dominguez, A. Ripoll, M.C. Turrientes, J.C. Galan, F. Baquero (Madrid, ES)

Objective: To describe a robust molecular protocol based on real-time PCR method able to discriminate strains in pairwise competition experiments, in order to estimate easily the relative fitness.
Methods: Two *E. coli* isogenic strains, REL606 and REL607, which differ in only one base pair (SNP) were used. This SNP, located in the arabinose operon, allowed us to discriminate between those strains by phenotypic (capacity to use arabinose as energy source) and genotypic methods (RT-PCR). Two forward primers (differing only in the SNP) and only one reverse primer were design to amplify specifically each one of the strains. The amplified product was detected with Sybr-green as detector dye. A calibration curve was made in each experiment using chromosomal DNA of known concentration. To validate the method, we quantified the relative fitness (W) in standard competition experiments between REL606 and REL607 wild-type strains. Moreover, two rifampicin-resistant spontaneous mutants, carrying the mutations S531F and L533P in RpoB obtained from REL606 and REL607 respectively, were coculture with their parental strains and between them during three days, plating each day and taking an aliquot for DNA extraction. Six replicates of each competition experiment were performed and W was calculated for both methods. The magnitude of the fitness difference corresponds to how much more slowly one strain grew relative to the other.
Results: The competition assay between REL606 (ara−) and REL607(ara+) wild-type strains did not yielded difference in fitness ($W=1.03\pm0.01$ by plates and 1.03 ± 0.05 by RT-PCR). REL606 rif-R(S531F) strain grew 25% and 33% slower than REL607 by plates and RT-PCR respectively ($W=0.75\pm0.13$ vs 0.67 ± 0.06). On the other hand REL607 strain grew 71% (plates) or 74% (RT-PCR) slower than REL606 ($W=0.29\pm0.04$ vs 0.26 ± 0.02). Finally, the competition experiments between REL607 rif-R(L533P) and REL606 rif-R(S531F) showed 83% ($W=0.17\pm0.08$) and 69% ($W=0.31\pm0.05$) by plates and RT-PCR respectively.
Concluison: Similar values of W were found using the classical method or the new protocol based in RT-PCR. We proposed the use of RT-PCR technology to estimate the fitness cost, because presents several advantages respect to the traditional one: i) the huge manipulation of lots of plates is completely reduced, ii) it is possible to carry out more than one experiment at the same time and iii) it is possible to specifically detect a strain inside an heterogeneous population.

Resistance from the middle of nowhere

S349 Erythromycin resistance plasmids isolated from the bacterial community of a municipal wastewater treatment plant encode an interesting spectrum of macrolide resistance determinants

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Wastewater treatment plants (WWTP) are a reservoir for bacteria harbouring antibiotic resistance plasmids. Genomics of resistance plasmids isolated from WWTP bacteria revealed a high diversity of plasmid replicons and accessory genetic modules carrying resistance genes. Five of the completely sequenced plasmids confer resistance to macrolides and other antimicrobial compounds. Two of these plasmids, namely pB4 and pRSB111, belong to the IncP-1 incompatibility group combining very promiscuous, self-transmissible broad-host-range plasmids. The pB4 tripartite multidrug efflux system consisting of a permease of the resistance-nodulation-division (RND) family, a periplasmic membrane fusion protein (MFP) and an outer membrane factor (OMF) confers resistance to erythromycin and roxithromycin. Plasmid pRSB111 encodes a macrolide phosphotransferase and a transmembrane transport protein that are both required for high-level macrolide resistance. Different variants of these enzymes are also encoded on the mobilisable multidrug resistance plasmid pRSB101 and the IncF-like plasmid pRSB107 which also contains virulence-associated modules. Finally, the IncP-6 erythromycin resistance plasmid pRSB105 harbours the resistance genes mel and mph, encoding, respectively, a predicted ABC-type efflux permease and a macrolide-2'-phosphotransferase. Many more macrolide resistance genes could be detected in plasmid-DNA preparations from WWTP bacteria by hybridisation experiments using a resistance gene microarray representing 192 different resistance genes. Among these are different ere genes encoding erythromycin esterases, erm genes for rRNA adenin N-6-methyltransferases and mef genes specifying macrolide efflux proteins. To get a more complete picture of the plasmid metagenome of WWTP bacteria an ultrafast sequencing approach applying the 454-technology was carried out. Bioinformatic analysis of the obtained sequence data set also confirmed that WWTP bacteria are a reservoir for different macrolide resistance determinants.

Reference(s)

Schlüter, A., Szczepanowski, R., Pühler, A. & Top, E.M. (2007). Genomics of IncP-1 antibiotic resistance plasmids isolated from wastewater treatment plants provides evidence for a widely accessible drug resistance gene pool. FEMS Microbiol Rev 31, 449–477.

S350 Beta-lactamase genes from environmental bacteria

L. Poirel (Le Kremlin Bicetre, FR)

β-Lactamase genes are of concern in clinical isolates since they hydrolyse β-lactams, the most commonly used antibiotics in human medicine. Four molecular classes of β-lactamases do exist, and they possess variable hydrolysis spectra, including penicillinases, expanded-spectrum β-lactamases, and carbapenemases. Some clinically-relevant species produce naturally β-lactamases (i.e. SHV in *Klebsiella pneumoniae*), but most natural producers are non pathogens. Some of these natural producers have been demonstrated to be the source of β-lactamase genes known to have spread in other species and thus at the origin of acquired resistance to β-lactams. Kluyvera spp. are good examples since those environmental species possess naturally on their chromosome those CTX-M-like encoding genes now emerging worldwide in Enterobacteriaceae. Another example is *Acinetobacter* radioresistens being the progenitor of the carbapenemase OXA-23-encoding gene known to be at the origin of carbapenem resistance in *Acinetobacter baumannii* worldwide.

Also possible is to observe some environmental species as intermediate reservoirs in dissemination of plasmid-mediated β-lactamase genes from their progenitor to the clinically-relevant species. The identification of the IMI-2 carbapenemase encoding gene on plasmids in *Enterobacter asburiae* strains from different US rivers is a good example of such threat. The identification of some plamids harboring β-lactamase genes from waste-water treatment plants, sewages or sludges is also of concern. The role of genetic structures like insertion sequences, transposons, and integrons in the mobilisation of those β-lactamase genes from environmental bacteria to clinical ones seems crucial, as well as that of plasmids and phages in their diffusion.

Studies are needed to better evaluate the epidemiology of β-lactamase genes in the environment, and of course to evaluate the factors favouris-ing their dissemination (antibiotic selection pressure, temperature, ?. . .).

Surveillance of healthcare-related infections

S355 Is it time for pan-European surveillance of healthcare-related infections?

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International comparisons yield interesting insights regarding quality of care, beyond the field of healthcare associated infection (HAI) prevention. Therefore, the exchange of experiences of national surveillance systems should be encouraged. However, the interpretation of differences of HAI rates should be made very carefully. Differences in healthcare systems, legal and cultural aspects, as well as differences in the methods of the surveillance systems, may have an enormous influence. A further most crucial aspect of surveillance data is their validity, and its evaluation is very difficult.

The European Centre for disease prevention and control has to decide in the future which level of European surveillance of HAI should be achieved. Of course there are several options:

1. The harmonisation process of the individual national surveillance networks should be continued to finally achieve a uniform European HAI surveillance system. This process already started with HELICS (Hospitals in Europe Link for Infection Control through Surveillance; since 1994) and was continued with IPSE activities (Improving patient safety in Europe, since 2005) for surveillance of surgical site infections and HAI surveillance in Intensive care units.
2. The harmonisation process should be stopped because it is not really feasible to create a useful European database, and the efforts should be concentrated on a regular exchange of experience between the national networks, on the organisation of validation studies, joint risk factor studies etc.
3. ECDC should create a pan-European surveillance for infections with minor problems in identification and application of the definitions, e.g. nosocomial CDAD/1000 patient days or nosocomial laboratory confirmed BSI per 1000 patient days (adjusted according to the frequency of diagnostics).
4. ECDC should start pan-European surveillance with a totally new European surveillance system with interesting patient groups at risk and without existing systems for these patient groups in most of the countries in order not to create problems due to the need for modification of the existing systems (e.g. bone marrow transplant patients, very low birth weight newborn etc.).

The talk will discuss advantages and disadvantages of the different strategies in order to stimulate further discussion.

Emerging viral and bacterial diseases

O359 Phylogenetic analysis of autochthonous and imported Italian Chikungunya virus strains

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Objective: A large outbreak of Chikungunya virus (CHIKV), a mosquito-borne viral disease, has begun in the Comoro islands in 2005. In few months many other countries of the Indian Ocean have experienced a dramatic increase of cases. During the last years many imported cases in returning travellers have been detected and autochthonous cases have been recently described in Italy (Emilia Romagna), where a suitable the vector is present. Molecular characterisation of 8 Chikungunya virus isolates, 6 imported to Italy from Mauritius and from India in 2006 and 2007, 2 coming from the 2007 Italian outbreak is reported.

Methods: CHIKV sequences, targeting partial nsP1 and E1 genes, were amplified directly from serum samples from 8 and 7 patients, respectively; almost whole E1 gene sequences were obtained from virus isolates on C6/36 cells from 6 patients. RT PCR, targeting both nsP1 and E1 regions, was performed by slight modification of a previously published method

Results: Phylogenetic analysis of E1 and nsP1 showed that all strains belong to Indian Ocean cluster within the East/Central/South Africa genotype, grouping with the Indian Ocean Islands and Indian subclusters, according to geografic provenance. All the strains carried in E1 the M269V and D284E signatures of the Indian Ocean outbreak, while the A226V mutation was present in all isolates imported from Mauritius, in those imported from India in 2007 and in those from the Italian outbreak, while it was absent in the isolates imported from India in 2006

Conclusions: Our findings indicate that, during 2006 and 2007, multiple strains have been imported to Italy from countries where explosive Chikungunya outbreaks were ongoing. The presence of A226V mutation in the isolate imported from India in July 2007 and in the isolates from the 2007 Italian outbreak, originating from a case imported from India, may suggest that the virus envelope sequence of Indian strains is changing towards the spread of this mutation, with possible impact on virus spread in the vector as well as in humans.

O360 Epidemiology of Puumula virus infections in Denmark, 1996–2007

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Nephropathia epidemica (NE) caused by Puumula virus has been recognised in Denmark as a clinical entity since 1958. It has remained highly focal in distribution with clinical cases limited to the island of Funen.

Previous studies on Puumla virus (PUUV) strains isolated from Danish bank voles (Clethrionomys glareolus), the major reservoir, have revealed that Danish PUUV strains form a distinct genetic linage with no particularly close relatedness to the other PUUV lineages in Europe.

Objectives: The aim of this study was to further characterise the epidemiology of Puumula virus infections in Denmark and describe the clinical manifestations in Danish Puumula virus patients.

Methods: Retrospective case description study among patients with serologically confirmed PUUV infections and a seroprevalence study in a high-risk population of 140 orienteers living and training on the island of Funen. The study period was 1996–2007.
Results: Serologically verified PUUV infection was found in 128 patients. Mean age 40 years (9–70y), with a male/female ratio 2.4/1. Dominant initial symptoms were acute onset of fever, abdominal and lower-back pain, while blurred vision was rare. 7% of patients required dialysis.
NE cases were found distributed throughout the whole year but with the highest number of cases in November–February. Marked regional difference within Funen was found with most cases occurring in rural parts of southern and South-eastern Funnel. Cyclic variation in the annual number of NE cases was seen with a periodicity of 3 years. Among orienteers 7% were seropositive, but none had a history of NE.
Conclusions: Our findings indicate that the clinical manifestations of PUUV infections in Denmark are similar to what has been described from Fennoscandinavia. A significant number of infections may be asymptomatic. The distribution of NE remains mainly focal and limited to a part of Funen. Further studies to clarify the ecological factors responsible for PUUV distribution are needed to enable better risk evaluation.

O361 International Circumpolar Surveillance of invasive bacterial diseases, 2000–2006

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Background: The International Circumpolar Surveillance (ICS) system conducts population-based surveillance of invasive bacterial diseases in Greenland (GN), Northern Canada (N Can), Northern Sweden (N Swe), Norway (Nor) and in the U.S. Arctic (Alaska [AK]).
Methods: Isolates from patients with invasive diseases caused by *Haemophilus influenzae* (Hi), *Neisseria meningitidis* (Nm), Group A *Streptococcus* (GAS), and Group B *Streptococcus* (GBS) were forwarded to reference laboratories in AK (2000–2006), N Can (2000–2006), GN (2001–2006), Nor (2005–2006) and N Swe (2003–2005) for confirmation and serotyping. Norway did not provide data for GAS and GBS. Clinical and demographic information were collected using standardised surveillance forms.

Table 1: Rates* of Invasive Disease, ICS Data, 2000–2006

Country	Hi	Nm	GAS	GBS
AK	2.1	0.8	4.7	3.6
GN	0	2.6	0.6	1.2
N Can	8.4	1.1	6.9	2.1
N Swe	0.7	0.1	1.3	3.7
Nor	1.6	0.8	N/A	N/A

*Annualised crude rate per 100,000.

Results: The total numbers of reported cases were 249 Hi, 128 Nm, 289 GAS, and 215 GBS. Crude annualised rates of invasive disease per 100,000 population varied by country and organism (Table 1). AK Native and N Can Aboriginal people had higher rates of disease [(AK Native: Hi=5.7/100,000, GAS=11.5/100,000), (N Can Aboriginal: Hi=11.5/100,000, GAS=10.2/100,000)] than non-Aboriginals [(AK non-Native: Hi=1.2/100,000, GAS=3/100,000), (N Can non-Aboriginals: Hi=1.1/100,000, GAS=1.1/100,000)]. Of the 221 Hi cases that were serotyped, 37 (17%) were Hib [AK 22 cases (rate 0.5), N Can 13 cases (rate 1.4), Nor 2 cases (rate 0.04] and age ranged from <1 to 69 years; Hib disease occurred in persons <2 years of age in AK (41%) and N Can (77%). Fifty-one (23%) Hi cases were serotype a (Hia) [AK 13 cases (rate 0.29), N Can 38 cases (rate 4.15)]. Non-typeable Hi comprised 37% of serotyped cases in AK, 26% in N Can and 63% in Nor. Age distribution of Hi cases differed between countries; 49% of AK cases and 89% of Nor cases were >40 years compared with 13% in N Can ($p < 0.001$, $p < 0.000$).
Conclusion: Aboriginal peoples of AK and N Can have higher rates of invasive bacterial disease caused by Hi and GAS than non-aboriginals. Overall rates of Nm disease are higher in GN than AK, N Can, N Swe and Nor. Cases of invasive Hib disease continue to occur in children <2 years of age in the North American Arctic. Hi cases are more likely to occur in older persons in AK and Nor and young children in N Can; age distribution differences warrant further study.

O362 Shiga toxin-producing *Escherichia coli* carrying stx2f as an emerging cause of diarrhoea in the Netherlands

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Objectives: Shiga toxin-producing *Escherichia coli* (STEC) that carry the stx2f gene are considered to be host-adapted to pigeons, and therefore of limited clinical relevance to humans. In fact, to date only 6 cases have been documented in the recent literature (1990–2007), and with the exception of a single case, all have been linked with uncomplicated diarrhoea. Due to this low incidence, routine diagnostic procedures have not been adapted to detect this variant. In this study we investigated the possible role of stx2f-carrying STEC in the aetiology of diarrhoea.
Methods: A total of 2155 stool specimens, for which routine screening for gastrointestinal pathogens was requested, were analysed between September 2006 and March 2007. DNA was extracted from the specimens using the NucliSENS easyMAG specific A protocol. STEC were detected by internally controlled multiplex real-time PCR (Schuurman et al J Microb Meth 2007:406–415) adapted for detection of the stx1c, stx1d, and stx2f genes. Positive specimens were partially subtyped using the monoplex real-time PCRs to identify stx1, stx1c, stx1d, stx2f, and the group containing stx2, stx2c, stx2d, stx2e, and stx2g (stx2cdeg). Isolation of STEC strains was performed from stx2f positive specimens by culture on sorbitol MacConkey agar and colony screening by PCR.
Results: A total of 37 specimens showed STEC specific amplification signals. Subtyping was successful in 35 of 37 specimens, and revealed stx2f (n = 4) as the third most prevalent genotype after stx2cdeg (n = 13) and stx1 (n = 6). Other genotypes detected included stx1 + stx1c (n = 2), stx1c (n = 2), stx1 + stx2cdeg (n = 1), and stx1/stx1c + stx2cdeg (n = 1). Patients positive for stx2f had uncomplicated diarrhoea (n = 2) and bloody diarrhoea (BD) (n = 2), although the BD could also be explained by co-infection/morbidity these patients. Isolation of an STEC strain was successful in 2/4 stx2f-positive specimens, and yielded an O63:H6 serotype, eae gene (intimin) positive in both cases. Both strains were also shown to be highly related based on pulsed field gel electrophoresis, although no epidemiological link was apparent between both patients.
Conclusions: STEC that carry the stx2f gene were the third most prevalent stx-genotype in The Netherlands during the study. Our results suggest that stx2f-carrying STEC may be an overlooked cause of diarrhoea. Diagnostic screening strategies need to be adapted to detect this variant in the routine diagnostic laboratory.

O363 First description of *Enterococcus faecalis* small colony variant in a patient with aortic valve endocarditis

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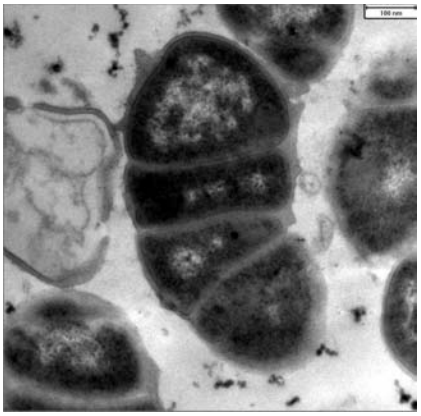
Objective: Small colony variants (SCV) constitute a slow-growing subpopulation of bacteria with distinctive phenotypic and pathogenic traits. To our knowledge, SCV are not yet described in enterococci. We discovered SCV of *Enterococcus faecalis* in blood cultures of a patient with aortic valve endocarditis and characterised the structural particularities of the phenotype.
Case report and results: A 74-year old female patient who underwent aortic valve replacement with a bovine bioprothesis three years ago

presented to the emergency department with persistent fatigue. By transoesophageal echocardiography vegetations on the aortic valve were documented. In two out of two blood cultures *E. faecalis* susceptible to ampicillin and imipenem, and intermediate susceptible to rifampicin was grown. In addition to typical *E. faecalis* colonies, a SCV phenotype consisting of small pin-point colonies was detected in both blood cultures. Typing by pulsed-field gel electrophoresis revealed clonal identity of both phenotypes. Antimicrobial susceptibility as tested on blood-containing Mueller-Hinton agar was comparable. In order to characterise the SCV further we performed light, scanning electron microscopy (SEM), and transmission EM (TEM) of both phenotypes. In the Gram-stain, the SCV showed Gram-positive, inhomogenous cocci with irregular size while the isogenic normal phenotype appeared homogenously. By SEM, SCV varied in size between one-fourth and up to eight times the size of the normal phenotype. Irregular and multiple divisions were observed in SCV. In addition, debris covered SCV isolates and an intercellular substance was more abundant in SCV than in the normal phenotype. By TEM, multiple and irregular cross walls, large swollen-appearing cells as well as empty cells were observed in the SCV (Fig. 1). Growth curves of both phenotypes revealed marked slower growth of the SCV. The patient was treated with imipenem and rifampicin for six weeks and suffered from a relapse of the endocarditis three weeks later, requiring replacement of the prosthetic aortic valve. After another six weeks of antimicrobial therapy with ampicillin and gentamicin the patient recovered.



Conclusion: *Enterococcus faecalis* is able to form a SCV phenotype with impaired growth characteristics and distinctive ultrastructural alterations observed by light microscopy and EM. Further studies are underway to investigate alterations in cellular metabolism in enterococcal SCV.

Technological innovation of bacterial typing

O364 Development of a molecular typing scheme by mass spectrometry

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Objectives: Comparative sequence analysis in large collections of microbial isolates utilising marker regions provides the framework in molecular typing and a combination of PCR, electronically accessible databases, standardised protocols and automated, high-throughput technologies make the analysis of these regions easily achievable.
We have recently developed a comparative sequence analysis tool based on mass spectrometry, which serves these needs. The technology has successfully been applied to 16S based typing (1) and multi-locus sequence typing (2). Here, we present the workflow, data analysis and statistical measures of the technology for the development of a novel molecular typing scheme for *Neisseria gonorrhoeae*.
Methods: Marker regions are amplified by PCR with a tagged primer system, which facilitates in vitro transcription of both DNA strands. Subsequent base-specific endonuclease digests of the two RNA transcripts at the bases cytosine and uracil result in four mixtures of RNA cleavage products. The high precision measurement obtained by a mass spectrometer is used to resolve the mixtures. Nucleic acid sequences are identified by correlating the acquired spectra with theoretical peak patterns predicted for in silico cleavages of each sequence contained in a reference sequence database. Microheterogeneities between the best reference and the sample sequence are identified and deliver new reference sequences.
Results: 32 marker regions of potential importance for the development of a molecular typing scheme for *N. gonorrhoeae* and a set of 267 phenotypically characterised samples were subject to comparative sequence analysis by mass spectrometry. 94% of the marker regions were converted into PCR assays and 83% of all samples resulted in high quality data throughout the set of 30 markers. Data analysis was a result of UPGMA peak pattern clustering for sample grouping, statistical analysis and sequence analysis by a time-efficient SNP Discovery algorithm. Dideoxy sequencing was used to resolve samples of high variance and showed concordance with the mass spectrometry data.
Conclusion: The introduced mass spectrometry based comparative sequence analysis tool provides a rapid alternative to dideoxy sequencing for microbial identification and the development of molecular typing schemes.

Reference(s)

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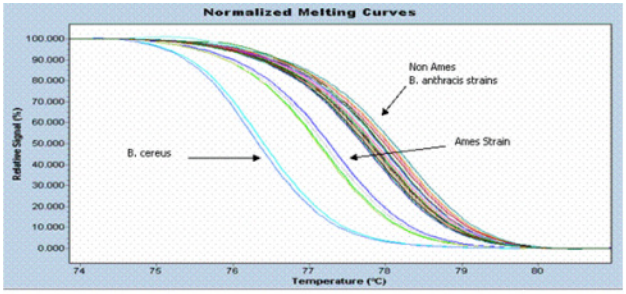
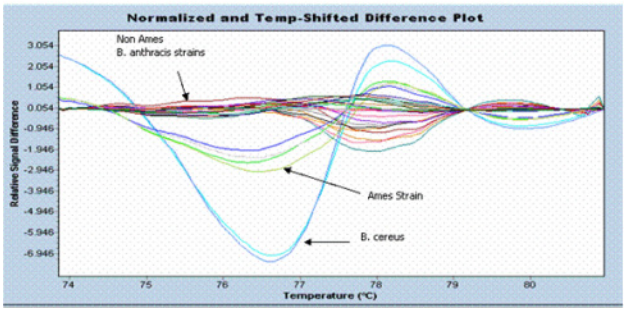
O365 High resolution melting curve analysis and strain-specific SNPs: a new method for differentiation of the Ames strain from other *Bacillus anthracis* strains

W. Ruppitsch, J. Calaway, M. Van Ert, T. Hadfield, A. Stöger, K. Grif, A. Pietzka, F. Allerberger (Vienna, AT; Palm Bay, US; Innsbruck, AT)

Objectives: The aim of this study was to assess the usefulness of high-resolution melting (HRM) curve analysis on the LightCycler 480 PCR system as a tool for the correct identification of *Bacillus anthracis* and to assess its utility as a method to recognise the Ames strain, the strain used in the 2001 anthrax attacks in the USA.
Methods: DNA from nine bacterial isolates [eight *B. anthracis* and one *B. cereus* DSM 4312], including one strain originating from an American diplomatic postal bag in December 2001 in Vienna [1], was isolated using the Qiagen Blood & Body Fluid Spin Protocol as described by the manufacturer. All *B. anthracis* isolates were subtyped by VNTR analysis as described previously using a 25 VNTR system [2]. For HRM curve analysis six specific primer combinations were chosen and tested for the identification of the Ames strain by specific single nucleotide polymorphisms [3] using the LightCycler 480 High Resolution Melting Master as described by the manufacturer. The obtained data were analysed with the HRM gene scanning software (Roche).
Results: VNTR analysis confirmed the 2001 'postal bag' isolate as a *B. anthracis* Ames strain and the other *B. anthracis* isolates as 'non-Ames' genotypes. HRM curve analysis allowed 1) the differentiation of *B. cereus* DSM 4312 from the *B. anthracis* isolates and 2) the rapid and accurate discrimination of the *B. anthracis* Ames strain from the other seven *B. anthracis* isolates. Of interest, the VNTR data from three *B. anthracis* isolates from Tyrol, Austria were shown to be European B2 strains and extremely similar to French and Italian B2 genotypes. Two *B. anthracis* isolates from the country of Georgia were also subtyped; one was identical to the vaccine strain 34F2 Sterne, and the other (411-G) was an A3a isolate.
Conclusion: We were able to show that HRM curve analysis is a fast, simple and cost effective screening method for identification of *B. anthracis* strains. HRM curve analysis, coupled with strain-specific SNP signatures, allows differentiation of the Ames strain from other *B. anthracis* strains using a single PCR. As the source of the intentional release of *B. anthracis* spores in 2001 has never been elucidated, the

ability to identify or exclude the presence of the Ames strain in clinical and environmental samples is of great importance.





Reference(s)

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[3] Van Ert MN, et al. (2007) J. Clin Microbiol. 45: 47–53

O366 Bacterial multistrain genomotyping arrays: optimal choice of control in two-colour experiments

F. Pinto, S. Aguiar, J. Melo-Cristino, M. Ramirez (Lisbon, PT)

Objectives: Comparative genomic hybridisation (aCGH) studies use microarrays to evaluate the distribution of genes of sequenced bacterial strains among unsequenced strains. The use of this technology to study different strains of the same species has led to important insights into the identification of the species "core-genome" as well as the distribution of the genes in the "accessory genome" in the population. As genomic sequences from multiple strains of the same species become available, multistrain microarrays are designed, containing spots for every unique gene in the sequenced strains. In two-colour aCGH experiments with multistrain microarrays, the choice of control sample can be the genomic DNA of one strain or a mix of the genomic DNA of all the strains used in the array design. This important problem has no universally accepted solution. The aim of this study was to evaluate the relative performance of each of the two types of control in two-colour aCGH experiments.
Methods: We performed a comparative study of the two control sample options with a microarray designed with three fully sequenced strains. We hybridised two of these strains as test samples using only the third strain as a control or a mix of the three strains as the control sample. The strain used in the single control was the one with more specific spots in the array. Resulting Log-ratios of test over control sample signal were used to classify genes as present or absent. We evaluated classification performance through the area under a receiver operating characteristic curve (AUC), accuracy, sensitivity and specificity. AUC measures the probability that the Log-ratio of a present gene is higher than that of an absent gene.
Results: We show that for both types of control sample it is beneficial to analyse spots in separate classes according to their expected control channel signal (0.05–0.15 AUC increase). The use of a mix control leads to higher accuracies (0.05 increase). This best performance is due to gains in sensitivity (0.21 increase, $p = 0.001$) that compensate minor losses in specificity (0.05 decrease, $p = 0.014$). Moreover, the use of a single strain control increases the error rate in genes that are not present in all reference strains, the set of genes where more variation across unsequenced strains is expected.
Conclusion: The use of a mix control in multistrain microarrays leads to better performances than using a single strain control.

O367 Rapid extended-spectrum β-lactamases detection using DNA chips

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Objectives: Conventional culture based bacterial identification and antimicrobial susceptibility testing methods are too slow to enable prospective therapy and provide limited information depth and there is a high demand for improved alternatives. Molecular methods such as DNA chips open the way for rapid testing and prospective therapy. A continuously emerging problem of microbial resistance is related to Extended Spectrum Beta-Lactamases (ESBL). The majority of clinically relevant ESBL variants derive from the genes blaTEM-1 or blaSHV-1 by mutations that alter the amino acid configuration and increasingly from the acquisition and mutation of genes from the CTX-M gene family. In the past decade further enzymes like the OXA lactamases and plasmid-mediated AmpC lactamases have emerged in the clinics as a new severe problem as they are likely to hydrolyse modern cephalosporins. The presentation will demonstrate developments made under the German PathoGenoMik programme on the example of the ESBL chips as well as future routes for rapid microbial diagnostics in the clinic with special focus on novel technologies for faster assay times and microfluidic integration.
Methods: Here we describe the development and application of fluorescence based diagnostic oligonucleotide microarrays for the genotyping of TEM, SHV, and CTX-M β-lactamase variants based on allele specific hybridisation. To increase the coverage, we developed chip modules to genotype plasmid-mediated AmpC and OXA-type beta lactamases. To enable a single all-in-one β-lactam resistance test for the clinic, we fully integrated individual chip modules (TEM, SHV, CTX-M) to a single ESBL chip platform.
Results: The ESBL chip was validated using 60 clinical samples taken over one year in the clinical routine of different Gram-negative organisms (phenotypically characterised as ESBL). The detected variants included TEM-1, SHV-1, SHV-5, SHV-12, CTX-M3, CTX-M9, CTX-M14 and CTX-M15. Analytically difficult samples were correctly identified such as mixed resistances of various genotypes. All ESBL phenotypes could be ascribed to the presence of a CTX-M variant (78%) or SHV variant (22%), whereas no ESBL TEM variant was found. As the total assay could be performed in 4h.
Conclusion: The ESBL chip presents a promising example in our development of a panel of diagnostic microbial chips. They can now be further integrated with the whole analytical chain to provide clinically meaningful and cost effective tools.

O368 Terminal restriction fragment length polymorphism as a diagnostic tool in gastrointestinal disease: a preliminary study

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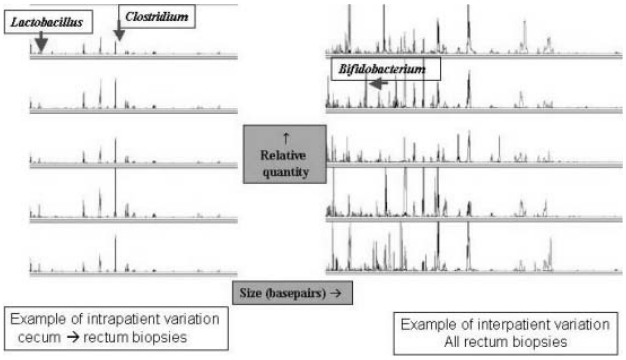
Objective: Terminal restriction fragment length polymorphism (T-RFLP) has been utilised as a typing method to examine microbial community structure in human faecal samples. Adherence of microbes to the gastrointestinal mucosa rather than luminal presence however is a prerequisite for development of intestinal disease.
To this date, the T-RFLP method has not yet been evaluated for the possibility to obtain a rapid, broad view of the (adherent) microbial composition of the human intestine and its dynamics in diseased states.

This evaluation might result in a screening tool for abnormalities in the adherent gastro-intestinal microflora by way of analysing changes in terminal restriction fragment (TR-F) profiles. This study was done to obtain evidence on the performance of the T-RFLP method.
Method: The technique involves amplification of the 16S rRNA gene with two differently labeled primers, digesting the amplicon with restriction endonucleases, and retrieving the molecular weight of the labeled terminal restriction fragments (the outer ends of the amplicon) in an automated DNA sequence apparatus. The labeling of both the forward and reverse primers might lead to a more accurate identification of species within a community profile since the presence of two peaks instead of one confirm the presence of a certain species.
Firstly, cultures of three common intestinal habitants of the human gut were subjected to T-RFLP analyses, alone and in mixtures, to determine if competition in the PCR between species has an effect on the T-RFLP profiles.
Secondly, mucosal biopsies from five colon locations per patient were gathered from 20 patients for analysis by T-RFLP. The three aforementioned species were quantified in the samples by quantative real-time PCR to determine if adequate amounts were present for detection by T-RFLP.
Results: It was shown that the T-RFLP method produces consistent, reproducible profiles. The patient samples showed little to no intrapatient varation in T-RFLP profile compared to interpatient variation.



Conclusion: For reasons of simplicity, similarity between T-RF sizes of different genera should be omitted or reduced to a minimum by way of careful restriction enzym selection. Furthermore, a database containing DNA sequences of the adherent human intestinal microbial community, combined with adequate computer software capable of performing meta-analyses of forward and reverse peak presence, is essential for obtaining rapid T-RFLP profile results.

In vitro susceptibility and mechanisms of resistance of antifungals

O369 Emergence of azole cross-resistance in *Candida glabrata* following exposure to azole antifungals in a surgical intensive care unit

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Objectives: Fluconazole (FLC) is widely used for prophylactic, preemptive and curative treatments of invasive candidiasis in surgical intensive care unit (SICU) patients. Acquired resistance to azoles in *Candida glabrata* (Cg) strains after exposure were not rare but data on its incidence were lacking.
The aim of this study was to assess the incidence of acquired FLC resistance and azole cross-resistance in Cg following azoles exposure in SICU.
Methods: Three-year prospective survey (02/2003–01/2006) with systematic mycological screening performed on all patients admitted to the SICU, immediately at admittance, then weekly until discharge. Patients with more than 2 weeks of Cg colonisation were retrospectively included in this study. For each patient, each isolate was genotyped using microsatellite-based MLVA method. Susceptibilities of Cg isolates to azoles were determined with NCCLS reference method (first and last isolate per strain per patient, susceptibilities to FLC, itraconazole, voriconazole VRC and posaconazole). Patient data (including azoles exposure during SICU stay) were retrospectively collected. Acquired FLC resistance was defined by MIC of the last isolate for FLC > 8 μg/mL and a 4x-fold increase of this MIC during SICU stay.
Results: Thirty nine patients out of 1218 admitted in SICU were included. Characteristics of patients included were: mean age 65 years (±15), median SICU stay 30 days [range: 18–160], mean SAPS II score 44 (±14). Main underlying disorders were abdominal surgery and polytrauma.
All patients harbored only one Cg strain, except one patient colonised with two different strains. Acquired azole resistance was not observed in six patients without azole exposure. Thirty three patients were given azole antifungals during SICU stay, all with high daily dose (DD) of FLC or VRC (mean DD: 470 mg/day). Nine out of 33 (27%) presented SICU-acquired resistance of their own Cg strain. Cg strains with FLC acquired resistance all showed cross-resistance to other azoles.
No significant risk factor of acquisition was identified among patient data, characteristics of azole regimens used (duration, total dose) and Cg genotypes.
Conclusion: Acquisition of cross-azole resistance following azoles exposure occurs frequently in SICU patients. Antifungal agents others than azoles (i.e. candins, polyenes) should be considered as initial therapy in SICU patients with suspected invasive candidiasis in the setting of prior azole exposure.

O370 Investigation of mutations in ERG11 gene of fluconazole-resistant *Candida albicans* isolates from Turkish hospitals

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Background: Life-threatening *Candida* infections have become an important clinical problem. Fluconazole is one of the azoles widely used for both prophylaxis and therapy of *Candida* infections. This widespread and repeated use of fluconazole has resulted in resistance in *Candida* isolates.
Objectives: The aim of our study was to investigate Y132H and other mutations in the ERG11 gene and to determine the possible contribution of efflux pumps in conferring fluconazole resistance to *C. albicans* isolates.
Methods: Seven fluconazole-resistant / susceptible dose-dependent (R/SDD) and ten fluconazole-susceptible (S) *C. albicans* isolates cultured from various clinical specimens were included in this study. The susceptibility of the strains against amphotericin B, fluconazole, itraconazole, ketokonazole, clotrimazole and flucytosine were determined by broth microdiltion method according to CLSI M27-A2 standards. Restriction enzyme analysis was performed by using PagI enzyme after PCR on all isolates for Y132H mutation, and sequence analysis was done for other mutations in the ERG11 gene. Overexpression of efflux pumps was also investigated by microdilution method M27-A2 using substrates and inhibitors.
Results: All seven fluconazole R/SDD isolates were found to be resistant to flucytosine except one, all were susceptible to amphotericin B and all isolates except one were resistant to itraconazole and ketoconazole and all of them except one were susceptible to clotrimazole. Y132H mutation was not detected in all strains. K143R, G464S, G465S and V488I mutations were determined in three of the R/SDD isolates. S412T and R469K mutations determined only in this group of strains by sequence analysis were thought to play a role in resistance but they should be evaluated with advanced studies. Possible overexpression of efflux pumps was determined especially in three fluconazole-resistant *C. albicans* isolates.
Conclusion: Mutations such as K143R, G464S, G465S and V488I in ERG11 gene and overexpression of efflux pumps were determined to be effective mechanisms in our R/SDD *C. albicans* isolates; however

other mechanisms of resistance, such as overexpression of ERG11 and mutations in the ERG3 gene should also be investigated.

O371 Azole resistant clinical *Aspergillus fumigatus* isolates are genetically clustered

E. Snelders, A.J.M.M. Rijs, H.A.L. van der Lee, J. Kuijpers, W.J.G. Melchers, P.E. Verweij (Nijmegen, NL)

Objectives: Resistance to triazoles was recently reported in *Aspergillus fumigatus* (AF) isolates cultured from patients with invasive aspergillosis. We investigated the genetic relatedness of these clinical isolates.
Methods: A collection of 26 triazole resistant AF isolates that were phenotypically and genotypically characterised was used. The isolates were resistant in vitro to itraconazole and exhibited elevated MICs of voriconazole and posaconazole. A combination of a tandem repeat of the promoter and a amino acid substitutiton (L98H) in the cyp51A gene was the predominant genetic change. These isolates had been cultured from patients admitted to our University Medical Centre between 2000 and 2006. Twenty-six azole susceptible isolates were randomly selected as controls. Genetic relatedness was investigated and phylogenetic trees were made.
Results: Genetical analysis indicated the presence of 3 clades within the collection of 52 AF isolates. All azole-resistant isolates were genetically distinct from each other, but 24 of 26 azole-resistant isolates clustered together in a single clade indicating genetic relatedness. The branches of the azole-resistant isolates had shorter genetic distances than the matched azole susceptible control isolates.
Conclusion: The clustering of the azole-resistant AF isolates into a distinct clade with short genetic distances suggests a close genetic relatedness between these resistant isolates. Since azole resistance appears to have emerged only recently, the evolution of this resistance mechanism needs to be further investigated.

O372 Environmental *Aspergillus fumigatus* isolates resistant to triazoles are genetically related to resistant clinical isolates

E. Snelders, R.A.G. Huis in't Veld, A.J.M.M. Rijs, H.A.L. van der Lee, J. Kuijpers, W.J.G. Melchers, P.E. Verweij (Nijmegen, NL)

Objectives: Recently the emergence of triazole-resistance was reported in clinical *Aspergillus fumigatus* (AF) isolates. It is unclear if azole resistance arises during therapy or that it is due to other causes of azole exposure. We investigated the possibility that azole-resistance is present in environmental AF isolates.
Methods: Soil, leaves, seeds and compost samples were obtained from local plant nurseries and a local garden centre. Soil was also sampled in the surroundings of the Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands. In addition AF isolates from the hospital indoor environment present in our fungus culture collection were tested for resistance. All samples were cultured on Sabouraud agar plates with and without itraconazole. Azole-resistant AF isolates were matched with representative azole-sensitive control isolates. Phenotypic susceptibility profiles (CLSI reference method) and molecular resistance mechanisms (cyp51A gene) were determined as well as molecular strain identification. Genotypical analysis was performed to determine if the environmental isolates clustered with clinical isolates.
Results: AF was cultured from 49 of 79 environmental samples, with 10 exhibiting an azole resistant phenotype. Resistant isolates were also cultured from soil from flower beds (6 isolates), seeds (1), compost from a plant nursery (1) and compost from the garden centre (2). Five of 248 isolates from the hospital indoor environment were azole-resistant: patient rooms (3) and hospital water (2). A tandem repeat and a L98H substitution were present in 13 of the 15 resistant isolates. This resistance mechanism was previously described to be the dominant change in triazole-resistant clinical isolates. Genotypical analysis showed that all isolates are unique but genetically clustered, 13 of the 15 environmental resistant isolates were clustered together in one clade. These environmental isolates also showed clustering with azole resistant clinical isolates.
Conclusion: Azole-resistant AF is present in the environment. Since azole-resistant AF was cultured only from the environment that had been manipulated by humans, a link with exposure to azole fungicides seems plausible. Molecular analysis of the azole resistant environmental isolates may suggest that patients acquire azole resistant aspergillus disease by inhaling resistant conidia from their environment.

O373 Epidemiological amphotericin B cut-off values in *Aspergillus* species using EUCAST methodology and an extended dilution series

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Objectives: Testing of amphotericin B for Aspergilli is problematic. Amphotericin B minimum inhibitory concentrations (MICs) for most *Aspergillus* species cluster between 0.5–2 μg/ml. MICs are not helpful in distinguishing between susceptible and resistant *Aspergillus* spp. with the exception of some isolates of *Aspergillus terreus* complex.
Methods: We performed a continuous 20 step dilution series of amphotericin B between 0.2 and 3 μg/ml in 0.2 μg/ml steps, and evaluated the MICs in 115 isolates of *Aspergillus* spp. Isolates of *Aspergillus fumigatus* complex (n = 63), *A. terreus* complex (n = 35), *Aspergillus flavus* complex (n = 26) and *Aspergillus niger* complex (n = 16) were obtained from patients suffering from invasive aspergillosis and stored in water at room temperature. Susceptibility testing was performed according the EUCAST methodology for conidia forming moulds. An inoculum size of 2×10^5–5×10^5 CFU/ml and RPMI 1640 supplemented with 2% glucose (RPMI 2% G) as culture medium was used. MIC reading was done after 48hours of incubation at 37ºC. The MIC value was defined as a no-growth visual endpoint. In addition, we studied the in vivo response of several isolates to amphotericin B. Outcome data were obtained earlier in a temporarily neutropenic disseminated mouse model of invasive aspergillosis.
Results: MIC distributions were as follows: for *A. fumigatus* complex ranging from 0.4 – 1.4 μg/ml, with modal MIC of 0.8 μg/ml; for *A. flavus* complex from 0.8 – 2.4 μg/ml with modal MIC of 1.2–1.4 μg/ml; for *A. terreus* complex from 1 – >3 μg/ml with a biphasic distribution with one modal MIC at 1.0 μg/ml and another at 2.4 μg/ml; and for *A. niger* complex from 0.4 – 1.2 μg/ml with modal MIC of 0.8 μg/ml. Only *A. flavus* and *A. niger* complex distributions were approximately Gaussian. Suggested epidemiological cut-offs are therefore 1 μg/ml for *A. fumigatus*, *A. niger* and *A. terreus* complex, and 1.4 μg/ml for *A. flavus* complex.
We were able to correlate in vivo and in vitro data. Mice infected with *Aspergillus* spp. showing MICs <1 μg/ml (n = 9) had a successful outcome, with MICs >1 μg/ml (n = 3) failed amphotericin B therapy ($p < 0.05$).
Conclusions: The extended serial amphotericin B dilutions allow for clear and different susceptibility patterns within the various aspergilli tested. Clinical studies are needed to evaluate the usefulness of the suggested epidemiological cut-off values for defining clinical breakpoints for amphotericin B.

Novel vaccine antigens and host response

O374 Do vaccines increase the risk of Guillain-Barre syndrome?

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Guillain-Barré Syndrome (GBS) is an acute neurologic syndrome which may lead to paralysis. It has a known association with infections (*Campylobacter*, *Mycoplasma*, etc.) In 1976 during the Swine flu vaccination programme, an increase in GBS cases was tied to vaccination. Since that time, there have been concerns about other vaccines (eg Menactra, Td).

Kaiser Permanente (KP) is a medical care organisation (MCO) with 3.2 million members in Northern California. They have complete electronic medical records on all members.
The VSD (Vaccine Safety Datalink), a collaborative project with the CDC Immunization Safety Office (ISO), is composed of 8 medical care organisations which collect medical and vaccination data on more than 5.5 million members annually. Data from these organisations is compiled to conduct studies of vaccine adverse advents.
Methods: In a preliminary analysis, we looked at GBS occurring within 3, 6, or 10 weeks after vaccination, in KP NCAL members of all ages from 1994–2005.
For the analysis we used a novel "case series"-like approach. Instead of looking at a vaccination and potential GBS in a time window related to it (the usual case series approach), we looked at all vaccinated persons with GBS, and examined the proportion of vaccinated cases that were vaccinated in the window vs. the remainder of the year. We used logistic regression to compare the proportion of vaccinated cases whose vaccination occurred in the window versus the expected assuming there is no association with the vaccine. For seasonal vaccines, like influenza, we estimate the expected odds from the proportion of vaccines given inside vs. outside the window. This new method controls for seasonality and other confounders.
In this exploratory analysis of multiple vaccines and risk windows, using a novel analytical method, we found a possible increase in the risk of GBS in the 6-week period after vaccination with Td. After verification by chart review we plan to extend the analysis to the entire VSD study population. As the analytical method controlled very well for confounders, including seasonality, this model may be applicable to other vaccine safety studies.

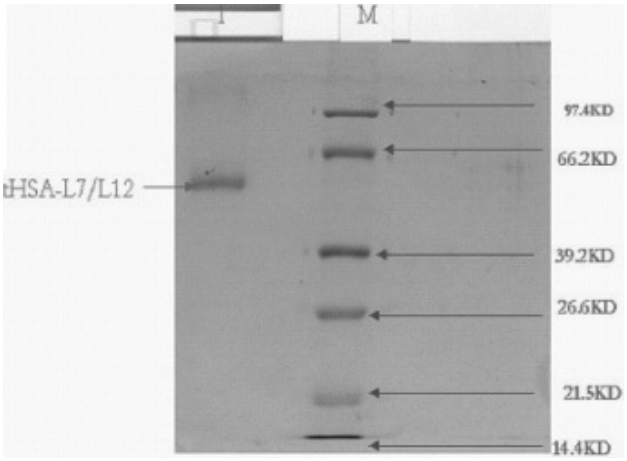
Table: Risk of GBS inside-versus outside the 6-week risk window among vaccinated GBS cases (only vaccines occurring in a 6 week window before the GBS are included)

Vaccine	Exposed inside risk window	Exposed outside risk window	Odds Ratio estimate	P-value	95% Confidence Interval
Td	8	27	2.271	0.043	(1.02, 5.03)
Influenza	29	113	1.422	0.443	(0.58, 3.50)
Hep A	2	3	5.241	0.072	(0.86, 31.92)
Pneumococcal	3	26	0.680	0.541	(0.20, 2.34)
Hep B	4	14	1.324	0.623	(0.43, 4.05)

O375 Immunological evaluation of recombinant human serum albumin–L7/L12 (*Brucella abortus* ribosomal protein) fusion protein in animal model

I. Pakzad, A. Rezaee, B. Tabaraiee, M. Rasaee, A. Zavaran Hosseinee (Ilam, IR)

Objectives: Brucellosis is the most important common bacterial zoonoses. Various vaccines such as subunit vaccine were proposed for prevention of this disease. The immunogenic *Brucella abortus* ribosomal protein L7/L12 is a promising candidate antigen for the development of subunit vaccines against brucellosis. In this research, protection of recombinant HSA–L7/L12 fusion protein in Balb/c mouse was evaluated.
Methods: The amplified gene was cloned in pYHSA5 vector then pYHSA5–L7/L12 construct was transformed in *Saccharomyces cerevisiae* and expressed protein from supernatant was purified by affinity chromatography column. Balb/c mouses were immunised in four groups by HSA–L7/L12 fusion protein (group 1), *Brucella abortus* S19 (group 2), HSA (group 3), PBS (group 4). ELISA, LTT tests and challenging one week after last injection were carried out. Bacterial count of spleen of immunised Balb/c mouse was done one month after challenging with virulent strain *B. abortus* 544.
Results: In ELISA test antibody titer HSA–L7/L12 fusion protein group was high. In LTT test the differnce of SI in HSA–L7/L12 compared with HSA and PBS was significant ($p \leqslant 0.002$). Bacterial count of spleen in goup 1 was decreased and the difference of bacterial count of spleen in goup 1 with groups 3 and 4 was significant ($P \leqslant 0.005$).
Conclusion: The results indicate that recombinant protein has the ability of proliferating lymphocytes, stimulating humoral immunity and protecting.



O376 Novel multiple epitopes containing vaccine against extraintestinal pathogenic *Escherichia coli*

A. Wieser, S. Schubert (Munich, DE)

Objectives: Extraintestinal pathogenic *Escherichia coli* (ExPEC) cause a wide variety of pathology such as sepsis, neonatal meningitis or urinary tract infections, and are responsible for a significant mortality and morbidity in humans and animals resulting in tremendous costs for the healthcare system. As ExPEC strains become increasingly resistant to antibiotics, preventive measures such as vaccination against these pathogenic *E. coli* strains are an achievable goal.
Methods: Based on previous findings in genome analysis we selectively target virulence factors and uropathogen associated proteins. To identify the relevant immunogenic regions, the unknown three dimensional structure of these proteins was simulated. Furthermore MHCI and MHCII epitopes as well as proteasome cleveage sites were predicted.
Considering these data two recombinant modular proteins were designed, containing several epitope bearing subfragments separated by linker sequences unlikely to be presented on MHCI or MHCII receptors. To express the recombinant vaccine proteins, two fully synthetic genes have been synthesised using an optimised codon bias to enhance protein expression in Enterobacteriaceae.
We evaluated two different application routes in the mouse model for these new multi-epitope vaccine proteins to obtain both, a high humoral and cellular immune response.
First, we used purified vaccine proteins and administered them nasally. Second we used a novel bacterial antigen delivery system which is efficient if administered orally, and targets the vaccine directly into the cytoplasm of mammalian cells in vivo to enhance the cellular T-cell mediated immune response.
Results: The elicited cellular and humoral immune response was evaluated using IFN-g ELISpot and sub-class specific antibody ELISA. Immunised mice showed titre increases of specific IgG in their serum as well as IgA antibodies in vaginal wash and an increase in IFN-g secreting T-cells specific for the recombinant vaccine proteins. In the challenge model of peritonitis a significant reduction of bacterial load could be achieved in immunised mice.
Conclusion: Subfragment vaccines containing multiple epitopes are effective against ExPEC in the mouse model. Further research has to be done to evaluate the potential of this approach in humans.

O377 Therapeutic immunisation with *Streptococcus sobrinus* enolase protects against dental caries in rats

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Virulence-associated immunomodulatory proteins produced by *Streptococcus sobrinus* were used as therapeutic vaccine against dental caries in rats. Enolase was identified as one of the *S. sobrinus* immunomodulatory proteins. In this study we used a recombinant *S. sobrinus* enolase (rEnolase) as a target antigen to assess its therapeutic effect in dental caries rat model.

Methods: Groups of 19-days-old Wistar rats were orally infected with approximately 10^9 *S. sobrinus* for five consecutive days and fed with a cariogenic solid diet. Five days later, they were orally immunised with rEnolase, or PBS (sham-immunised) plus alum as adjuvant, and re-immunised 3 weeks later. Throughout the study the *S. sobrinus* colonisation was assessed. The caries lesions were evaluated when rats completed 120-days and the levels of salivary IgA, IgG and serum IgG antibodies against the recombinant enolase were determined and the antibodies produced in rEnolase-immunised animals were tested against human enolase. Moreover, several organs where collected for histological observation.

Results: Higher levels of salivary IgA and IgG specific for the recombinant protein were detected in immunised rats than in controls. A significant decrease in sulcal, proximal enamel, and dentin caries scores was observed in the rEnolase-immunised animals. Histopathological analysis of various organs showed that oral immunisation with rEnolase did not induce any detectable tissue modification. Moreover, the antibodies produced by immunisation with rEnolase showed no cross-reactivity with recombinant human enolase.

Conclusion: The therapeutic vaccination with recombinant *S. sobrinus* enolase confers protection against rat dental caries and seems to have no harmful physiological effect on these animals rats. These results indicate that *S. sobrinus* rEnolase could be a promising and safe candidate to be tested in vaccination trials against dental caries in humans.

a) Portuguese patent nº102907 and USA Patent pending

This work was supported by Fundação para a Ciência e a Tecnologia (FCT) and FEDER, grant nº POCTI/SAU-IMI/60014/2004.

O378 Broad-coverage infant vaccine offering protection against invasive meningococcal group B disease

P. Oster (Siena, IT)

Although MenB is the predominant strain in many region of the world today, there is no truly global vaccine available to prevent this particular meningococcal infection.

In 2006, a two-year nationwide vaccination campaign in New Zealand with a OMV MenB vaccine developed specifically for the clonal outbreak of one specific serosub type (B:4 P1.4) responsible for a 10-year epidemic in that country was successfully concluded.

However to date, a broad-coverage MenB vaccine has been elusive because unlikely other serogroups the MenB polysaccharide producing only a poor response from the immune system.

Vaccine development is applying their technical expertise to develop safe and effective meningococcal vaccines to overcome these obstacles and to pioneer new recombinant vaccines against MenB (rMenB).

In a technique known as "reverse vaccinology", potential vaccine candidates were created by analysing the entire genome sequence of a highly powerful MenB strain. Through genetic engineering and from the investigation of 350 potential antigens, scientists identified those strains with surface proteins that best induced an antibody response.

Building on this genomics approach, Novartis is developing a broad-coverage vaccine to offer protection against multiple strains of MenB. Phase II trials have demonstrated satisfactory safety, tolerability and immunogenicity. In addition, rMenB is the first recombinant MenB protein vaccine to induce an immune response in infants.

Water in hospitals: a reservoir for nosocomial pathogens? (Symposium arranged with EFWISG)

S379 Nosocomial legionellosis

G. Wewalka (Vienna, AT)

In most European countries the number of notified cases of Legionnaires' Disease (LD) increased during the last decade because of easier diagnostic and more reliable reporting. Data collected from 35 countries by EWGLI (European Working Group for *Legionella* Infections) showed a 4.7 fold increase of cases of LD from 1997 (1344 cases) to 2006 (6280 cases). On the other hand the number cases of nosocomial LD increased (1.4 fold rise) much less from 215 cases (16% of all) in 1997 to 309 cases (4.9% of all) in 2006. This decrease of the proportion of the nosocomial cases is discussed as result of intensive control measures to prevent LD in healthcare facilities (HCF) in many countries. In Austria the situation is similar. In 1997 7 (35%) out of 20 cases of LD and in 2006 11 (15.9%) out of 69 cases were nosocomial cases.

A total of 410 cases of LD were reported to the Austrian reference centre of *Legionella* infections during 1996 and 2005 including 66 (16%) nosocomial cases, 131 (32%) travel associated-cases and 214 (52%) community acquired cases. In these 10 years 27 different Austrian HCF (25 hospitals, 2 elderly care facilities) were affected by nosocomial LD. Among these a university hospital in Western Austria accounted for 25.7% of all the nosocomial cases (18/66) as the most likely source of infection. Microbiological and epidemiological investigations indicated aerosolised potable water (tap water) as the most likely source of infection in the majority of the Austrian nosocomial cases.

A comparative analysis of the nosocomial ($n = 66$) vs. non-nosocomial ($n = 344$) cases was performed: Nosocomial cases suffered significantly more frequently from neoplastic disease (33.3% vs. 6.9%) or were more frequently immunosuppressed (15.0% vs. 6.3%). The median age was 64 years (interquartile range: 54–74yrs) in nosocomial cases vs. 53 years (interquartile range: 41–64yrs). No difference in sex distribution between nosocomial and non-nosocomial LD was observed. Mortality was significantly higher among the nosocomial cases (48% vs. 9.7%, $p < 0.001$).

Thirty-seven patients (56.1%) among the nosocomial cases and 36 patients (16.3%) among the non-nosocomial cases were diagnosed by culture of the organism. For 33 isolates the species serogroup and monoclonal subgroup were determined. Nosocomial LD is more often caused by less virulent *Legionella* strains, such as L. non-*pneumophila*, L. *pneumophila* non-serogroup (sg) 1 and L. *pneumophila* sg 1 belonging to Mab 3/1-negative subgroups.

S380 Hospital water and invasive fungal infections

P. Gaustad (Oslo, NO)

Invasive mould infections present problems in terms of diagnosis and therapy. They are increasingly common in the nosocomial setting. The predominant fungal pathogens in hospital water, in most cases detected as spores, are moulds such as *Aspergillus* spp., Mucorales and *Fusarium* spp. Immunocompromised patients are at risk. Mould infections cause high morbidity and mortality despite antifungal therapy. The incidence of nosocomial mould infections continues to increase despite the widespread use of air filtration systems, suggesting that other hospital sources for moulds exists. Water systems worldwide have been shown to be colonised with pathogenic moulds. The moulds are present the hospital water system, and the same genera are recovered from the municipal water. The number of species isolated in surfaced-sourced water is higher than the number isolated in groundwater water. The moulds are probably part of biofilms in the water system. Mould spores and hyphal fragment may be released into the water. Aerosolisation by showerheads, taps or toilet cisterns, aspiration or drinking of contaminated water may lead to patient exposure.

In the literature only a few reports connect recovered moulds from hospital water to patient isolates (Anaissie EJ CID 2001, Anaissie EJ Blood 2003, Warris J. Hosp. Incect 2001, Warris J Clin Microbiol 2003). Molecular epidemiological investigations of strains are hampered by the genetic diversity of moulds. The amplified fragment length polymorphism and the short tandem repeat analysis seem to be the most discriminative typing methods.
Early initiation of antifungal therapy and reversal of underlying host defects remain the cornerstones of treatment for nosocomial fungal infections. The mortality of nosocomial fungal infections remains high, and new therapeutic and preventive strategies are needed.
The current water treatment is insufficient in removing moulds from the hospital water. Prevention of waterborne nosocomial mould infections in high risk patients will include point-of-use filtration, sterile water for drinking, and bathing and not showers to avoid aerosolisation. Investigations of the water and its tubing system should be done properly to identify the water quality of the hospital. The surface-sourced water is more likely to contain pathogenic moulds than underground water and moulds are more likely to be present in cold water and showers than in hot water.
At this stage more data are needed to assess the exact role of water in nosocomial fungal infection.

S381 *Pseudomonas*, *Stenotrophomonas* and *Burkholderia* – are they in your hospital's water and does it matter if they are?

K.G. Kerr (Harrogate, UK)

Debilitated patients on intensive care units and individuals rendered profoundly neutropenic following chemotherapy for haematological malignancy are at risk of developing infections including those associated with Gram-negative bacteria of environmental origin. Infections caused by these bacteria are difficult to manage, partly because of inherent or acquired antimicrobial resistance manifested by these microorganisms and partly because of deficits in patients' host defences as a result of neutropenia or other underlying condition. There is thus a pressing need to develop effective interventions to prevent exogenous acquisition of infection from environmental sources. In this respect a wide range of aquatic sources, including ice-making machines, aqueous solutions of disinfectants and nebulizer apparatus have been considered as being of importance in outbreaks of infection associated with Gram-negative bacteria such as *Pseudomonas* and *Stenotrophomonas* spp.
The application of discriminative molecular typing techniques, such as pulsed-field electrophoresis, to characterise the relatedness of environmental and patient isolates has highlighted the increasing importance of hospital water supply systems, including potable water and water faucets, in the epidemiology of infections caused by environmental Gram-negatives. More recently, prospective studies conducted in non-outbreak situations have suggested that hospital water systems may also be of significance in endemic or sporadic infections associated with these bacteria.
However, isolation of environmental bacteria from potable water supplies which are indistinguishable from patients' clinical isolates cannot, in itself, answer the question as to whether patients acquire the bacteria from water faucets or whether faucets are directly or indirectly contaminated by the patients. Nevertheless, there is now accumulating evidence to suggest that interventions, such as point-of-use water filtration, to minimise or eliminate Gram-negative bacteria from aquatic sources in the patient environment may be effective as infection control interventions. These findings should now provide the impetus for further systematic prospective studies to establish whether these interventions can be adopted as integral components of infection control programmes for facilities which provide care for patients at risk of Gram-negative infection.

Issues in the way of treating chronic viral hepatitis (Symposium arranged with ESGVH & EASL)

S385 How to treat HBV mono-infected patients?

D. Salmon (Paris, FR)

In the recent years, there has been considerable progress in the treatment of chronic hepatitis B. Interferons (conventional then PEG) were the first drugs used. Although they have the advantage of a finite duration of therapy, a persistent response (loss of DNA-HBV and of AgHbe) is obtained after the end of therapy in only one third of cases. They should only be proposed in patients with factors known to influence positively the outcome. A large number of nucleoside/nucleotide analogues are, at present, available to treat hepatitis B. The efficacy of lamivudine alone, the first nucleoside analogue used, is limited by the high rate of resistance. Adefovir has efficacy comparable to that of lamivudine, but with low resistance rate. Entecavir and tenofovir are particularly active in the control of hepatitis B virus replication and are associated with minimal resistance development, even with long treatment duration. Other drugs, such as telbivudine, emtricitabine and clevudine, will become new treatment options in the near future. First-line therapeutic regimen usually include either a monotherapy with a potent agent that has a low rate of resistance; or could in the future include a combination of 2 nucleos(t)ide analogues. These agents have been shown to be effective in improving virological, biochemical, and histological features in high proportion of the patients with chronic hepatitis B. However, they can not eliminate hepatitis B virus and only suppress HBV replication. Furthermore, the emergence of drug resistant HBV is becoming problematic over the long-term. Therefore, physicians should be careful in selecting whom to treat, when to start treatment, how to monitor patients before, during and after the treatment.

MDR efflux pumps of Gram-negative bacteria: their development, genetic regulation and control

S387 Bacterial membrane drug efflux and receptor proteins

P.J.F. Henderson, D. Leng, J. Liu, P. Ma, G. Szakonyi, I. Nes, A. Kolsto, P. Roach, H. Yuille, V. Blessie, M.K. Phillips-Jones (Leeds, UK; Oslo, NO)

Multidrug (Mdr) efflux proteins (1) are widespread amongst microorganisms, including pathogens. These membrane proteins contribute to emerging antibiotic resistance. Two-component system (TCS) receptor membrane proteins (2) play key roles in metabolism and sensitivity to antibiotics of many microorganisms. The design of novel antibacterial drugs would be greatly facilitated by knowledge of the structures of these membrane proteins, which are poorly understood because of the difficulties of obtaining purified protein and crystals of quality. We describe a structural genomics strategy for the amplified expression, purification and characterisation of such proteins.
Over thirty Mdr and TCS membrane proteins have been purified from *Bacillus cereus*, *Bacillus subtilis*, *Brucella melitensis*, *Campylobacter jejuni*, *Escherichia coli*, *Enterococcus faecalis*, *Helicobacter pylori*, *Neisseria meningitidis*, *Staphylococcus aureus*, and *Streptomyces coelicolor*. Proteins from *B. cereus*, *E. faecalis*, *H. pylori* and *S. aureus* will be used as detailed examples to illustrate the strategy.
The success of this strategy is an important step towards reproducible production of transport and receptor proteins for the screening of drug binding and for optimisation of crystallisation conditions to enable subsequent structure determination and drug design.
Acknowledgements: This research is supported by the BBSRC, the EU European Membrane Protein Consortium EMeP, EU COST Action B16, and the Wellcome Trust.

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S388 Response of regulatory, efflux pump transporter and Omp genes during prolonged antibiotic stress and development of MDR in Gram-negative bacteria

L. Amaral, M. Viveiros, L. Rodrigues, M. Martins, G. Spengler, A. Martins, J-M. Pages, S. Fanning (Lisbon, PT)

Multi-drug resistance of Gram-negative bacteria is now known to be the result of over-expressed efflux pumps that extrude a variety of unrelated antibiotics prior to their reaching their targets. Although a number of methods have been developed for the assessment of over-expressed efflux such as the use of the universal efflux pump substrate ethidium bromide (EB) or the use of radiolabeled antibiotics in the presence and absence of an inhibitor of efflux pumps, these methods are labour intensive, are relatively imprecise and do not lend themselves for inter-laboratory use. We have developed a number of methods that A) identify over-expressed efflux activity of mdr pathogenic bacteria without the use of specialised instrumentation (1); B) assess specific activity of genes that regulate or code for transporter component of RND efflux pumps (2); C) assess and evaluate over-all efflux pump activity via a semi-automated system that distinguishes accumulation of EB from its efflux, can also identify agents that inhibit efflux activity as well as define conditions that control accumulation and efflux (3). Method C will be fully described for the assessment and evaluation of intrinsic and over-expressed efflux activity of mdr *E. coli*, *Enterobacter aerogenes* and *Salmonella*.

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S389 Regulation and control of membrane permeability (Influx and Efflux) in Enterobacteriaceae

J.M. Pages (Marseille, FR)

In Enterobacteriaceae, membrane permeability plays a "key" role in the level of susceptibility/resistance to antimicrobials. In recent years, modification of the bacterial envelope by decreasing the production of porins or increasing the expression of efflux pump systems has been reported. These modifications are frequently associated with other resistance mechanisms such as alteration of antibiotic molecules or modification of the drug targets, in various clinical isolates showing a MultiDrugResistant (MDR) phenotype.

In *Enterobacter aerogenes*, *Klebsiella pneumoniae* and *Salmonella enterica*, several genes and various chemical factors are involved in the emergence/selection of MDR isolates. These bacterial isolates exhibit a noticeable reduction of the number of functional porins per cell (strong decrease, no synthesis or expression of a functionally altered porin) and a high-level expression of efflux systems (e.g. over-expression of the AcrAB-TolC pump). The simultaneous presence of these mechanisms confers efficient resistance to the strain ensuring its dissemination, the colonisation of the patient and favours the acquisition of additional mechanisms of resistance. marA, ramA, and ompX are involved in a complex regulation cascade controlling membrane permeability and actively participate in triggering the MDR phenotype. Certain mutations in regulator genes induce the overexpression of efflux system and the downregulation of porin synthesis. In addition, specific molecules such as salicylate, imipenem or chloramphenicol are able to activate the MDR response. This phenomenon has been observed in vitro during culture of bacteria in the presence of drugs and during antibiotic treatment of colonised/infected patients. These effectors trigger the expression of specific genes, marA, ramA, or target some other genes located downstream in the regulation cascade.

The redundancy of regulators, the overlap in control pathways and the effect of external inducers actively contribute to the selection, dissemination and preservation of *Enterobacter aerogenes* MDR strains.

S390 Influence of local and global regulators on efflux-mediated resistance of *Salmonella enteritidis*: contributions to a pleiotropic phenotype

E. O'Regan, T. Quinn, S. Fanning (Dublin, IE)

Salmonella Enteritidis is the most common aetiological agent of foodborne salmonellosis worldwide. Fluoroquinolones are the most widely used family of antibiotics in the treatment of life threatening salmonellosis. Of significant public health concern is the emergence of *Salmonella* isolates with reduced fluoroquinolone susceptibility. Resistance to fluoroquinolones is largely attributible to mutations in the quinolone resistance-determining regions (QRDRs) in target genes and overproduction of efflux pumps. In this paper we present data describing the role of local (eg: acrR) and global regulators (including marA, soxS, rob and ram) on efflux-mediated resistance (to nalidixic acid and ciprofloxacin) in field isolates and in in-vitro selected ciprofloxacin resistant Salmonella Enteritidis isolates. Gene expression was assessed by 'real-time' PCR and invasiveness was determined by co-culture of bacterial and CaCo-2 cells. The fitness cost of antibiotic resistance was examined and a high-throughput phenotype microarray was used to assess any pleiotropic effects.

Insertional mutants were constructed by P22 phage transduction using a number of these global regulators. The geno- and phenotypes of these mutants were re-examined to determine what (if any) effects these insertions had.

Based on this analysis, our findings do not support the adoption of a unifying functional model to describe regulation. Rather, these data suggest that the evolutonalry history of a strain may be a consideration in determining the influence that these regulators have in controlling gene expression of the RND efflux pump in S. Enteritidis along with some pleiotropic phenotypes.

Meningococci and meningococcal invasive disease

S391 New insights into the pathogenic mechanisms

A. Jonsson (Uppsala, SE)

Neisseria meningitidis is a human-specific pathogen that causes sepsis and meningitis with high mortality. We have used a transgenic mouse model and bioluminescently labelled meningococci to monitor bacterial spread after infection. Bacteria accumulated in the nasal mucosa, and the colonisation was dependent of meningococcal pili and of GNA992, an outer membrane protein. Lethal disease was accompanied by bacterial enrichment in the thyroid gland, and by decreased thyroid hormone

T4 levels. Bacterial visualisation demonstrated waves of bacterial clearance and growth, which selected for bacteria expressing the phase variable outer membrane protein Opa, indicating the importance of this bacterial factor. Meningococcal lipooligosaccharide (LOS) has long been identified as a major inflammatory mediator of fulminant meningococcal sepsis and meningitis. However, both wild-type meningococci and an LOS mutant induced equivalent disease severity and similar proinflammatory responses in TLR4+/+ mice, but failed to cause fatal sepsis in TLR4$^{-/-}$ mice. Taken together, these data reveal novel disease dynamics and organ targeting during meningococcal disease. Further, fatality associated with meningococcal sepsis in mice is induced by the proinflammatory host response by recognition of one or more unidentified non-LOS components by TLR4.

S392 Role of virulence, antimicrobial resistance and host susceptibility for the outcome of meningococcal disease

R. Read (Sheffield, UK)

Across Europe the incidence of meningococcal disease varies from between 1 per 100,000 up to 14.3 per 100,000 population per annum. The case fatality rate overall is approximately 8%, but amongst survivors there is a relatively high frequency of severe sequelae including hearing impairment, neuro-cognitive abnormalities, severe skin and soft tissue abnormalities including loss of limbs, and renal failure. Physicians and paediatricians who treat meningococcal disease observe a range of severity from relatively benign disease to severe physiological disruption leading to death. In general terms, those who present with meningitis tend to have a better prognosis, whilst those who present with severe sepsis syndrome have the worst prognosis. Study of factors associated with the severity of meningococcal disease is difficult and confounded by factors such as the sporadic nature of the disease and marked differences in access to health systems between individuals. Many studies have shown that the outcome of meningococcal disease is associated with the phenotype of the infecting organism. The odds of death from disease are highest for certain sequence types, for example ST-11/ET-37 complex and ST-32/ET-5 complex. The precise phenotypes responsible for the enhanced virulence of these sequence types has not been precisely defined. The major virulence determinants of *Neisseria meningitidis* include the polysialic capsule, LPS immunotypes, sialylation, and outer membrane proteins including Opa and Opc. Whilst there is no consistent segregation of virulence determinants in clonal groups associated with the most severe disease, organisms expressing serogroup C have been associated with fatal outcome.

A number of host factors have been identified as important in determining severity of disease. Chief amongst these is age at presentation, with the worst prognosis being associated with adults. Some of this may be explained by the increasing sophistication of paediatric intensive care. Twin studies have indicated that death from severe infectious disease has a familial component and in the case of meningococcal disease a large number of studies have been conducted to investigate the role of genes encoding components of the immune system, the inflammatory response and coagulation pathways. The difficulty faced by geneticists is that death is the most easily verifiable end point for the purpose of studying genetic modifiers of severity of disease. Because of the death rate of only 8% this means that very small cohorts are available for study. However, association studies have revealed a relationship between a number of genes and the likelihood of death in meningococcal disease. These include Fc receptors, polymorphisms of plasminogen activator inhibitor type 1, properdin deficiencies, and polymorphisms within interleukin 1 and the interleukin 1 receptor antagonist.

In several countries, there have been increasing reporting of isolates of *Neisseria meningitidis* with reduced sensitivity to penicillin. Treatment failure has been reported extremely rarely and studies have failed to demonstrate any association between reduced penicillin susceptibility and fatal outcome. Most doctors consider that emergency treatment of newly-presenting cases with penicillin in the community is mandatory. Controversially, a recent study has suggested that treatment in the home with injected penicillin may be associated with increased severity of disease. This will be discussed.

S393 Immunological issues in anti-meningococcal vaccination

D. Goldblatt (London, UK)

Meningococcal infections continue to cause significant morbidity and mortality, especially in young children and adolescents in Europe where *Neisseria meningitidis* serogroups B and C cause the majority of disease. While no effective licensed vaccine exists for Group B, vaccines consisting of the purified capsule of serogroup C (and A, W and Y) have existed for many years. Their use has been limited to outbreak control and for travellers due to (i) the limited duration of protection afforded after a single dose and (ii) their poor immunogenicity in young infants. Conjugate meningococcal C vaccines, which overcome these limitations, were the first meningococcal conjugate vaccines to be licensed and entered use first in the United Kingdom in 1999 and subsequently introduced in many European countries where they have had an impressive impact on reducing the incidence of infection. Ongoing surveillance of meningococcal infection following introduction of the vaccine together with immunological studies has provided insights into the mechanism of immune protection and the role of immunological memory. These will be discussed and the areas that remain poorly understood highlighted.

S394 Trends in the management of meningococcal meningitis

D. van de Beek (Amsterdam, NL)

Neisseria meningitidis most commonly causes (meningococcal) meningitis in young adults and is a major cause of endemic meningitis.

Identification of patients with meningococcal meningitis can be difficult. Typical sings and symptoms of bacterial meningitis may not be present. Petechial skin rash has traditionally been considered the hallmark of meningococcal disease, but is absent in one third of patients presenting with meningococcal meningitis and can also occur in viral meningitis. Physical examination alone may not perform well enough to accurately diagnose or rule out meningitis. Examination of cerebrospinal fluid is important; it is required to confirm the diagnosis, identifies the causative organism, allows the testing of antibiotic sensitivities, and so helps to rationalise treatment. In view of the urgent nature of this testing, one of the issues physicians are faced with in an emergency department setting is whether cranial computed tomography is required before lumbar puncture.

Timing is critical in the management of patients with suspected meningococcal meningitis: the first step in the management of meningococcal meningitis is to obtain blood cultures and start antimicrobial therapy, and adjunctive dexamethasone when indicated. Prehospital parenteral antibiotic therapy is recommended in many countries but its use remains controversial. A first difficulty in this setting is how to identify a patient with meningococcal meningitis. A second dilemma is whether patients benefit from such pre-hospital treatment. And if general practitioners decide to treat patients with suspected bacterial meningitis with parenteral antibiotics, should dexamethasone be given before or with this first dose?

The management of the critically ill neurological patient with meningococcal meningitis can be difficult and poses important dilemmas. Signs of systemic disease occur frequently in patients with meningococcal meningitis and are associated with mortality and unfavourable outcome. Systemic disease frequently necessitates cardiopulmonary support and admission on an intensive care unit. Meningococcal sepsis is frequently associated with coagulation disorders such as disseminated intravascular coagulation. Intracranial complications as raised intracranial pressure, hydrocephalus, and brain infarction do occur. Arthritis occurs in 12% of patients; bacterial arthritis requires prolonged antibiotics and joint drainage. Adjunctive treatment strategies with dexamethasone, anti-endotoxin antibodies (HA-1A), recombinant bactericidal permeability increasing protein (rBPI21), and activated protein C have been described.

These important and controversial areas will be reviewed and relevant literature will be discussed in the framework of current treatment guidelines.

Use it and lose it? Antibiotic usage

O395 Usage of carbapenems significantly increase the rate of new colonisation due to antibiotic-resistant bacteria in hospitalised patients

E. Tacconelli, G. De Angelis, M. Cataldo, E. Mantengoli, T. Spanu, A. Pan, G. Corti, A. Radice, S. Antinori, F. Paradisi, G. Carosi, M. Antonelli, G. Rossolini, R. Cauda (Rome, Siena, Brescia, Florence, Milan, IT)

Objectives: Accurate assessment of risk factors for colonisation with antibiotic resistant bacteria (ARB) is often confounded by scanty data on antibiotics use. The aims of the study were: to prospectively define the incidence of new colonisation due to ARB per 1,000 days of antibiotics; to determine mean time of acquisition; to measure patients'risk factors for acquiring ARB; and to compare genotypic patterns of the strains.
Methods: A 12-month prospective multicentre cohort study including all in-patients (pts) starting antibiotics was planned. Samples of nose and rectum were taken before and after starting antibiotics (day 2, 4, 7, 15, and 30). ARB included: meticillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), and ciprofloxacin-resistant *Pseudomonas aeruginosa* (CR-PA). Pulsed-field gel electrophoresis was done to define genetic relatedness of the strains.
Results: In total, 6245 swabs from 864 pts were processed. The overall carriage rate of newly acquired ARB was 5%. The mean duration (days±standard deviation) of antibiotic therapy prior to the detection of a new colonisation was 8 (±4) for MRSA (range, 2–16), 6 (±6) for VRE (range, 2–19) and 9 (±8) for CR-PA (range, 2–28). The incidence of ARB per 1,000 days of therapy was 14 for carbapenems, 9 for glycopeptides, and 6 for cephalosporins of 3rd gen. and quinolones. Highest rates were observed for carbapenems in dialysed (29) and diabetic (28) pts and for 3rd gen. cephalosporins in pts with cronic renal impairment (27). Specific rates for MRSA were 8.2 per 1,000 days of macrolides, 7.9 of carbapenems, 3.2 of glycopeptides and 2.4 of cephalosporins. In the multivariate analysis length of hospitalisation >16 days (odds ratio [OR] 2.5, 95% confidence interval [CI] 1.2–5.1), HIV infection (OR 2.1, 95% CI 1.1–4.4), use of carbapenems (OR 2, 95% CI 1–3.8), and age >70 (OR 1.5, 95% CI 1.1–2) were significant predictors for new ARB acquisition ($P < 0.05$). All strains were genetically unrelated except for serial isolates from single patient.
Conclusions: The risk of ARB colonisation deeply varies during exposure to different antibiotics and is mainly related to pts underlying conditions and length of hospitalisation. Periodic screening of high risk pts undergoing antibiotics, in particular carbapenems, might be suggested.

O396 Temporal effects of antibiotic use and hand-rub consumption on incidence of MRSA and *Clostridium difficile*

N. Vernaz-Hegi, H. Sax, D. Pittet, P. Bonnabry, J. Schrenzel, S. Harbarth (Geneva, CH)

Objectives: To determine the temporal relation between use of antibiotics and alcohol-based hand rubs (ABHR) on the incidence of meticillin-resistant *Staphylococcus aureus* (MRSA) and *Clostridium difficile* at the Geneva University Hospitals.
Methods: Using interventional time-series analyses, we (1) evaluated the impact of 2 hand hygiene campaigns in 2003 and 2005 on the consumption of ABHR; (2) combined this model with a transfer function model on aggregated data on antibiotic use; and (3) assessed their effect on the incidence of clinical isolates of MRSA and *C. difficile* from February 2000 through September 2006. The WHO ATC/DDD classification was used as reference, normalised per 100 patient-days (PD).
Results: From the 84% of total antibiotic use (average, 33 DDD/100PD) analysed, 38% showed to have a temporal relation with MRSA incidence while no association was detected for *C. difficile* (except usage of broad-spectrum cephalosporins). The model indicated a significant impact of consumption of ABHR on MRSA, but not on *C. difficile*. The intervention model integrated to a transfer model showed the efficiency of the second hand hygiene campaign and an additional temporal effect of the use of five broad-spectrum antimicrobials, explaining 57% of the incidence of MRSA over time. An increase of 1DDD/100PD of antibiotic use increased the incidence of MRSA isolates per 100 PD from the current level, i.e. 0.01 for fluoroquinolone (significant impact 1 month later), 0.03 for macrolides (1 and 4 m), 0.03 for 3rd generation cephalosporins (4 and 5 m), 0.014 for cefepime (3 m) and 0.04 for piperacillin/tazobactam (3 m). Conversely, the hand hygiene campaign in 2005 reduced MRSA incidence; 1L of ABHR/100PD decreased MRSA by 0.03/100PD.
Conclusion: We observed an aggregate level relation between trends in MRSA incidence and the use of ABHR and different antibiotic classes, while no association with ABHR use was detected for *C. difficile*. Modelling drug-use-versus-susceptibility relations using time-series analyses is a useful tool complementing traditional epidemiologic approaches.

O397 The use of geographical information system to map antibiotic consumption

F. Marra, S. Mak, M. Chong, D. Patrick (Vancouver, CA)

Background: Antibiotic consumption in human populations is a factor in the emergence of resistant organisms. As such, it is important to track population-based consumption data on an ongoing basis and to explore the determinants for regional variations in antibiotic consumption for the province of British Columbia (BC).
Methods: We obtained data from the BC PharmaNet database on all outpatient oral antibiotic prescriptions for 2005. Prescriptions were expressed as their defined daily dose (DDD) per 1,000 inhabitants according to the 2006 World Health Organization Anatomical Therapeutic Chemical system. Geographic Information Systems (GIS) mapping was used to display the spatial variations of antibiotic consumption in BC. We explored the relationships between antimicrobial consumption and socioeconomic and climatic factors using Pearson's correlation. Overall and class-specific rates of consumption were described by the Health Service Delivery Area geography.
Results: Overall antibiotic consumption was highest in the northern health regions and lowest in the interior health regions. Correlations were found between antibiotic consumption and socioeconomic and climatic factors. Higher rates of antibiotic consumption were associated with the aboriginal population, higher family income, and July total precipitation. An inverse relationship was found between consumption and July average temperature. Further analysis of class-specific antibiotic consumption identified different geographic patterns of consumption and socioeconomic associations. Higher rates of penicillin, β-lactam, and macrolide consumption were seen with the aboriginal population, younger population (age <15 years), higher ratio of physicians to population, higher family income, and greater July total precipitation. An inverse correlation was found between antibiotic consumption and older age (age >65 years), mortality rate, and warmer July temperatures.
Conclusions: Different rates of antibiotic consumption exist within the province. Appropriate policies affecting antibiotic consumption in the community can be designed by looking at the relationships between antibiotic consumption and socioeconomic determinants, and their related impact.

O398 Hospital antibiotic prescribing in hospitals from 18 European countries 2000–2005: longitudinal analysis with comparison of adjustment for changes in clinical activity using admissions or occupied bed days

F. Ansari, H. Goossens, M. Ferech, A. Muller, H. Molana, P.G. Davey on behalf of the European Surveillance of Antimicrobial Consumption Project

Objective: To collect data about hospital antibiotic use with standardised methods in different European countries. The data were used to answer two research questions:
1. What is the trend in hospital antibiotic use over time?
2. What effect does adjustment for bed days or admissions have on trends in hospital antibiotic use?

Methods: A total of 18 hospitals participated in the study, one hospital from each of 18 countries. We collected monthly data about total antibiotic use over 6 years starting from January 2000. Crude data about antibiotic use was converted to the ATC drug classification and Defined Daily Doses (DDD).

Results: Antibiotic use measured in DDD increased in 14 hospitals and decreased in 4 hospitals. There was an underlying trend of reducing length of stay in 16 of the 18 hospitals. Consequently annual changes in DDD per 100 occupied bed days (DBD) were also greater than annual changes in DDD per 100 admissions (DAD) in 16 of the 18 hospitals. Overall there were five distinct patterns of antibiotic use over time:
1. Increasing antibiotic use that was not fully explained by increased clinical activity (11 hospitals). Increases in DDD over time remained after adjustment with either bed days or admissions.
2. Increasing antibiotic use was entirely explained by increased clinical activity (1 hospital). The increase in DDD over time reversed to a decrease in DBD and DAD. Hence the apparent increase in antibiotic use was entirely due to a large increase in clinical activity.
3. Divergence between DBD and DAD (2 hospitals). The increase in DDD was reversed when adjusted for admissions but persisted when adjusted for bed days. The divergent results were a consequence of an increasing number of admissions combined with reducing length of stay. The apparent increase in antibiotic use was likely to be explained by increase in clinical activity.
4. Decreasing antibiotic use that was not fully explained by decreased clinical activity (3 hospitals).
5. Decreasing antibiotic use that was entirely explained by decreased clinical activity (1 hospital).

Conclusions: Interpretation of longitudinal surveillance data about antibiotic use is facilitated by presentation of changes in DDD without adjustment for clinical activity in addition to adjusted data. Antibiotic use is influenced by number of admissions and by length of stay; consequently adjustment for clinical activity should be done with both admissions and occupied bed days.

O399 Performance of the TREAT decision support system in an environment with low prevalence of resistant pathogens

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Objectives: The decision support system for antibiotic treatment TREAT [1] has been shown to improve appropriateness of antibiotic therapy and reduce cost in regions with intermediate or high prevalence of resistant bacterial strains. The purpose of this study was to explore if TREAT can achieve similar improvements in Denmark, which has a low prevalence of resistant bacterial strains.

Methods: A retrospective trial of TREAT has been performed at Copenhagen University, Hvidovre Hospital. The system was calibrated by local data (e.g., distribution of pathogens, resistance to antibiotics, various administrative factors). The study was based on a database with detailed clinical data on adult patients with suspicion of moderate to severe infections, collected in 2005–2006. The data included risk factors for infections and pathogens, clinical and microbiological data, physicians chosen therapy and results for susceptibility tests of isolated pathogens. TREAT was tested empirically, i.e. in all cases (with exception of 2) the morphology/identity of the isolate was neither known to the physician nor to the system. Coverage (defined as the percentage of antibiotic treatment matching the susceptibility of isolated pathogen) and cost of treatment obtained by TREAT were compared to clinical practice.

Results: Out of 171 patients in the database, 161 fulfilled the inclusion criteria previously applied in clinical trials of TREAT. Significant clinical isolates were found in 65 (40%) cases, among them 25 isolates from the blood. Coverage achieved by TREAT in 65 patients with significant clinical isolates was 86%, while coverage achieved by the first attending clinical physician was 66% (OR 3.2, 95%CI 1.3–7.6, $p = 0.009$). The mean costs (in Euro) per episode for TREAT were 76 for direct expenses for antibiotics and administration, 96 for side effects, 310 for future resistance; and in clinical practice 54, 126 and 289, respectively.

Conclusion: Coverage achieved by TREAT was significantly higher than coverage achieved by the physician. The costs obtained by the system are lower in regard to side effects, and higher for direct expenses and the pressure for future resistance. These results suggest that TREAT can markedly improve appropriateness of antibiotic therapy and reduce cost for side effects in regions with low prevalence of resistant pathogens.

Reference(s)

[1] The TREAT Working Group. J Antimicrob Chemother 2006; 58: 1238–45.

O400 European Surveillance of Antimicrobial Consumption (ESAC): outpatient parenteral antibiotic treatment in Europe

S. Coenen, A. Muller, N. Adriaenssens, V. Vankerckhoven, E. Hendrickx, H. Goossens and the ESAC Project Group

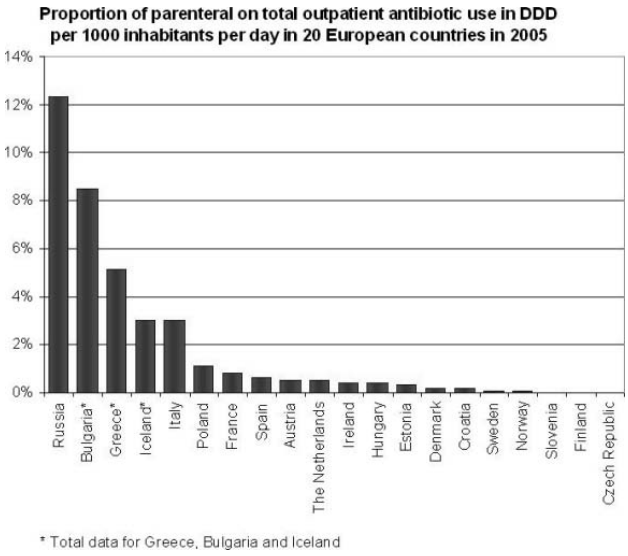
Objectives: To assess the proportion of outpatient parenteral antibiotic treatment on the total outpatient antibiotic use in Europe, and to identify the antibiotic groups and substances most commonly administered this way.

Methods: The European Surveillance of Antimicrobial Consumption (ESAC; www.esac.ua.ac.be) project, now funded by the European Center for Disease Control and Prevention (ECDC; agreement number 2007/001), continues to collect data on antimicrobial consumption for all Member States, candidate countries and EFTA-EEA countries using the anatomic chemical therapeutic (ATC) and defined daily dose (DDD) classification. For 2005, data on outpatient use of antibacterial for systemic use (ATC J01), aggregated at the level of the active substance and expressed in DDD per 1000 inhabitants per day (DID; WHO version 2007) were extracted by route of administration and by country. The primary outcome measure is the total parenteral use expressed as a percentage of the total outpatient antibiotic use in DID.

Results: The average proportion of outpatient parenteral antibiotic treatment in 20 (total use data for Greece, Bulgaria and Iceland) European countries in 2005 is 1.77%, ranging from 12.33% in Russia to 0.01% in Czech Republic (see figure), while the total outpatient use ranges from 28.94 DID in France (34.73 DID in Greece is total use) to in Russia 9.16 DID. The three most commonly used antibiotic groups for parenteral treatment are the cephalosporins (J01D; 43.36%, from 73.68% in Finland to 0% in Norway), the penicillins (J01C; 24.44%, from 74.49% in Hungary to 0% in Norway) and the aminoglycosides (J01G; 13.81%, from 75.28% in Norway to 0% in Estonia). The three most commonly used antibiotic substances for parenteral treatment are cefazolin (J01DB04; 12.02%, from 35.89% in Russia to 0% in more than one country), ceftriaxone (J01DD04; 10.89%, from 50.04% in France to 0% in more than one country) and cefuroxime (J01DC02; 7.32%, from 48.35% in Poland to 0% in more than one country).

Conclusion: Outpatient parenteral antibiotic treatment only represents more than 1% of the total outpatient antibiotic use in 6 out of the 20 European countries studied. However, as for the total outpatient antibiotic use and the use of different antibiotic groups and substances, there is a striking variation in the proportions of parenteral antibiotic use in Europe

as well. More in-depth data on outpatient antibiotic use are needed to explain this variation.



O401 Prevalence of antibiotic prescriptions in healthcare facilities, France, 2006

S. Maugat, L. Lacavé, F. L'Hériteau, C. Gautier, H. Tronel, M.H. Metzger, P. Jarno, J.M. Thiolet, B. Coignard on behalf of the RAISIN Study Group

Background: Antibiotic (ATB) consumption in healthcare facilities (HCF) in France is one of the highest among European countries. In June 2006, we conducted a prevalence survey of nosocomial infections (NI) in French HCF in which we also assessed the prevalence of ATB prescriptions, described their characteristics and by comparison to the 2001 survey assessed the impact of the national ATB programme.
Methods: The survey was proposed to public and private HCF and used ATC5 codes. Data were collected in a standardised manner by trained personnel in HCF and sent by encrypted e-mail to regional infection control coordinating centres (CClin) and the French Institute for Public Health Surveillance (InVS). The prevalence of patients with an ATB prescription (PPATB) was compared between 2001 and 2006 among HCF that participated in both surveys.
Results: Among 358 353 patients included from 2 337 HCF (accounting for 95% of all French hospital beds), 55 624 (15.5%) received an ATB and 74 515 compounds were given to patients. PPATB was higher in acute care (24.8%), especially intensive care units (49.0%), than in rehabilitation (9.9%) or in long term care (4.3%). PPATB varied with treatment indication: community-acquired infection (7.4%), NI (3.9%), surgical antibioprophylaxis (2.4%), prophylaxis of opportunistic infections (1.3%) or multiple indications (0.5%). The five more prescribed molecules were amoxicillin-clavulanic acid (PPATB= 4.4%), ofloxacin (1.8%), amoxicillin (1.5%), ceftriaxone (1.4%) or ciprofloxacin (1.3%). In the 1 351 HCF participating in both surveys, PPATB slightly increased from 2001 to 2006 (16.4% vs. 16.7%), especially in intensive care units, but decreased in obstetrics, rehabilitation, long term care or psychiatry. PPATB decreased for NI (4.5% vs. 4.1%) or prophylaxis (4.1% vs. 3.8%). For community-acquired infections, it evolved differently according to patients' age, decreasing (10.2% vs. 8.6%) before 6 years, and increasing (8.2% vs. 8.8%) after 44 years. PPATB for fluoroquinolones or third-generation cephalosporins increased (+9% and +15%, respectively).
Conclusion: The overall prevalence of patients with an ATB prescription in French HCF did not decrease from 2001 to 2006. Further studies are needed to understand the reasons. Prudent use of ATB remains a priority in France.

O402 Restriction of cephalosporins do not alter the burden of cephalosporin resistant *Klebsiella pneumoniae* and *Escherichia coli* in a surgical ICU

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Objective: To test whether a reduction of third-generation cephalosporin (3GC)use has a sustainable positive impact on the high endemic prevalence of 3GC-resistant *K. pneumoniae* and *E. coli* in a surgical intensive care unit (ICU).
Intervention: Switch from 3GC to piperacillin in combination with a β-lactamase-inhibitor as standard therapy for peritonitis and other intraabominal infections in 7/2004.
Methods: Segmented regression analysis of interrupted time series was used to analyse antibiotic consumption and resistance data 30 months before and 30 after the intervention. Antimicrobial usage density (AD) was expressed as daily defined doses (DDD) and normalised per 1000 patient-days. The proportion of resistant isolates (RP) is calculated by dividing the number of resistant isolates by the total number of the isolates of this species tested against this antibiotic multiplied by 100. The resistance densities (RD) are expressed as the number of resistant isolates of a species/1000 pd.
Results: The intervention was associated with a significant and sustainable decrease in the use of 3GC. Use decreased from 178.9 before to 68.7 DDD/1000 pd. The intervention resulted in a mean estimated reduction of total antibiotic use of −375.0 DDD/1000 pd, which is equivalent to a 27% reduction. Total antibiotic use showed no significant month to month change before and after the intervention. Piperacillin and piperacillin/tazobactam showed a significant increase in level of 64.4 DDD/1000 pd and continued to increase by 2.3 DDD/1000 pd per month after the intervention.
The intervention was not associated with a significant quarterly change in the RD of *K. pneumoniae* and *E. coli* resistant to 3GC. In contrast, the reduced use of 3GC and the switch to piperacillin was followed by a continuous increase in the RD of *E. coli* resistant to piperacillin by 0.5 per 1000 pd per quarter and by a continuous decrease of *P. aeruginosa* resistant to piperacillin of 0.1 per quarter.
Conclusion: We conclude that concentratiing on the reduction of 3rd generation cephalosporins is not necessarily followed by a positive impact on the resistance situation in the ICU setting. Replacement with piperacillin with β-lactamase inhibitor might likewise provide a selection pressure on 3GC-resistant *E. coli* and *K. pneumoniae*. To improve resistance it might not be sufficient to restrict interventions to a risk area, rather, it may be essential to include the whole hospital and even the community.

O403 Consensus development of quality indicators for hospital antibiotic use: the ABS International Quality Indicators Sub-Project

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Objectives: To develop valid Quality Indicators (QI) to assess clinical performance of antibiotic use, as part of the Antibiotic Strategy International, an EU-Project aiming at improving antibiotic therapy in hospital care.
Methods. An international team of 12 experts in four disciplines developed and selected QIs for structure (hospital organisation and resources), processes of care (diagnostic, treatment and prophylactic practice) and outcome (drug use). The development of QIs was achieved in 3 steps: a) identification of QIs in the literature; b) multi-criteria scoring and ranking based on scientific value and applicability; c) general discussion and final consensus QIs selection.
Results: Based on 105 potential QIs, 55 structural, 13 process and 3 outcome QIs were selected and developed. Structural QIs

described the organisation and resources as well as communication and evaluation tools available at hospital level for implementing a multimodal, multidisciplinary antibiotic stewardship programme. Process QIs focussed on four care processes: 1) surgical prophylaxis (indication, drug choice, timing and duration of administration); 2) management of community-acquired pneumonia (blood culture and *Legionella* antigen tests and drug choice for empirical treatment); 3) management of *Staphylococcus aureus* bacteraemia (echocardiography, IV catheter removal and duration of effective therapy); and 4) IV-PO switch for treatment with equivalent bio-available antibiotics. Outcome indicators focussed on antibiotic use.
Conclusions: This international consensus development of structural and process QIs provides tools for evaluating the intensity of hospital antibiotic stewardship programmes and auditing key treatment and prophylactic practices. Process QIs will be tested for feasibility, reliability and sensitivity to improvement in pilot hospitals.

O404 Changes in patterns of antimicrobial use in Swedish hospitals from 2003 to 2006 following the introduction of large-scale nationwide point prevalence studies

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Objectives: The objective of the studies was to perform descriptive point prevalence studies (PPS) of antimicrobial use in relation to diagnoses in Swedish hospitals.
Method: The protocol was designed to present demographic data as well as the amounts and indications for antimicrobial agents. Treatments were recorded in relation to diagnoses, prophylactic use, community acquired (CAI) and hospital acquired infection (HAI). Three nation-wide PPS were performed in November 2003, 2004 and 2006. Three areas were identified for intervention before the last PPS; duration of peri-operative prophylaxis, treatment of community-acquired pneumonia, use of fluoroquinolones in community-acquired cystitis in women. Direct information to all hospital physicians and feed-back of earlier results was used as the intervention strategy.
Results: 13,420 patients treated with antimicrobial agents in 54, 49 and 64 hospitals were included in the three PPS. The number of admitted patients corresponds to 50–75% of all admitted patients in Sweden during one day. 32.5–34.9% of the admitted patients were treated with antimicrobials. The indication for treatment was CAI in 17.0–18.0%, HAI in 9.2–9.9% and prophylaxis in 6.3%. For adults cultures were taken before oral treatment in 60% and before parenteral treatment in 70%. Only minor changes in the patterns of the overall use of antimicrobials were observed. However, in lower urinary tract infections of women the relative use of mecillinam and trimethoprim increased from 36 to 44% and from 22 to 26%, respectively, while the use of fluoroquinolones decreased from 25 to 13%. For community acquired pneumonia no significant changes were observed. The 2006 PPS result shows a decrease of peri-operative treatments longer than one day from 47% in 2003 to 31% in 2006. The one-dose peri-operative prophylaxis in the lower gastrointestinal tract has increased from 62% to 77%. The total amount of antimicrobials used for adults was 40.3, 43.1 and 43.3 DDD/100 admitted patients, respectively.
Conclusions: The PPS method has become a valuable tool in Sweden to describe the patterns of antibiotic use. The 2006 PPS showed after the interventions during 2005 and 2006 changes of antimicrobial use in peri-operative prophylaxis with shorter therapies and less fluoroquinolones in treatment of community acquired cystitis in women. However, in treatment of community acquired pneumonia cephalosporins still dominates and are included in more than 35% of all therapies.

Travel medicine, tropical and parasitic diseases

O405 Molecular methods for accurate diagnosis and epidemiological picture of imported malaria

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Objectives: Malaria is the most frequent imported infection in Italy, related to the increasing number of travellers and migratory flows from endemic countries.
Microscopic examination has poor sensitivity and may give problems in the differentiation of *Plasmodium falciparum* (Pf), *P. malariae* (Pm), *P. ovale* (Po), and *P. vivax* (Pv), especially in cases of low parasitaemia or mixed infections: to circumvent these limitations molecular assays based on 18S-rDNA were developed by several research groups, including us. Our study aimed to accurately and promptly diagnose cases of malaria and to describe their occurrence in our area comparing the results of microscopy and molecular assays, in order to assess the usefulness of these assays in the diagnostic practice.
Methods: Blood samples from 701 patients presenting to the University Hospital of Parma from 2000 to 2007 with clinical suspicion of malaria were subjected to microscopic examination and to 6 different PCR protocols targeting plasmodial 18S-rDNA alternatively used during 2000–07, including nested- and Real-time PCR assays.
Results: By microscopy 153 cases of malaria were diagnosed [129 Pf (84.3%), 7 Po (4.6%), 10 Pv (6.5%), 5 P. spp. (3.3%), 1 Pf/P. spp. (0.65%), 1 mixed infection (0.65%)], whilst 159 were diagnosed by PCRs [129 Pf (81.1%), 14 Po (8.8%), 6 Pv (3.8%), 3 Pm (1.9%), 7 mixed infections (4.4%)].
Conclusion: Despite microscopy remains the reference diagnostic method (rapid and inexpensive), in some cases molecular assays are the only ones allowing a correct diagnosis of malaria, particularly to detect infections by species other than Pf and mixed infections.
However, in our study only one PCR assay developed by us showed the higher accuracy in Po detection due to specific primer design done to recognise all the variants in Po 18S-rRNA gene.
PCR proved to be more sensitive and specific than microscopy and changed the picture of malaria epidemiology in our area detecting 5 single and 1 mixed infections missed by microscopy, revealing 5 single and 2 mixed infections incorrectly diagnosed by microscopy and giving speciation in 6 cases in which microscopy had limited the result to genus identification.
The most prevalent malaria cases in our area as well as in Italy were imported from Africa and due to Pf, followed by Po and Pv.
In our experience a rapid and accurate diagnosis of malaria allowed to administer a prompt and targeted therapy with positive impact on the clinical management of the patients.

O406 Seroprevalence of Chagas' disease in a general university hosiptal in Valencia, Spain

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Introduction: Chagas' disease is caused by infection with the protozoan agent *Trypanosoma cruzi* (*T. cruzi*).
Represents a serious blood safety problem due to increasing inmigration from Latin America. Outside of endemic areas, Chagas disease may be transmitted from the transfusion of infected blood components, congenital infections and organ transplantations.
The aim of this study is to determinate the seroprevalence of the *T. cruzi* antibodies in samples of people from endemic countries, people that travelling at these countries and sons from affected mothers born in Spain.
Methods: In 246 samples of 234 patients from 11 countries, we were tested by enzyme-linked inmunoassay (ELISA) (Dade

Behring CHAG0560DB) for IgG antibodies against *T. cruzi*, and indirect inmunofluorescence (IFI) (Biocientífica SA Inmunofluor Chagas NF09–60) for IgG (Bio-Mérieux 75 692) and IgM (Bio-Mérieux 75 672) antibodies.

Results: The percentage of positive samples was 58 (23.58%)and their distribution as follows: 40 Bolivia's patients (69%), 2 from Ecuador (3.5%), 2 from Colombia (3.5%), 1 from Chile (1.75%) and 13 from Spain (22.25%).

Between the 13 Spanish's patients 7 of them were children (age between 0 and 6 years old); and the other 6 remaining patients were adult women. These 6 women, fitting of endemic areas were obtained nationality in Spain.

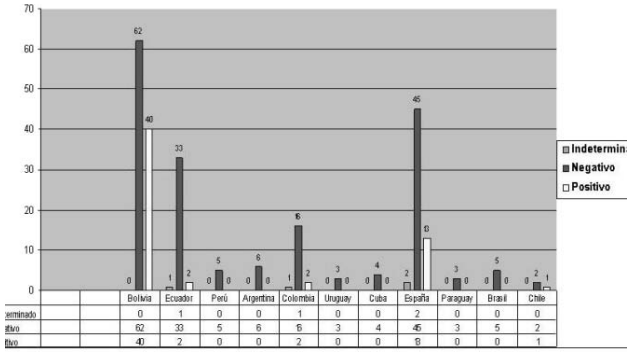
We were detected IgM antibodies in 4 of the 7 children and also one child we were found a positive result by PCR.

Conclusion: The seroprevalence that we found in our considered samples was about 23.58% and the country with higher seroprevalence was Bolivia.

Many other countries showed relevant seroprevalence especially Latin-American countries; and that suppose migrations are a risk factor in destinations countries.

For this reason, Spain could be in a near future in Chagas's disease world distribution.

It's remarkable one case of vertical transmission with IgG and IgM antibodies and positive PCR. According to vertical transmission; children become a high risky population sector and this fact may be considered, not only about infected mothers.



O407 10 years of cutaneous leishmaniasis treatment in Liverpool. Outcomes and complications with sodium stibogluconate

D. Sloan, H. Williamson, A. Young, T. O'Dempsey, A. Miller, N. Beeching (Liverpool, UK)

Background: The incidence of cutaneous leishmaniasis (CL) in the UK is rising. Adverse events associated with intravenous sodium stibogluconate (SbV) therapy include biochemical, haematological and cardiac toxicity, and intravenous catheter problems. An audit of CL in-patients receiving parenteral SbV in Liverpool from 1998–2007 was conducted to assess the cure rate and identify treatment complications.

Methods: CL was diagnosed by impression smear, culture, histology or PCR. SbV was given for 10–20 days, often via a Peripherally Inserted Central Catheter (PICC). Blood and ECG monitoring was done thrice weekly. Case-notes were reviewed retrospectively. Drug delivery and treatment outcomes were recorded for every patient. PICC complications with SbV infusion were compared with those for other drugs on the same ward. Abnormal blood results and ECG changes were analysed. Treatment interruptions due to adverse events were documented.

Results: 72 patients were studied. 23 (32%), mainly with Old World CL, received 10mg/kg SbV for 10–20 days (Group A). 49 (68%), mainly with New World CL, received 20mg/kg for 20 days (Group B). 71 (99%) patients were cured or clinically improved by discharge. 5 (7%) subsequently developed disease recurrence. 14/58 (24%) patients receiving SbV via PICCs required premature line removal with thombophlebitis. Line infection was microbiologically confirmed in 6 (10%) cases. SbV was associated with significantly more PICC complications than other drugs (Relative Risk 3.53, 95% Confidence Interval: 1.68–7.40). Hyperamylasaemia, thrombocytopenia and QTc prolongation were statistically more common amongst Group B patients but never required cessation of therapy. All patients developed biochemical transaminitis but this resulted in treatment interruption on only 4 (5.9%) occasions.

Conclusions:

1. Intravenous SbV achieves a high cure rate in CL.
2. Biochemical, haematological and cardiac toxicity is well reported but rarely requires interruption of therapy.
3. PICC lines for SbV infusion are associated with more complications than those inserted for other drugs. Frequently changed peripheral cannulae may be preferable.

O408 A water filtration method using river sand and activated carbons for the removal of intestinal protozoan parasites in contaminated drinking water at a point-of-use treatment

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Introduction: Drinking water that is contaminated with micro-organisms causes an estimated 6 to 60 million cases of gastrointestinal illness annually, worldwide. WHO and UNICEF estimate that approximately 2.2 million die of waterborne diseases each year. Clean safe water is mendatory to every household. Chlorine which is used for water disinfection does not kill protozoan parasites.

Objectives: To analyse sand filters and different types of activated carbons, experimentally in their capability in removing protozoan parasites from drinking water.

Materials and Methods: Known human intestinal protozoan parasites were put in water which was poured in to either a metal or plastic bucket with a mesh screen at the bottom. The bucket contained either coarse or very fine sand. The water collected after filtration was then spun and analysed for protozoan parasites. Activated carbons; namely baobab shells, macadamia nut shells, amarula stones, local wood carbons, commercial activated carbons and the activated carbons used by the Zimbabwe National Water Authority (ZINWA) at their water treatment plants, were analysed separately. Each activated carbon was placed in distilled water that had known amounts of protozoan parasites and incubated for 24 hours with constant shaking at room temperature. The water was then sieved using a tested cotton cloth that allowed parasites to pass through, spun and then analysed for protozoan parasites.

Results and Conclusions: Very fine sand was more efficient in capturing protozoan parasites (99%) than coarse sand (80%). Activated local wood charcoal reduced the number of parasites in water by 17%, whilst commercial activated carbon adsorbed all other intestinal protozoan parasites except for *Entamoeba coli* that was reduced by 98%. Amarula stones reduced parasites by 33.5%, activated carbon from ZINWA reduced parasites by 99.6% whilst activated carbons from baobab shells and macadamia nut shells completely adsorbed parasites by 99.99%. All the activated carbons were capable of adsorbing *Giardia lamblia* by 99.99% except for the ZINWA activated carbon (99.6%). Very fine sand in combination with either commercial carbon, activated wood carbon or activated carbons from baobab shells and macadamia nutshells are potential useful media to use as household point of use treatment of water for the removal of parasites before application of another method such as chlorination in order to kill bacterial cells.

O409 Implementing molecular detection of gastro-intestinal protozoa and implications for an algorithm for complete parasitological diagnostics in the microbiological laboratory routine

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Introduction: Molecular assays for detection of *Entamoeba histolytica*, *Giardia lamblia*, *Cryptosporidium hominis/parvum*, and, respectively, *Dientamoeba fragilis* have already been described. They were more

sensitive and/or specific than microscopy but are to date not routinely applied as replacement of the time-consuming microscopic analysis for protozoal gastrointestinal (GI) pathogens.
An internally controlled molecular assay for the simultaneous detection of all four protozoa in a single faeces sample was validated and compared to the Triple Feces Test (TFT) microscopic analysis. Also, an algorithm for the complete parasitological diagnosis after implementation of the real-time PCR assay in the laboratory routine was created.
Methods: TFT sets (2 fixed and 1 unpreserved faecal sample), were collected from 397 consecutive patients. The complete TFT set was examined for GI parasites by microscopy and compared to multiplex real-time PCR for *E. histolytica*, *G. lamblia*, *C. hominis/parvum*, and *D. fragilis*, applied on the unpreserved faeces samples. Faecal DNA was extracted by Nuclisens easyMAG (Biomerieux) with Phocid Herpes Virus added as internal inhibition control.
Also, microscopic results and clinical patient information of 2887 TFT sets were analysed retrospectively to determine local risk-factors for infection with non-protozoal GI parasites.
Results: Real-time PCR of 397 unpreserved samples yielded 169 (43%) positives (44 (11.1%) samples positive for *G. lamblia*, 122 (30.7%) positive for *D. fragilis* and 3 (0.8%) positive for *C. hominis/parvum*), while microscopy of the TFT samples yielded 100 (25%) positives (29 (7.3%) samples positive for *G. lamblia*, 69 (17.4%) positive for *D. fragilis* and 2 (0.5%) positive for *C. hominis/parvum*, respectively).
Analysis of the 2887 TFT sets showed eosinophilia, elevated IgE and travelling to (sub) tropical areas to be risk-factors for non-protozoal GI parasites. The resulting diagnostic algorithm includes application of real-time PCR on all samples, adding microscopy on an unpreserved faecal sample only in case of presence of a risk factor.
Conclusions: 1) Application of real-time PCR improved the diagnostic yield with 18%. 2) A single unpreserved faecal sample is sufficient for complete parasitological diagnosis when an algorithm based on clinical patient information is applied.

O410 Travel characteristics and main diagnoses observed in ill returned travellers

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Objectives: To study the main diagnoses among returning travellers with respect to the geographical area visited, length and type of travel
Methods: The Tropical Medicine Unit at the Ramon y Cajal Hospital, is a referral unit where immigrants and ill returned travellers are attended since year 1989. A comprehensive microbiological study was performed in all the cases.
Length of travel was divided into three groups: <30 (Short), 30–180 (Medium) and >180 days (Long-term); whereas type of travel was classified into four groups: professional high and low risk (PHR; PLR), tourism high and low risk (THR, TLR).
Discrete variables were compared by means of Chi squared or Fisher exact test. For quantitative data an independent samples t-test was used.
Results: 2993 travellers consulted our department during 1989–2006, with a progressive increase in the number of patients over the years. The mean age of the travellers was 35 years (interquartile range: 28–40) and there were 47.9% of women.
The main diagnoses were: suspected bacterial gastrointestinal infections (16.3%), 16.7% of them had bacterial growth confirmation (*S. sonnei*: 36.5%, *Salmonella* sp: 29.2%, *Campylobacter* sp: 13.4% and *C. difficile*: 9.7%) and were observed mainly in travellers to North Africa and South-West Asia. The second most frequent diagnosis was parasitic intestinal infection (12.8%): *G. lamblia*: 28.2%, *E. histolytica*: 14.9%, *T. saginata*: 6.2%, *A. lumbricoides*: 3.3% and *S. stercoralis*: 2.6%; in 42.7% of the cases more than one parasite was found. The third diagnosis in order of frequency was malaria (9.5%. produced by *P. falciparum*: 53.7%, followed by *P. vivax*: 17.5%), acquired mainly in Subsaharan Africa (16.9%)
Conclusion: Both type and length of travel were associated with bacterial GI infections (TLR and <30 days). Only travel length was a determinant for parasitic GI infections (increased risk for travel >30 days) Malaria was only associated with type of travel (less risk in TLR).

	Type of Travel				
Diagnose (%)	PLR	TLR	THR	PHR	p
Bacterial GI infection	12.5	20.0	17.8	13.9	<0.001
Parasitic GI infection	12.1	10.9	14.3	14.4	ns
Malaria	12.1	6.3	10.2	11.1	<0.001

	Travel Length			
Diagnose (%)	<30 days	30–180 days	>180 days	p
Bacterial GI infection	19.7	15.1	10.0	<0.001
Parasitic GI infection	11.2	14.5	14.8	0.02
Malaria	9.0	10.3	10.0	ns

O411 Cutaneous myiasis in two Dutch patients with and without a history of recent travel

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Objectives: Myiasis is caused by infestation of the skin by larvae of several dipterous (2-winged) flies and occurs mainly in tropical climates, predominantly affecting animal hosts. Human infection can occur through wearing egg-contaminated clothes or through an insect bite, when the insect carries eggs. After penetrating the skin painlessly, stage I larvae mature subcutaneously and emerge finally through the skin. This leads to erythematous nodules containing a single larva. Gradually a central hole develops, which may discharge fluid or pus and the white body of the larva may protrude.
Cutaneous myiasis is endemic to South and Middle America (*Dermatobia hominis*) and Sub-Sahara Africa (*Cordylobia antropophaga*; tumbu fly).
Methods: We describe two patients with furuncular myiasis, one with and another without a history of travel to an endemic area. Myiasis is rarely seen in the Netherlands.
Results: *Case 1*. A Dutch patient with no history of recent travel presented at his general practitioner with two furuncular lesions at his back, which were itchy and painfull. Four weeks earlier, the patient had noticed an insect bite at the same location. When the patient did not respond to one week of doxycycline, the diagnosis cutaneous myiasis was established.
Case 2. A 50-year-old man travelled through the Amazon region for several months. Two months after his return to the Netherlands, he visited a dermatologist as he had a persistent skin lesion on his left shoulder and was worried about having acquired Leishmaniasis. This was ruled out by microscopy and culture of a skin biopsy. At his second visit to the dermatology department, a central hole had occurred in the lesion and a *Dermatobia hominis* larva was noticed and removed. Afterwards, he reported having felt movements in his skin, but had contributed this to his imagination.
Conclusion: Cutaneous myiasis is rarely seen in the Netherlands and should not only be considered in a traveller returning from an endemic area, but also in patients with persistent skin lesions after an insect bite and no history of travel.

O412 Schistosomiasis in Sheffield 2002–2007. Our experience

R. Gowda, A. Vedio, M.W. McKendrick (Sheffield, UK)

Objectives: Schistosomiasis is a parasitic infection acquired in the tropics by exposure to freshwater. Chronic infection may result in serious

sequelae such as portal hypertension & bladder cancer. Early diagnosis & therapy is thus crucial.
We describe the epidemiology, clinical features & management of schistosomiasis in Sheffield
Methods: Pharmacy records were used to identify all patients treated with Praziquantel between 2002–2007. Case notes of patients treated for Schistosomiasis were reviewed retrospectively. Best practice guidelines were devised following a literature review and then data on country of infection, appropriate history, clinical features, laboratory data, HIV status and details of treatment were collected.
Results: 90/105 cases treated with praziquantel during the period were for schistosomiasis. A proven diagnosis of schistosomiasis was made in 13 patients by ova detection; others were diagnosed by serology. 69 (77%) cases were migrants and only 19 (23%) were travellers. The majority were from Africa. Only 29 (32%) cases had a full history and only 42 (47%) were investigated fully. Clinical presentation was often subclinical or non-specific. Eosinophilia was present in only 32/87 (37%) cases tested and only 15/32 (47%) of these cases resolved after treatment. 86 (96%) cases were treated with the appropriate doses of praziquantel but only 22 (24%) had a repeat course 6 months later. 18 of 34 (53%) cases of schistosomiasis tested for HIV were positive although 56 cases were untested.
Conclusions: Schistosomiasis is a huge global burden affecting 200 million people. Increasing migration from and travel to endemic areas has lead to an increase in incidence in Sheffield.
In our study, history and investigation was often incomplete with a risk of cases being missed. Eosinophilia was inconsistent and not reliable marker.
Only 24% of cases had the recommended repeat course of praziquantel. Although HIV was common in our patients, our HIV patients are routinely screened for schistosomiasis and thus this may represent a bias. Other study limitations are that diagnosis was usually made by serology and only patients identified by praziquantel treatment are included. Nevertheless, this study raises important issues of screening, awareness of epidemiology and the importance of prompt effective treatment of even subclinical infection. Other issues such as the best method of diagnosis and sensitivity of serology will also be discussed.

O413 Travel-associated enteric fever in England, Wales, and Northern Ireland: results from an enhanced surveillance pilot

J. Lawrence, J. Jones, E.J. Threlfall (London, UK)

Objectives: On average, 178 cases of typhoid and 203 cases of paratyphoid (181 A, 22 B) have been diagnosed in England, Wales, and Northern Ireland (EWNI) each year. Routine laboratory reporting systems only capture two thirds of travel history information, and other information such as reason for travel, ethnicity, country of birth, and vaccine history is not routinely collected. To learn more about enteric fever reported in EWNI, a pilot study of enhanced surveillance of cases began 1 May 2006. The main findings from this pilot after one year of data collection are outlined.
Method: An enhanced questionnaire was completed for each laboratory-confirmed case of enteric fever as part of routine follow up according to United Kingdom (UK) guidelines. The information was collected in a MS Access database and cases known to have travelled abroad from the UK, with onset dates between 1 May 2006 and 30 April 2007, were extracted and analysed using MS Excel.
Results: During the report period, there were 163 cases of paratyphoid A, two cases of paratyphoid B, and 129 cases of typhoid associated with travel abroad. The majority (93%) had travelled to the Indian sub-continent (ISC) (India, Pakistan, or Bangladesh). The highest rate of infection per 100,000 visits was in all cases who had travelled to Bangladesh (29.09 per 100,000 visits), followed by Pakistan (22.31), and India (14.17). The rates increased in those who travelled to visit friends and relatives (VFRs). Of 252 VFRs, 87% (219/252) were of Indian, Pakistani, or Bangladeshi ethnicity, of which 34% were UK born and 51% were non-UK born, the majority of which were born in their country of ethnic origin and had travelled back as VFRs.
Fifty-four cases (12 with typhoid and 42 with paratyphoid A) had received typhoid vaccine within the three years before travel. This varied by ethnicity; of those of Indian, Pakistani, or Bangladeshi ethnicity, 18% received typhoid vaccine compared to 47% of cases of white ethnicity.
Conclusion: The pilot has provided evidence that the majority of typhoid and paratyphoid cases reported in EWNI are in those of Indian, Pakistani, or Bangladeshi ethnicity (either UK born or non-UK born), who have travelled to the ISC as VFRs. Ethnicity may affect whether a traveller is vaccinated against typhoid before travel. Enhanced surveillance of enteric fevers in EWNI promises to be a useful tool for providing an evidence base on which targeted pre-travel health advice can be developed.

O414 Isolation of late-stage *Plasmodium falciparum* infected red blood cells using a new cost- and time-saving magnetic cell separation kit

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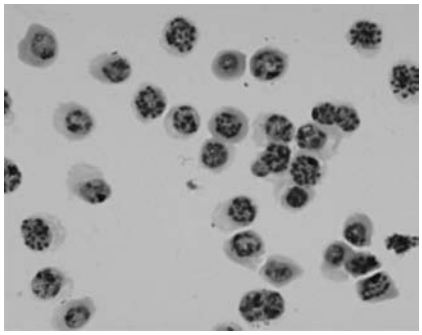
Objectives: In malaria research, a large variety of assays require highly purified infected red blood cells (iRBCs). Magnetic separation relies on intrinsic magnetic properties of trophozoite and schizont (late-stage) iRBCs and offers an alternative to conventional separation by Percoll® gradients. Concentration of late-stage iRBCs by high gradient magnetic separation (HGMS) was first reported in 1981. In the nineties, commercial HGMS columns with a polymer coated matrix became available for high purity separation of malaria-iRBCs. Unfortunately, high costs render their use unattractive for many laboratories. We adapted a new low-cost HGMS kit for isolation of late-stage iRBCs from *Plasmodium falciparum* cultures and compared costs to polymer-coated HGMS columns and separation with Percoll® gradients.
Methods: Isolation of late-stage iRBCs from *P. falciparum* TM 267 cultures was performed in a Skypure HGMS system. 70–500 µl of packed RBCs with late-stage parasitaemias of 1.7–17.2% were used (total parasitaemias 8.5–29.9%). This corresponded to app. $7–50\times10^8$ total RBCs containing $2.6–49.4\times10^7$ late-stage iRBCs. Purification results were analysed by flow cytometer and microscopy. Viability was evaluated by calculation of infection rate after re-culture of isolates.
Results: Purity of iRBC isolates consistently ranged from 93.2% to 99.0% (mean 95.4%). Under optimised conditions, over 90% of isolated iRBCs contained segmented schizonts (Figure 1). Maximum column capacity was found to be 1 to 1.2×10^7 iRBCs. Processing time was less than 45 min. Infection rate ranged from 21.0% to 56.4%. Cost comparison showed 1.5–3 Euros for Percoll® gradient separation and 18–20 Euros for HGMS systems employing polymer-coated columns, compared to 5–6 Euros for the HGMS system tested in this study (cost of consumables per separation of 200 µl packed cells from malaria cultures).
Conclusion: Compared to separation by Percoll® gradient, the new kit is about 30% more time efficient and offers highly consistent results, even for isolation of segmented schizont iRBCs. When compared to polymer coated columns, it offers app. 70% cost savings for consumables. Therefore, the HGMS kit examined is considered highly suitable for purification of late-stage iRBCs.



New treatment options for multidrug-resistant Gram-positive infections (Symposium organised by Astellas)

S415 Dual mode of action of telavancin against *Staphylococcus aureus*

P. Courvalin (Paris, FR)

Glycopeptides, vancomycin and teicoplanin, bind to the C-terminal D-alanyl-D-alanine (D-Ala-D-Ala) of lipid II peptidoglycan precursors and block transglycosylation and transpeptidation reactions. Acquired VanA- and VanB-type resistance in enterococci results from the production of modified precursors ending in D-Ala-D-lactate (D-Lac) to which glycopeptides exhibit low binding affinities, and the elimination of the D-Ala-D-Ala ending precursors synthesised by the host Ddl ligase. VanA-type strains show high-level inducible resistance to both glycopeptides, whereas VanB-type strains have variable levels of inducible resistance to vancomycin only, as teicoplanin is not an inducer. Six VanA-type meticillin resistant *Staphylococcus aureus*, that have acquired the glycopeptide resistance vanA operon from enterococci, have been isolated in North America. In contrast to VanA-type resistant strains, *S. aureus* with intermediate level resistance to vancomycin (GISA) and heterogenous GISA (hGISA) are becoming prevalent worldwide. The mechanism of intermediate resistance in *S. aureus* is not well understood. Telavancin is a lipoglycopeptide that possesses a lipophilic decylaminoethyl sidechain added to the vancosamine sugar of vancomycin; this substituent confers a dual mode of action: 1)Like glycopeptides, telavancin binds to the C-terminus D-Ala-D-Ala of lipid II intermediates in peptidoglycan synthesis. Molecular studies have indicated that telavancin binds with 35-fold enhanced affinity to the membrane-embedded lipid II compared to the soluble target, thus providing an explanation for the enhanced transglycosylation inhibition. 2)Direct interaction of the drug with lipid II leads to disruption of the barrier function of the bacterial membrane. Exposure of *S. aureus* to telavancin results in time- and concentration-dependant loss of membrane potential and increased membrane permeability. Quantitative flow cytometry assays indicate that the majority of *S. aureus* are fully depolarised within 1 hour following exposure to clinically relevant telavancin concentrations. These data provide a mechanistic basis for the potent activity of the drug against GISA/hGISA clinical isolates, observed both in vitro and in vivo. In microorganisms with acquired Van-type glycopeptide resistance, telavancin, like teicoplanin, is not an inducer of the vanB gene cluster. Thus, VanA-type isolates have reduced susceptibility to telavancin whereas VanB-type strains remain fully susceptible.

S416 Telavancin: a novel lipoglycopeptide for treatment of complicated skin and soft tissue infections

S.L. Barriere (South San Francisco, US)

Meticillin-resistant *Staphylococcus aureus* (MRSA) is a common pathogen isolated from complicated skin and soft tissue infections (cSSTIs). Telavancin is an investigational, rapidly bactericidal lipoglycopeptide antibiotic with a multifunctional mechanism of action against Gram-positive bacteria, including MRSA. Two large, international, Phase 3 studies (ATLAS 1 and 2) have compared the efficacy and safety of telavancin (10 mg/kg IV every 24 hours) with vancomycin (1 g IV every 12 hours) in 1,867 patients with cSSTIs. Combined data from ATLAS 1 and 2 have consistently shown that telavancin is non-inferior to vancomycin for the treatment of patients with a cSSTI (clinical cure 88.3% versus 87.1%), including 579 patients with a cSSTI caused by MRSA (clinical cure 90.6% versus 86.4%). Furthermore, telavancin had an acceptable adverse event profile for the treatment of serious infections caused by resistant bacteria.

A subgroup analysis of the ATLAS studies compared clinical and microbiological responses to treatment with telavancin or vancomycin in 194 patients with surgical site cSSTIs. Within this subgroup, telavancin showed numerically superior efficacy when compared with vancomycin (clinical cure 86% versus 71% in MRSA-infected patients), however, these differences did not reach statistical significance.

Geographic differences were noted in the genotype of *S. aureus* isolates from clinically and microbiologically evaluable patients with cSSTIs enrolled in Phase 2 trials. Meticillin-susceptible *S. aureus* (MSSA) isolates from South Africa were significantly more likely to carry certain toxin genes, including pvl, than MSSA isolates from the USA. Of the MRSA isolates in the study, 86% were SCCmec IV and contained the pvl gene. Patients infected with strains containing the pvl gene were significantly more likely to be cured of their cSSTI. In the ATLAS studies, the pvl gene was detected in isolates from 64% (557/870) of patients, with a higher rate of pvl-positivity in patients with MRSA (459/543 [85%]) than MSSA isolates (98/327 [30%]). The outcome of treatment with telavancin was unaffected by pvl status with clinical cure rates similar for telavancin and vancomycin regardless of pvl status.

Telavancin may provide a useful alternative to vancomycin for treatment of patients with cSSTIs, including those caused by MRSA, independent of the expression of toxin genes such as pvl.

S417 Exploring new therapeutic options for hospital-acquired pneumonia

A. Torres (Barcelona, ES)

Hospital-acquired pneumonia (HAP) is the most common healthcare-acquired infection contributing to death. An increasing body of evidence demonstrates that delayed HAP treatment or use of an inappropriate antibiotic dramatically increases mortality. HAP is defined as a respiratory infection developing more than 48 hours after hospital admission. In a proportion of patients, HAP is associated with mechanical ventilation, in which case it is termed ventilator-associated pneumonia (VAP). In patients with VAP there is a 20–55% mortality rate, which increases to >70% if infection is caused by multidrug-resistant pathogens.

Over the past 15 years, the incidence of nosocomial pneumonia due to Gram-positive relative to Gram-negative organisms has increased. *Staphylococcus aureus*, especially meticillin-resistant *Staphylococcus aureus* (MRSA), and penicillin-resistant pneumococci are replacing Gram-negative bacteria as predominant causes of nosocomial pneumonia. Telavancin is a novel, investigational, rapidly bactericidal lipoglycopeptide with a multifunctional mechanism of action against Gram-positive bacteria, including inhibition of cell wall peptidoglycan synthesis and disruption of the functional integrity of the bacterial membrane. Telavancin is rapidly bactericidal against a broad range of clinically relevant Gram-positive bacteria, including MRSA.

Telavancin was recently evaluated in two large Phase 3 studies involving patients with HAP, including patients with VAP, caused by Gram-positive bacteria such as MRSA. These identical, multinational, multicentre, randomised, double-blind studies compared the efficacy and safety of telavancin 10 mg/kg IV every 24 hours with vancomycin 1 g IV every 12 hours (dosing optimisation allowed per individual site guidelines) for 7–21 days. Eligible patients included men and women, aged 18 years or older with HAP caused by suspected or confirmed Gram-positive pathogens. The primary efficacy analysis in each study was non-inferiority of telavancin compared with vancomycin in clinical cure rates in clinically evaluable (CE) patients at the test-of-cure visit, 7 to 14 days after the end of study treatment.

Severe community-acquired pneumonia update: mortality, mechanisms and medical intervention (Symposium organised by Novartis Pharma)

S418 Epidemiology, guidelines, processes of care and clinical experience in severe community-acquired pneumonia

J. Rello (Tarragona, ES)

Mortality in CAP patients is <1% in outpatients, 5% in inpatients and up to 25% in intubated patients. A recent study estimated mortality near 50% in ICU patients requiring inotropes. Site of care assessment based on severity of illness is key, affecting diagnostic workup and empirical antibiotics.

The Pneumonia Severity Index (PSI) can predict patients who can be discharged home safely, but occasionally underestimates severity, particularly in young patients without comorbidities who have severe respiratory failure. The PSI has been useful in documenting outcomes equivalence with empiric antibiotics, whereas delaying appropriate antibiotics worsens survival in classes IV-V pneumococcal bacteremic pneumonia.

CRB-65 is easy to use and identifies patients at high risk of mortality (who might benefit from early ICU admission).

Recently updated IDSA/ATS guidelines for CAP (2007) include both major and minor criteria indicative of the need for ICU admission.

These scores do not generally account for comorbidities, a potential problem in older patients. Biomarkers may complement scores in predicting outcomes.

Severity assessment based on the PIRO concept includes comorbidities (COPD, immunocompromise), age >70years; multilobar opacities; shock, severe hypoxaemia; acute renal failure; bacteraemia and ARDS. PIRO score was obtained at ICU <24 h from admission and 1 point given for each present feature (range 0–8 points). Mean PIRO score was significantly higher in non-survivors than in survivors (4.6 ± 1.2 vs 2.3 ± 1.4). Higher scores were significantly associated with higher mortality, prolonged length of ICU stay, and days of mechanical ventilation (all $p < 0.001$). ROC curves showed PIRO score (AUC=0.88) outperformed APACHE II (AUC=0.75, $p < 0.01$) and ATS/IDSA criteria (AUC=0.80, $p < 0.001$) to predict 28-day mortality. CURB-65 achieved an AUC of only 0.79.

As a predictor of admission to hospital, and HDU or ICU care, it achieved an AUC of 0.88 and 0.64 respectively. We believe this highlights the limitations of CURB-65 as a track and trigger tool. Postponing oxygenation assessment >1h is associated with a significant delay (>6h) in initiating antibiotics. A delay in oxygenation assessment >3h is associated with increased mortality (HR 2.06). Therefore we suggest implementing a simple care bundle to improve management of CAP in the ED, using 3 or 4 evidence-based variables, with immediate pulse oxymetry and O2 assessment being the cornerstone and initial step.

S419 Infection as an initiation of coagulation and inflammation: targeting the lung

T. van der Poll (Amsterdam, NL)

Patients with severe infections almost invariably show evidence of activation of the coagulation system. The lungs are amongst the most frequently affected organs during severe infection and sepsis. The abundant presence of intravascular and extravascular fibrin appears to be a specific hallmark of acute lung injury following sepsis and is much more obvious than the fibrin deposition in other organs. Tissue factor (TF) is regarded as the primary initiator of coagulation in severe infection. Effective blocking of the tissue factor pathway by either recombinant tissue factor pathway inhibitor (rTFPI) or anti-tissue factor antibodies in experimental sepsis attenuates lung injury and partially prevents pulmonary dysfunction. In addition, inhibition of tissue factor activity prevents local activation of coagulation in models of pneumonia. Another mechanism that contributes to fibrin deposition in the lung is the local depression of fibrinolysis, due to the increase of plasminogen activator inhibitor type I. These effects on pulmonary coagulation and fibrinolysis are regulated by various pro-inflammatory cytokines. This lecture will discuss the regulation and impact of pulmonary coagulation during severe infection.

S420 Rationale and the role of biologic adjunctive therapy in sCAP

P.-F. Laterre (Brussels, BE)

Tissue factor pathway inhibitor (TFPI) is an endogenous molecule having both anti-inflammatory and anticoagulant activity. However, TFPI is overwhelmed by increased expression of tissue factor (TF) in sCAP. Tifacogin (recombinant TFPI) replenishes endogenous TFPI and restores haemostasis.

A retrospective analysis of a large, randomised clinical trial of tifacogin in patients with severe sepsis (OPTIMIST) revealed that patients with CAP who had a documented microbial source of infection, who did not receive concurrent heparin, or the combination of both, had lower mortality compared with patients receiving placebo, even though no significant effect was seen in the overall severe sepsis population (Laterre et al, Critical Care, submitted). These results suggest that tifacogin may reduce mortality in patients with sCAP who did not require heparin.

This potential benefit of tifacogin is currently being tested in the Phase III CAPTIVATE study, in approximately 2100 patients. CAPTIVATE will compare current sCAP standard of care with a combination of sCAP standard of care plus adjunctive tifacogin, (either 0.025 mg/kg/hr or 0.075 mg/kg/hr in a 96 hour continuous infusion). The study objective is to determine whether treatment with tifacogin early in the disease process can reduce incidence of mortality by preventing the development of disseminated intravascular coagulation and multiple organ system failure. Study entry criteria were based on the 2007 IDSA / ATS guidelines plus a clinical diagnosis of community-acquired pneumonia. Specifically required are the presence of either 1 major criterion (requirement for mechanical ventilation, or need for therapeutic vasopressors), or 2 or more minor criteria (hypotension despite adequate fluid resuscitation, PaO_2/FiO_2 ratio <250, respiratory rate $\geqslant$30/min, non-invasive ventilatory support, BUN > 7mM, new onset confusion, multilobar pneumonia, platelet count <100,000 cells/mm^3 or a fall of >25% in previous 48 hours to <120,000, leukopenia or hypothermia).

The primary endpoint of the study is 28 day all cause mortality. Secondary endpoints include treatment failure, need for mechanical ventilation, incidence of disseminated intravascular coagulation, duration of ICU admission, and length of hospitalisation. If an overall treatment benefit is observed with tifacogin, then pharmacoeconomic analysis will also be performed. Survival data will also be collected at 90 days, 6 months, and 1 year to assess long-term safety.

S421 The role of biomarkers – predicting patients at risk of decompensation and responders to adjunctive therapy

J.-P. Mira (Paris, FR)

Patients with severe sepsis show substantial abnormalities in several biomarkers of inflammation and coagulation. These abnormalities have been inconsistently correlated with syndrome severity, because of both a major heterogeneity in the sources of infections (lung, abdomen, urine, ...) and variability in causative microorganisms. Community-acquired pneumonia (CAP) is the most common cause of community-acquired severe sepsis in ICU, and represents a well characterised disease with protocolised treatments. Hence, analysis of biomarkers in this homogeneous population may be important to define disease severity and candidates for new therapeutic approaches.

Subgroup analysis of results from the OPTIMIST study of tifacogin in severe sepsis patients revealed a decrease in mortality in sCAP patients treated with tifacogin who had a documented microbiological infection and who were not concurrently treated with heparin. A trend toward

reduced mortality was also seen in sCAP patients with procalcitonin $\geqslant$ 2 ng/mL (Laterre et al, Critical Care, submitted) and in the overall severe sepsis population with evidence of active coagulation (PF $\geqslant$ 3nM) at baseline (Opal et al. IDSA 2004) who were treated with tifacogin. These results warranted further investigation, and led to the initiation of the CAPTIVATE study of tifacogin in 2100 patients with sCAP. Moreover, resolution of the human genome has led to the discovery of new genomics biomarkers associated with sCAP mortality.
It is hoped that by investigating both genomics markers and "classic" biomarker level changes over the clinical course of the disease, that it will be possible in the future to predict which patients are at potential risk of decompensation, and which patients are most likely to benefit from early treatment such as tifacogin.

Oropharyngeal candidiasis: impact of new treatment approaches on patient outcome (Symposium organised by SpeBio)

S425 Oropharyngeal candidiasis in immunocompromised patients: which burden? Importance of appropriate antimicrobial treatment strategy

E. Bouza (Madrid, ES)

Oropharyngeal candidiasis (OPC) displays a prevalence of up to 70% in oncology patients and 20% in HIV infected (HIV) patients. Although the prevalence of OPC in HIV patients is decreasing, OPC still occurs in all subjects who are not treated with HAART, develop AIDS or are in therapeutic failure. In patients with oncology/haematological diseases, the prevalence of OPC is conversely increasing, depending on the type of cancer and anticancer treatments (chemotherapy, radiotherapy, ...). *Candida albicans* is the main species associated with OPC but non-*albicans* are increasing, while mutiresistant *Candida albicans* and non-*albicans* strains have emerged. These changes are a consequence of long term prescriptions of narrow spectrum antifungal agents and repeated prescriptions of oral systemic antifungal agents. Moreover, in this heavily treated immunocompromised population, it is important to limit the potential for drug-drug interactions. Therefore, national and international guidelines have been issued and recommend using a local antifungal therapy with a spectrum extended to all *Candida* species as first line treatment of OPC. In clinical practice, the treatment compliance of the immunocompromised patients represents a challenge and should be favoured by a local antifungal agent easy to use.

S426 Oropharyngeal candidiasis: what to do to face resistance of *Candida* species?

M.A. Ghannoum (Cleveland, US)

The widespread and recurrent use of oral systemic antifungal agents for the treatment of candidiasis in immunocompromised patients had led to the emergence of resistance of *Candida* either *albicans* or non-*albicans* to some antifungals. Miconazole (MICON) has long been used for the topical treatment of oropharyngeal candidiasis (OPC), so that it was important to generate data on recent clinical isolates and using the recent CLSI methodology.
In a first study, we established the susceptibility profile for MICON against recent *Candida* isolates and compared its antifungal activity to that of other antifungals. In a subsequent study, we determined the potential of resistance development to MICON. Minimum Inhibitory Concentrations (MICs) were determined for amphotericin B, caspofungin, clotrimazole, fluconazole (FLU), itraconazole, nystatin, voriconazole and MICON against 25 strains of 6 species, including *C. albicans*, *C. krusei*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis* and *C. dubliniensis*. MICON demonstrated potent inhibitory activity against all of the *Candida* spp. strains tested. The overall MIC range for MICON was 0.004–1.0 μg/ml, while MICON MIC90 (0.5 μg/ml) and MIC50 (0.03 μg/ml) for all species were comparable to that of other antifungal agents except for FLU to which they were 4 dilutions lower (Minimum inhibitory concentration that inhibits 50 and 90% of the strains tested respectively). No strain obtained from recent clinical isolates were resistant to MICON among any of the species tested, and importantly, MICON demonstrated overall MIC range 0.06–1 μg/ml against strains showing elevated MICs for FLU (8–64 μg/ml).
To date, resistance to MICON has not been reported. The objective of our second study was to determine whether repeated exposure of *Candida* spp. to MICON leads to resistance against this antifungal. Two clinical *Candida* isolates from oral cavity were selected and included one FLU-resistant and one FLU-susceptible strain each of: *C. albicans*, *C. glabrata*, and *C. tropicalis*. The initial MIC of MICON against each isolate was determined before a repeated exposure to different concentrations of MICON (from 0.5x MIC to 4x MIC) for a total of 15 passages. In general, there was no increase in MICs with the highest final MIC equal to 0.5 μg/ml.
Our data showed that MICON demonstrated a potent inhibitory activity against all of the recent *C. albicans* or non-*albicans* isolates tested, including those with known FLU resistance. These data suggest that MICON could have utility as a first line treatment of OPC.

S427 A new targeted and innovative treatment of oropharyngeal candidiasis in immunocompromised patients

P. Attali (Paris, FR)

Despite the recommendations of international guidelines, topical antifungal agents are hardly used in the treatment of oropharyngeal candidiasis (OPC) in immunocompromised (IC) patients because of the need for 4 to 6 daily dosing, poor taste acceptance that compromise patient compliance and lessen efficacy.
The pharmacokinetic of once daily miconazole 50mg mucoadhesive buccal tablet (MBT) (Loramyc®) was compared to that of miconazole 375mg gel administered in three divided doses in 18 healthy volunteers. Salivary miconazole concentrations after MBT application were higher (15.1±16.2mg/ml) than those of miconazole gel (1.6± 1.6mg/ml, $p < 0.001$) and persisted over the MIC (1 μg/ml) for 13.4±5.2 hours vs 1.2±2.6h ($p < 0.001$). Miconazole plasma concentrations were rarely detected with MBT (5/162 samples vs 10/162 with gel). Finally, 17 of 18 volunteers preferred miconazole MBT.
Twenty five HIV+ patients with documented OPC were enrolled in a group-sequential dose-adaptative trial with stopping rules and treated with MBT once daily for 14 days. The trial was halted prematurely according to the predefined stopping rules because of higher than expected efficacy. Clinical success was observed in 84% of patients with 52% complete response. Clinical cure was obtained in 52%. Relapses within 30 days post treatment (32%) only occurred in untreated or uncontrolled HIV+ patients. Plasma miconazole concentrations were not detected.
Single daily dose of miconazole 50mg MBT or miconazole 500mg oral gel (MOG) administered in 4 divided doses for 14 days were randomly allocated to 282 head and neck cancer patients with documented OPC. The success rate was statistically not inferior in the MBT group to that observed in the MOG group (56% vs 49%) with complete response in 52.5% vs 45.4% of patients respectively. After adjustment for the extent of lesions and salivary secretions because of an uneven distribution of patients at baseline in favour of MOG, a trend toward superiority was observed in favour of MBT ($p = 0.13$), particularly among patients with multiple lesions ($p = 0.013$). Clinical cure was obtained in 39%. Relapses at 45 days post treatment were observed in around 20% (p=NS).
In both trials MBT adhered for more than 6 hours in more than 90% of applications. MBT was well tolerated.
These trials demonstrated that once daily miconazole MBT is an efficacious, safe and well accepted topical treatment of OPC in IC patients. The reduction in drug dose precludes the risk of systemic exposure, of drug-drug interactions and liver toxicity.

S428 Challenge of oropharyngeal candidiasis in immunocompromised patients: the need for an optimised treatment

O.A. Cornely (Cologne, DE)

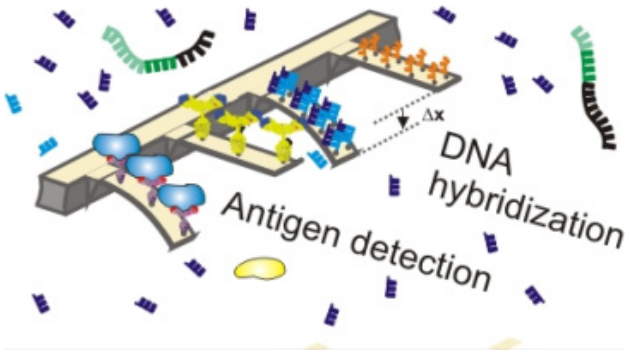
Occurrence of oropharyngeal candidiasis in immunocompromised patients carries a high burden, not only in terms of patient discomfort. Since mucosal colonisation is a risk factor for systemic dissemination, oropharyngeal candidiasis implies an early treatment initiation.
Systemically active antifungals carry the challenge of drug-drug interactions as well as the potential for a variety of organ toxicities. Although topical treatment is clearly preferable for localised superficial infections, systemically active antifungals are used frequently. Most likely these will be azoles.
Different patient groups will benefit from additional topical treatment options for oropharyngeal candidiasis. These are in particular patients with HIV/AIDS and patients with neoplastic disease. In HIV infected subjects receiving multiple concomitant medications and suffering from oropharyngeal candidiasis, the risk of interaction of systemic antifungal treatments with HAART needs to be minimised and thus guidelines do recommend first line local antifungal therapy.
The incidence rates of oropharyngeal candidiasis vary within the highly complex cancer patient population and depend on the type of tumour, treatment modalities and cytostatics used. Consequences of oropharyngeal candidiasis may be: decreased food and sometimes even liquid intake, loss of weight, and reduced general state and quality of life. Potentially subsequent decreased chemotherapy doses and increased intervals between cycles may have a negative impact on therapeutic success.
For both groups of immunocompromised patients, it is important to detect and treat oropharyngeal candidiasis from the start. The availability of a new local antifungal agent with a limited systemic exposure and a once daily dosing favouring patients' compliance should optimise the management of oropharyngeal candidiasis in immunocompromised patients.

Innovative diagnostics

S437 Microcantilever biosensors and diagnostics

H.P. Lang (Basel, CH)

We have established a new type of biological sensor based on microfabricated silicon cantilevers arrays. Each cantilever is 500 micron long, 100 micron wide and less than 1 micron thick. They are miniaturised, ultrasensitive and fast-responding sensors for application in chemistry, physics, biochemistry and medicine and respond by bending due to absorption of molecules or by shift in resonance frequency. They can be operated in different environments such as gaseous environment or liquids. When operated in liquid, microcantilever sensors are able to detect biochemical reactions. Each cantilever is functionalised with a specific biochemical probe receptor, sensitive for detection of the corresponding target molecule. Applications lie in the fields of label- and amplification-free detection of DNA hybridisation, detection of proteins as well as antigen-antibody reactions and detection of larger entities, such as bacteria and fungi. We present detection of fungal growth within two hours using resonating microcantilevers coated with nutrition media.
When operated in gaseous environment, polymer-coated cantilever array sensors can be applied as kind of electronic nose for characterisation of vapors. Medical application fields include fast characterisation of exhaled patient's breath samples for detection of diseases, based on the presence of vapors such as acetone in exhaled air, as characteristic of diabetes II patients. Similarly, patients suffering from uraemia can be identified by detection of dimethylamine in exhaled air. Further examples include characterisation of patients with respiratory tract infections.



Key publications:
– J. Zhang et al., "Rapid and label-free nanomechanical detection of biomarker transcripts in human RNA", Nature Nanotechnology 1, 214–220 (2006).
– N. Backmann et al., "A label-free immunosensor array using single-chain antibody fragments", Proc. Nat. Acad. Sci. USA 102, 14587–14592 (2005).
– R. McKendry et al., "Multiple label-free biodetection and quantitative DNA-binding assays on a nanomechanical cantilever array", Proc. Nat. Acad. Sci. USA 99, 9783–9787 (2002).
– J. Fritz et al., "Translating Biomolecular Recognition into Nanomechanics", Science 288, 316–318 (2000).
– H.P. Lang et al., "An Artificial Nose Based on a Micromechanical Cantilever Array", Analytica Chimica Acta, 393, 59 (1999).
– H.P. Lang et al., "An Artificial Nose Based on Microcantilever Array Sensors", Journal of Physics: Conference Series 61, 663–667 (2007).

S440 From lab on a chip to a lab-in-a-cell: the impact of nanotechnology on biomedicine

A. van den Berg (Enschede, NL)

Over the past 15 years, the concept of Lab on a Chip (LOC) has been developed to a great level of maturity. These microfluidics systems are nowadays employed in a variety of applications in physics, analytical chemistry, medical applications and recently more and more in biological ones. We will show that there is an increasing interest in experiments with single cells, and it will be shown that there are great opportunities for doing so.
In this presentation, first an example will be given of a LOC for the analysis of lithium, a drug frequently used by manic-depressive patients, in whole blood [1]. Subsequently, we will present a sensor system for the monitoring of cultivation parameters such as temperature, oxygen content, pH and cell growth. This system is intended to be used for monitoring of micro-cell cultures, and is compatible with the standard 96 well-plate format [2]. An another example, a million-chamber Petri-dish will be shown for cultivation of bacterial cultures [3].
In a following example, a microfluidic cell trap enabling time-lapse experiments with U937 cells undergoing apoptosis studied with confocal microscopy [4]. The use of chemically modified Qdots is shown for photo-stable cell-imaging of the same apoptotic process [5]. This is particularly important if long time-lapse experiments are carried out, that would normally suffer from bleaching of conventional fluorescent probes.
Finally, a cell-trap chip is shown for gene transfection into individual cells using electroporation. More specifically, the transfection of the GFP-ERK1 construct and subsequent translocation from the cytosol to the nucleus of human mesenchymal stem cells under influence of external bFGF signals is demonstrated [6]. The last example demonstrates that there is an important future role for experimentation with single cells on a chip, or using the cell as experimentation space in a new paradigm: the Lab-in-a-Cell.

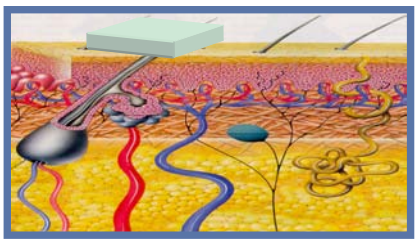


Fig. 1. Schematic presentation of microneedle array sampling blood for lithium analysis.

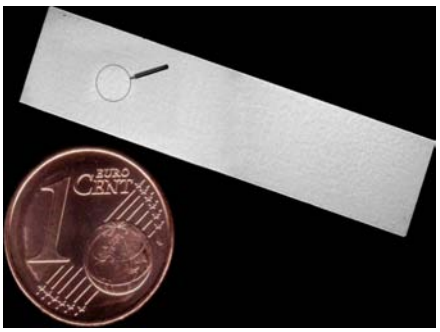


Fig. 2. Picture of the anapore substrate, subdivided in > 1 million, 7x7 um cultivation chambers.

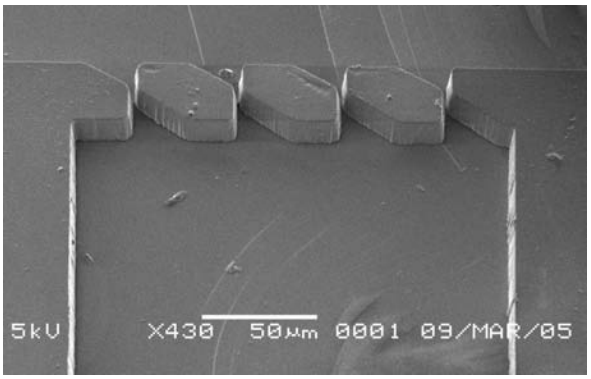


Fig. 3. Microfabricated cell trap for time-lapse analysis of apotosis induced in suspension cells.

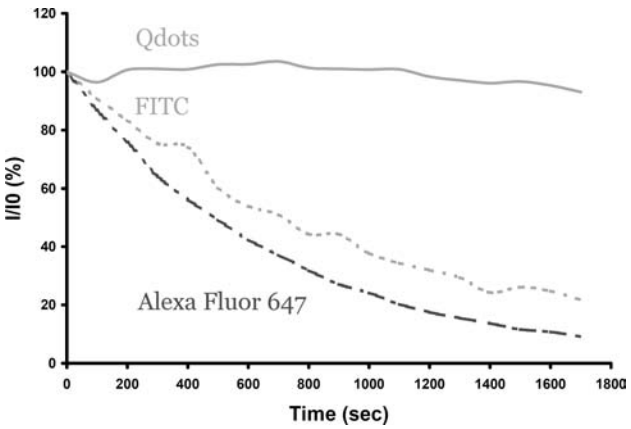


Fig. 4. Intensity vs. time curves for Qdot probes (orange) and two fluorescence probes.

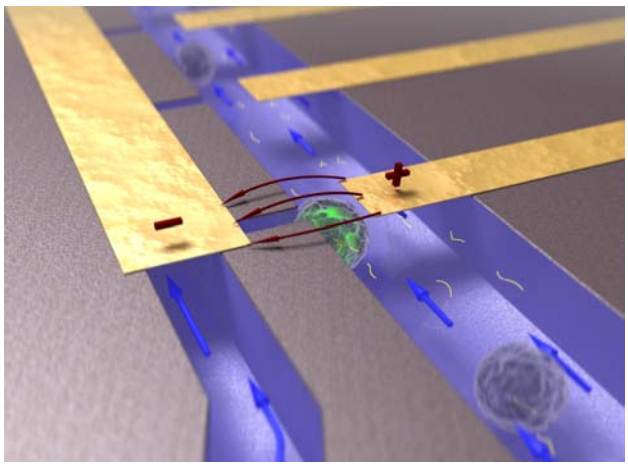


Fig.5. Artists' impression of single cell electroporation setup.

Reference(s)

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[5] S. Le Gac et al., Nano Letters, 6(9), (2006), 1863–1869.
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Unforgettable trips and unwanted souvenirs: issues in travel medicine

S441 Infections and other problems on ferries and cruise ships

E. Dahl (Bergen, NO)

Passenger ships are isolated communities with a high population density, crowded public rooms and living accommodation, shared sanitary facilities, and common water and food supplies. Hence, infectious diseases are easily transmitted aboard. Outbreaks of measles, rubella, varicella, meningococcal meningitis, hepatitis A, Legionnaires' disease, influenza and gastroenteritis among passengers and crew have been reported and are feared both for their health and publicity reasons. However, consequences are usually less serious for ferries, carrying people between two close ports, than for cruise ships. Vessels doing cruises that last weeks to months often visit remote areas with varying sanitation standards, and evacuation may not be possible for days. While ferries often carry no medical personnel, cruise ships as a rule have 1–2 physicians and 1–3 nurses.

Cruise companies emphasize illness prevention through pre-employment medical screening and vaccination programmes for their crew, as well as detailed and rigid ship sanitation procedures. Over one hundred outbreaks of infectious diseases, particularly gastrointestinal disease, were reported to be associated with ships between 1970 and 2000. Despite good performance on health inspections, outbreaks of gastroenteritis per 1000 cruises increased almost tenfold from 2001 to 2004. The increase was mostly attributable to norovirus, and highlights the inability of environmental programmes to fully predict and prevent risk factors common to person-to-person and fomite spread of disease. The much publicised outbreaks and global threats have led the cruise companies to cooperate closely with local, national and international public health authorities – and with each other. These efforts have resulted in detailed protocols on how to deal with every aspect of shipboard sanitation: under normal circumstances, when there is a threat of an outbreak, and during an outbreak. They include mandatory hand disinfection for all persons entering the ship, and strict isolation demands for all cases of gastroenteritis for a minimum of 24 hours after the last

symptom. Isolation aboard is difficult and risky. A bedside test to quickly diagnose or rule out norovirus is therefore high on the cruise industry's wish list.
As it is almost impossible for ships to comply with different requirements in each port, it is a pressing international challenge for all port countries to agree on a uniform vessel sanitation programme for passenger ships.

S442 Sex, sun, sea and sexually transmitted infections

K.E. Rogstad (Sheffield, UK)

Holidays provide an ideal opportunity for increased sexual contact, whether taken at home or abroad. Travellers are released form the normal social restraints of their own community and are more likely to drink alcohol and take illegal drugs whilst away.
Travel advice usually includes information on prevention and prophylaxis for traditional infectious diseases but rarely addresses the risks of planned or unplanned sexual activity whilst abroad. As well as being at risk of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections, travellers are also at risk of HIV, Hepatitis B, syphilis and the tropical sexually transmitted diseases (STDs) such as chancroid, granuloma inguinale and lymphogranuloma venereum. The need for advice prior to travel, prophylaxis and access to care on their return according to risk and symptoms should be part of the routine consultation in travel clinics. This is particularly relevant to those who may be sex tourists, who may also require additional information on potential legal issues.

S443 Fever after travel: what are the causes?

T. Jelinek (Berlin, DE)

Selected diagnosis and individual national surveillance systems apart, quantity and quality of imported infectious diseases in Europe are largely unknown. Experience of the last decades has shown that their impact is increasing on European health systems and, indeed, societies due to hugely increased travel activities and immigration. Current estimates assume that in Germany alone, 300,000 patients fall sick every year after a journey outside of Europe. In addition, health systems have to cope with an unknown number of migrants that need treatment for a variety of conditions. Overall, information about relevant infectious diseases in endemic areas is comparatively easy to get. Information about the same diseases in returning travellers are much more difficult to acquire. Only few international studies have been done in regards of this question. As a result, only limited data are available on imported infectious diseases in Europe, especially those that are not notifiable in all countries. TropNetEurop, the network on the surveillance of imported infectious diseases in Europe, has been founded in 1999. The network is trying to foster collaboration of clinicians throughout the continent in order to increase information exchange and knowledge about imported infections. Selected diagnosis are reported regularly in anonymised form. Several outbreaks among travellers and immigrants have been detected since the foundation of the network (www.tropnet.net).
Fever is the most important clinical sign of imported infectious diseases post travel. Common causes include life threatening diseases like malaria and less dangerous, but quite common diagnoses as dengue fever. The presentation gives an overview of epidemiological and clinical data. The most frequent causes of fever post travel are disucussed.

S444 Travellers' diarrhoea: vaccination, chemoprophylaxis or treatment

R. Steffen (Zurich, CH)

Three fundamental options exist to prevent Travellers' diarrhoea (TD): reduction of exposure, chemoprophylaxis, and immunisation.
According to a recent review evidence is missing that avoidance of 'dangerous' food and beverages reduces the risk of TD.
Among the many drugs suggeted for chemoprophylaxis, probiotics showed no or at best a low protective efficacy rate. Bismuth subsalicylate is modestly effective. Among antibacterial agents, trimethoprim-sulfamethoxazole, doxycycline and others are considered obsolete because of antimicrobial resistance by prevalent enteric bacterial pathogens. Antimicrobial resistance has also become an increasing problem with widespread use of fluoroquinolones and fear of systemic reactions has limited the prescription of such medication. Poorly absorbed antibiotics, mainly rifaximin, are far more attractive, but this agent is not available in most European countries.
The whole cell/B-subunit (WC/BS) cholera vaccine has repeatedly been shown to prevent TD by LT-ETEC strains, possibly even by other pathogens. Similarly a new heat labile LT-ETEC transcutaneous patch vaccine has been effective beyond just LT-ETEC. If such vaccines are recommended, it is paramount to underline that the protective efficacy is far from complete and that TD may still occur en route.
As no current prophylactic measure is truly satisfactory, (self)-therapy of TD remains an important strategic option against TD. Quinolones seem still to be effective in most parts of the world, but there is a lack of recent data on the frequency of resistance from analysis of TD stool samples on all continents. There are indications that azithromycin is the drug of choice in SE-Asia. Often a single antimicrobial dose will be sufficient to cure TD. With increasing concerns about post-infectious irritable bowel syndrome we should consider a more liberal approach with respect to inclusion of antimicrobials in the travel kit.

Pros and cons of glycopeptides

S447 Vancomycin is not obsolete

G. Eliopoulos (Boston, US)

Vancomycin is an imperfect agent, like every other antibiotic. Nevertheless, each day it is used successfully in hospitals, nursing facilities and homes, as the first line agent for treatment of infections due to meticillin-resistant *Staphylococcus aureus* and for infections caused by a broad array of other Gram-positive bacteria in individuals allergic to or otherwise intolerant of β-lactam antibiotics. This enormously successful clinical experience comes in spite of its widespread use for non-approved indications, based on breakpoints that antedate the discovery of isolates with reduced susceptibility, without detailed appreciation of its tissue penetration, and sometimes under circumstances of suboptimal ancillary intervention (drainage, debridement or removal of foreign material). Most patients tolerate vancomycin well; not surprisingly, adverse effects do appear to be more frequent as higher than usual doses are utilised against infections due to organisms at the high end of the MIC distribution. Randomised, double-blind prospective clinical trials have largely failed to identify newer agents that are superior in clinical efficacy to vancomycin, despite tantalising signals of possible advantages of other agents against MRSA. Which, if any, potential advantages would support the anticipated differential in cost of the newer agents remains to be determined. With time, better antibiotics than vancomycin will be introduced, and they will assume their rightful place in therapy. However, as long as we do not expect vancomycin to achieve the impossible – to treat infections due to non-susceptible organisms at sites where it does not penetrate – this antibiotic will remain an attractive therapeutic option.

Fungal infections

O449 Fungal biomass is a key factor affecting polymorphonuclear leukocyte-induced hyphal damage of filamentous fungi

C. Antachopoulos, J. Demchok, E. Roilides, T. Walsh (Bethesda, US; Thessaloniki, GR)

Objectives: Previous studies of polymorphonuclear leukocyte (PMN)-induced hyphal damage (HD) of filamentous fungi have used the effector-target (E:T) ratio as the only variable for assessment or comparison of results. However, we hypothesised that the overall hyphal biomass of organism may also be an important determinant in host-pathogen

interaction. We therefore investigated the effect of fungal biomass on PMN-induced HD.

Methods: An inoculum of 2×10^4 conidia/ml of one isolate each of *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus terreus*, *Rhizopus oryzae*, *Rhizopus microsporus*, *Cunninghamella bertholletiae*, Scedosporium prolificans and *Fusarium solani* was incubated for 6 one- to two-hourly different time periods in order to yield biomass values ranging between 0.01–0.1 optical density (OD, measured at 405 nm). PMNs from healthy volunteers were then added at E:T ratios of 5:1, 10:1, 20:1, 50:1 and 100:1, and HD was assessed by XTT metabolic assay. For each E:T ratio, the relationship between HD and OD was studied using nonlinear regression analysis.

Results: HD decreased with increasing biomass following a sigmoid pattern described with the Emax model (median R^2: 0.87). HD at 0.01 OD exceeded HD at 0.1 OD ($P < 0.01$ for all E:T ratios) by >2-fold in 64 of 80 comparisons (8 isolates x 5 E:T ratios x 2 replicate experiments). The sigmoid curves were shifted to the right with higher E:T ratios; the EC50 values (i.e., the model-derived OD at which HD is reduced half-way between the maximum and minimum HD values for a given E:T ratio and isolate) obtained for 50:1 or 100:1 were higher than those obtained for 5:1 ($P < 0.01$). Using the same E:T ratio, interspecies differences were observed; for 5:1, lower EC50 values were obtained for *A. flavus* and the three zygomycete species.

Conclusion: PMN-induced HD decreases with increasing biomass at rates that are species-dependent and that decrease with higher E:T ratios. This biomass effect may have important implications for understanding systemic host defences and can be a useful factor in the design and interpretation of studies of PMN-induced HD of filamentous fungi.

O450 Development of a nested PCR assay for the detection of *Fusarium solani* DNA and its evaluation in the diagnosis of invasive fusariosis using an experimental mouse model

Z. Khan, S. Ahmad, A. Theyyathel (Kuwait, KW)

Objective: The *Fusarium* species are common environmental fungi that enter the body through the lungs or through a cutaneous source and disseminate through the bloodstream in immunosuppressed/immunocompromised patients. Fusariosis is now the second most common mould infection in immunocompromised patients and *Fusarium solani* accounts for nearly half of these infections. The aim of this study was to develop a specific nested PCR (nPCR) assay for the detection of *F. solani* DNA in culture and in clinical specimens.

Methods: The nPCR assay was developed by using genomic DNA isolated from *Fusarium* species and from other common pathogenic and environmental fungi. The nPCR assay was evaluated by using DNA isolated from bronchoalveolar lavage (BAL) and serum samples from mice infected intravenously with *F. solani* conidia and sacrificed on day 1 and then on every third day up to 25 days post-infection. The lung homogenate, BAL and blood samples were also cultured for *F. solani*.

Results: The nPCR assay was specific for *F. solani* and the lower limit of detection was 450 fg of template DNA corresponding roughly to 11 *F. solani* cells. Cultures of lung homogenate of infected animals up to day 16 yielded *F. solani* with decreasing fungal load and were negative thereafter. The nPCR positivity in BAL was 100% concordant with culture results. Although detection of *F. solani* DNA in serum was less sensitive than in BAL, it could be detected for longer duration, i.e. up to 22 days.

Conclusions: We have developed a sensitive and specific nPCR assay for the detection of *F. solani* DNA. Our data from experimental mouse model show that detection of DNA in BAL and to a lesser extent in serum by nPCR offers a sensitive and specific diagnostic approach for invasive *F. solani* infection.

Supported by KURA grant MI 04/02.

O451 Detection of *Aspergillus* DNA by a nested PCR assay with high sensitivity and specificity rates is able to improve the diagnosis of invasive aspergillosis in paediatric patients

M. Hummel, B. Spiess, J. Roder, G. von Komorowski, M. Duerken, K. Kentouche, H. Laws, H. Moerz, D. Buchheidt (Mannheim, Jena, Dusseldorf, DE)

Background. Fungal infections are a leading cause of morbidity and mortality in severely immunocompromised patients with an increasing incidence in recent years. IA is the most common filamentous fungal infection and is, in adults as well as in children, difficult to diagnose. To improve the outcome of patients with invasive aspergillosis (IA), early diagnosis and treatment initiation is crucial.

Several PCR assays to detect *Aspergillus* DNA have been established, but so far, studies on molecular tools for the diagnosis of IA in children are few.

We evaluated the results of a nested PCR assay to detect *Aspergillus* DNA in clinical samples from paediatric and adolescent patients with suspected IA.

Methods. Blood and non-blood samples from immunocompromised paediatric and adolescent patients with suspected invasive fungal infection were sent for processing *Aspergillus* polymerase chain reaction (PCR) to our laboratory and investigated with our previously described nested PCR assay. PCR results from consecutive patients from three university children hospitals investigated between November 2000 and January 2007 were evaluated. Fungal infections were classified according to the EORTC classification on the grounds of clinical findings, microbiology and radio-imaging results.

Results. 291 samples from 71 patients were investigated for the presence of *Aspergillus* DNA by our previously described nested PCR assay. Two, 3 and 34 patients had proven, probable and possible IA, respectively. *Aspergillus* DNA was detected in blood, cerebrospinal fluid (CSF) and in bronchoalveolar lavage (BAL) samples. Sensitivity and specificity rates of the PCR assay were 80 and 81%, respectively

Conclusion. Our nested PCR assay is able to detect *Aspergillus* DNA in blood, CSF and BAL samples from paediatric and adolescent patients with IA with high sensitivity and specificity rates. PCR for *Aspergillus* DNA contributes essentially to improve the diagnosis of IA.

O452 Surveillance of aspergillosis by galactomannan testing, culture and histopathology in lung transplant recipients

M.C. Arendrup, K.L. Mortensen, M. Iversen, N. Milman (Copenhagen, DK)

Background: The clinical utility of *Aspergillus* galactomannan detection (GM) in bronchoalveolar lavage (BAL) fluid for the diagnosis of aspergillosis in lung transplant recipients has recently been described.

Methods: 68 paired BAL fluid and serum samples from consecutive lung transplant recipients undergoing routine bronchoscopy were prospectively analysed for GM and results compared with culture, imaging and histopathology. Underlying diseases were chronic obstructive pulmonary disease in 24 patients, cystic fibrosis in 19, sarcoidosis in 10, alpha-1-antitrypsin deficiency in 8, pulmonal fibrosis in 8, and others in 2 patients. Patients were classified according to clinical findings during the bronchoscopy as having high risk for aspergillosis (patches involving the site of anastomosis and the airways of the lung), medium risk (patches restricted to the site of anastomosis) or low risk (no or minor patches at the site of anastomosis).

Results: A total of 68 BAL fluids from 51 recipients of single (37) or double (31) lung tranplants were investigated. 12 patients were classified as high risk patients (18%), 17 as medium (25%) and 39 as low risk patients for aspergillosis (57%). In 22/68 cases (32%) the patient received antifungal prophylaxis, which in 21/22 cases was voriconazole and in 1 case caspofungin.

The GM index in BAL fluid was >0.5 in 5/68 cases (2/39 low risk (5%), 2/17 medium risk (12%) and 1/12 high risk (8%) cases). 4 of these 5 patients were recipients of double lung transplants. In 2 cases the GM

index was >0.7 (1 medium (6%) and 1 high risk patients (8%), both double lung transplant recipients) and in 1 >1.0 (high risk episode in double lung transplant recipient). No patients had positive GM in serum at any time. Six patients had a positive BAL fluid culture for aspergillus, none of whom had positive GM and 4 of whom were classified as low risk patients. Five patients had histopathological findings suggestive for aspergillus 4 of whom were classified as high risk patients. The BAL GM index in these patients was 1, 0.4, 0.3, 0.3 and 0.1 and none had positive cultures from the BAL fluid.

Conclusions: In the setting of lung transplant recipient with a frequent use of anti-aspergillus prophylaxis, BAL fluid GM appeared to correlate better with the clinical risk classification than culture, but not as good as histopathology. BAL fluid GM testing can not be regarded as a stand alone test in this setting.

O453 Fungiscope – the Global Rare Fungal Infection Registry

M.J. Rueping, J.J. Vehreschild, C. Beisel, U. Auerbach, C. Mueller, C. Wickenhauser, O. Cornely (Cologne, DE)

Introduction: The incidence of invasive fungal infections increases worldwide, and rare fungi – neither belonging to the genera *Aspergillus*, *Candida*, *Pneumocystis* or *Cryptococcus*, nor being endemic, such as *Histoplasma* spp. or *Coccidioides* spp. – are increasingly identified as causative pathogens.

Methods: We are coordinating a global registry for cases of rare invasive fungi. Our objective is to broaden the knowledge on epidemiology, to determine the clinical pattern of disease, to describe and improve diagnostic procedures and therapeutic regimens, as well as to facilitate exchange of clinical isolates among the contributors.

Entry of retrospective data occurs via a web-based registration system (MACRO) that focuses on demographic information, underlying diseases, risk factors, details on the infection (pathogen, localisation, specimen collection) therapy and outcome.

Inclusion criteria include cultural, histopathological, antigen, or DNA evidence of invasive fungal infection. Infection due to *Aspergillus* spp., *Candida* spp., *Cryptococcus neoformans*, *Pneumocystis jirovecii* or any endemic fungal infection, such as coccidioidomycosis or histoplasmosis, as well as mere colonisation or other non-invasive infection are exclusion criteria.

Each subset of this cohort will be published at a time. Authorship will be restricted to those centres, contributing patients or translational work to the subset.

For evaluable patient documentations filled in by the participating centre a compensation of € 100 each will be paid. For isolates made available to the central laboratory an additional € 50 will be paid.

Results: By now, 28 cases of rare invasive fungal infections have been identified, including *Absidia corymbifera*, *Cunninghamella bertholletiae*, *Penicillium marneffei*, *Rhizomucor pusillus*, as well as *Acremonium* spp., *Fusarium* spp., *Coccidioides* spp., and *Trichoderma* spp. Clinical results are partly pending. Most patients were in an immunocompromised state as a result of their underlying disease, chemotherapy or transplantation.

Discussion: The clinical relevance of invasive fungal infections by rare fungi is increasing steadily. In a short period of time, actual cases from Germany, Austria, Italy and the United Kingdom could be documented, showing the broad spectrum of pathogens.

Further investigators and coordinators are cordially invited to contribute to the success of Fungiscope.

O454 Candidiasis in patients with haematological malignancies, in a tertiary care cancer centre (2001–2007): stable incidence but changing epidemiology of a still frequently lethal infection

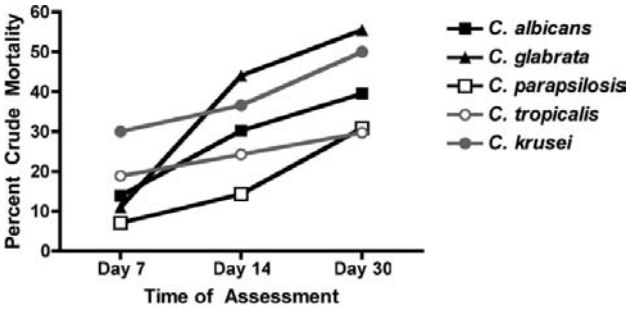
N.V. Sipsas, R.E. Lewis, D.P. Kontoyiannis (Houston, US)

Objective: In the era of ever expanding anti-*Candida* armamentarium, there has not been recent large series on the incidence, microbiology, resistance patterns of candidiasis in high risk patients with hematologic malignancy and or stem cell transplantation.

Methods: We retrospectively reviewed the records of adult patients with candidaemia and or candidiasis (EORTC criteria), treated for hematological malignancies (March 2001-February 2007). Demographic characteristics, clinical and microbiological data, outcomes, as well as MIC of antifungal agents (CLSI method), were recorded.

Results: A total of 173 episodes of candidaemia (170 patients) were analysed. *C. albicans* and *C. parapsilosis* were isolated most commonly (25% and 24% respectively). However, frequently azole-resistant *Candida* species such as *C. glabrata* or *C. krusei* accounted for only 22% of all episodes (5% and 17% respectively). The incidence of candidaemia (per 100.000 inpatient days) remained relatively stable from 13.9 in 2001 to 19.2 in 2006 (p=NS). 72% of episodes were breakthrough candidiasis to prior antifungal prophylaxis. Twenty five percent of the 173 isolates were resistant to fluconazole (67% of *C. glabrata*, or 6 out of 9 isolates), and 7% resistant to voriconazole. Of the 59 *Candida* isolates with caspofungin MICs, 4 (7%) had an MIC > 2. In contrast, resistance to amphotericin B (MIC > 2) was uncommon (1%). There was no trend in prevalent *Candida* species or patterns of resistance over the study period. Overall 30 day mortality was 38% and the 30 day attributable mortality was 17%. Factors associated significantly 30-day overall mortality (multivariate analysis) were age >50 years, intercurrent infection and neutrophil count <100/ml at diagnosis of candidiasis; for 30-day attributable mortality age >50 years and sustained candidaemia. The *Candida* species associated with the highest mortality (44%) was *C. glabrata*.

Conclusion: Despite widespread use of antifungal prophylaxis in high risk hematology patients, the incidence of candidiasis remained stable. More over, breakthrough infections, frequent in vitro resistance (especially to fluconazole), the predominance of non-*albicans Candida* species and high crude mortality continue to pose significant challenges.



O455 Epidemiology, management and risk factors for death of invasive *Candida* infections in critical care units: a multicentre, prospective, observational study in France (2005–2006)

O. Lortholary, J.P. Gangneux, P. Montravers, J.P. Mira, F. Gouin, J.P. Sollet, J. Carlet, J. Reynes, B. Régnier, M. Rosenheim, O. Leroy for the AmarCand Study Group

Objective: To describe the evolving epidemiology, management and risk factors for death of invasive *Candida* spp. infections in intensive care units (ICUs) in France

Methods: This country-based, prospective, observational study (AmarCand study) was carried out in 101 ICUs. 271 adult patients with proven invasive *Candida* spp. infection who received a systemic antifungal therapy were included between October 2005 and May 2006.

Results: The study included 107 (39.5%) patients with isolated candidaemia, 87 (32.1%) with invasive candidiasis without candidaemia, and 77 (28.4%) with invasive candidiasis and candidaemia. With regards to risk factors, 76% of patients had mechanical ventilation, 97% were catheterised, 59% had had prior antibiotherapy, 21% were immunocompromised, and 34% had a malignancy. *C. albicans* accounted for 57.0% of the causative species, followed by *C. glabrata* (16.7%), *C. parapsilosis* (7.5%), *C. krusei* (5.2%) and *C. tropicalis* (4.9%). In 17.1% of the cases, the causative species was less susceptible or resistant to fluconazole. Fluconazole was the empiric treatment

most commonly initiated (65.7%), followed by caspofungin (18.1%), voriconazole (5.5%), or amphotericin B (3.3%). The case fatality ratio at the end of ICU stay was 45.9%. Multivariate analysis showed that factors independently associated with death in ICU were: diabetes mellitus (odds ratio [OR] 4.51, 95% confidence interval [CI] 1.72–11.79, p = 0.002), immunosuppression (OR 2.63, 95% CI 1.35–5.11, p = 0.0045), invasive mechanical ventilation (OR 2.54, 95% CI 1.33–4.82, p = 0.0045), and body temperature >38.2ºC (reference: 36.5–38.2ºC, OR 0.36, 95% CI 0.17–0.77, p = 0.008).

Conclusion: The AmarCand study results show that 68% of patients with systemic candidiasis in ICU present with candidaemia. *C. albicans* remains the most frequent causative species. Reduced susceptibility to fluconazole is observed in a large number of *Candida* spp. isolates. 84% of ICU adult patients receive fluconazole or caspofungin as first line therapy and mortality of invasive candidiasis in ICU remains dramatically elevated.

O456 Comparison of the risk factors for *Candida albicans* versus other *Candida* spp. bloodstream infections in intensive care unit patients

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Objectives: *Candida* species are the fourth leading aetiologic agent of bloodstream infections causing high morbidity and mortality rates. The aim of this study is to determine the risk factors for candidaemia including *Candida albicans* and non-*albicans* strains in intensive care unit patients.

Methods: Intensive care unit patients who had candidaemia between the period of January 2004 and October 2007 were included in this study. A structured form was used to collect data from the patient records about demographic characteristics, clinical and laboratory values and interventions for each patient. Chi-square test and logistic regression methods were used for statistical analysis.

Table: Adjusted odds of covariates, associated with non-*albicans* candidaemia, multivariable analysis

Characteristic	*p*	OR (CI 95%)
Age	0.400	0.987 (0.956–1.018)
APACHE II score at onset of candidaemia	0.099	1.111 (0.981–1.259)
CVC	0.452	1.679 (0.435–6.482)
TPN	0.322	1.718 (0.588–5.015)
Entubation	0.149	0.478 (0.175–1.304)
Renal failure	0.089	0.374 (0.120–1.163)
Haematologic malignancy	0.177	5.006 (0.483–51.890)
Solid-organ malignancy	0.278	1.895 (0.597–6.011)
Neutropenia	0.606	0.422 (0.016–11.221)
Solid-organ transplant	0.557	1.755 (0.269–11.453)
Burn	0.792	1.276 (0.209–7.806)
Prior antifungal therapy	**0.023**	**4.174 (1.215–14.345)**

CVC: Central venous catheter; TPN: Total parenteral nutration.

Results: A hundred and two candidaemia patients were inluded in the study. Fifty-nine (57.8%) of the episodes were caused by *C. albicans* and 43 (42.2%) were caused by non-*albicans Candida*. The distribution of non-*albicans Candida* spp. were as follows; 12.7% *Candida tropicalis*, 8.8% *Candida glabrata*, 7.8% *Candida famata* and 12.9% other *Candida* spp. (*Candida kefyr*, *Candida lusitaniae*, *Candida humicola*, *Candida parapsilosis*, *Candida* spp.). There was no statistically significant difference in the distribution of *Candida* isolates between the years 2004 and 2007 (p > 0.05). The risk factor determined for *C. albicans* candidaemia was renal failure (acute or chronic) and risk factors for non-*albicans* candidaemia were solid organ transplantation and prior antifungal therapy. The statistical analysis regarding risk factors was summarised in the Table. There was no statistically significant difference between the rates of mortality among the two groups.

Conclusion: *C. albicans* is still the most common *Candida* species and there was no major difference in the distribution of the candidaemia isolates among our patients for the last three years. Renal failure was found to be the only risk factor associated with *C. albicans* candidaemia and prior antifungal therapy, either prohylactic or empirical, was found to be associated with non-*albicans* candidaemia. As it is well known that appropriate empirical antifungal therapy is the most important factor contributing to low morbidity and mortality rates, each centre should have their own surveillance data.

O457 Treatment of invasive *Candida* infections – Systematic review and meta-analysis

A. Gafter-Gvili, L. Vidal, E. Goldberg, L. Leibovici, M. Paul (Petah-Tiqva, IL)

Background: Invasive candidiasis (IC) is now the fourth leading cause of nosocomial blood stream infections. New drugs for IC have been developed and tested.

Objective: To compare between the available antifungal treatments, in terms of mortality and efficacy.

Methods: Systematic review and meta-analysis of randomised controlled trials comparing between different antifungal drugs for the treatment of candidaemia and other forms of invasive candidiasis. Two reviewers independently appraised the quality of trials and extracted data. We used all-cause mortality as the primary outcome; and microbiological failure, treatment failure and adverse events as secondary ones. Relative risks (RR) with 95% confidence intervals (CIs) were pooled.

Results: Fifteen trials were included, 9 of which compared fluconazole to any other drug (amphotericin B, itraconazole, or combination of fluconazole and amphotericin B), 4 compared echinocandins to other drugs (fluconazole, amphotericin B, liposomal amphotericin B), one compared between micafungin and caspofungin, and one compared between amphotericin B plus fluconazole and voriconazole.

There was no difference in mortality when fluconazole was compared to amphotericin B (RR 0.92 [95%CI 0.73–1.17], although there was an increase in microbiological failure in the fluconazole arm (RR 1.77 [95%CI 1.18–2.65]. Anidulafungin decreased microbiological failure compared with fluconazole (RR 0.50 [95% CI 0.29–0.86]) and with less adverse events. Caspofungin was comparable to amphotericin B in mortality and efficacy but with less adverse events (RR 0.56 [95% CI 0.44–0.71]. Micafungin was comparable to liposomal amphotericin B in mortality and between micafungin and caspofungin demonstrated no significant differences in efficacy or adverse events.

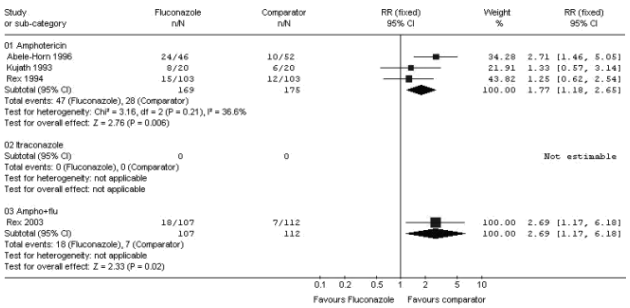

Figure 1. Microbiological Failure for trials comparing fluconazole vs. any other antifungal drug.

Conclusions: Currently available data support either fluconazole, amphotericin B and all echinocandins for primary treatment of IC. For empiric treatment in areas with a high prevalence of non-*albicans Candida* species, echinocandins should be considered as first line. When the isolate is identified, fluconazole may still be considered as first line therapy for *Candida albicans*, while echinocandins, liposomal

amphotericin B or amphotericin B should be chosen for the non-*albicans Candida* species.

O458 Conversion of intravenous echinocandins to oral azoles for invasive candidiasis: impact on outcomes and resource utilisation

S. Davis, A. Kouza, T. Wiegand, J. Vazquez (Detroit, US)

Objectives: Echinocandins are increasingly used in the initial management of invasive candidiasis (IC); however these agents are more costly than alternatives. IV/PO conversion programmes reduce cost for use of antimicrobials with high oral bioavailability, but conversion to an agent of a different class is a new concept. The objective of the present study was to determine the effect of an IV/PO conversion programme from echinocandins to oral azoles on clinical outcomes and resource utilisation.

Methods: 160 consecutive patients receiving echinocandins were evaluated over 2 study periods. Phase 1: data collected retrospectively, caspofungin and micafungin available on formulary, no IV/PO intervention.

Phase 2: data collected prospectively, anidulafungin only on formulary, formal IV/PO intervention implemented. Outcomes were compared among the clinically evaluable (CE) population (documented or suspected invasive candidiasis and meeting PO conversion criteria at any time during therapy).

Results: 97 pts met CE population (57 Phase 1, 40 Phase 2). Patient characteristics: (median or % in Phase1, Phase 2): age (55, 57 years), APACHE (17, 15), prior antifungals (60, 55%), prior ICU (5, 25%; P = 0.017), prior surgery (0, 30%; p = 0.007), diabetes (9, 30%; p = 0.013), prior antibacterials (96, 80%; p = 0.014). Indication for therapy: documented candidaemia/invasive candidiasis (44, 28%), suspected candidiasis (56, 72%). *Candida* species: none (28, 18%), *albicans* (39, 50%), *glabrata* (16, 10%), multiple (10, 15%), other (7, 7%). Clinical success rates were 82% Phase 1, 85% Phase 2. All cause in-hospital mortality (28, 15%, p = NS) PO conversion rates during hospitalisation: 23% vs 48% (p = 0.011), PO conversion at any time: 38% vs 53% (p=NS). Post implementation of IV/PO intervention, antifungal therapy was completely discontinued in 13 patients upon recommendation of IV/PO switch. Duration of total and intravenous antifungal therapy was reduced in Phase 2. (Figure 1) Fluconazole was the most common agent used for PO switch followed by voriconazole.

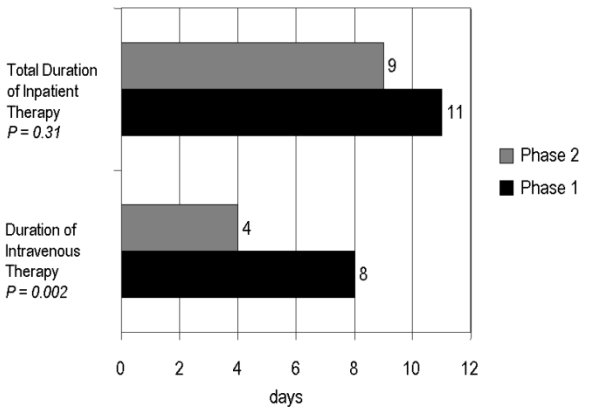

Figure 1. Median duration of therapy.

Conclusions: Patients initially treated with IV echinocandins and switched to PO azoles achieved favourable outcomes. An intervention to implement IV/PO switch therapy is useful for reducing resource utilisation associated with the treatment of invasive candidiasis.

Clostridium difficile – Changing epidemiology and treatment options

O459 Mini state-of-the-art lecture: Recurrent *Clostridium difficile*-associated disease: an emerging problem requiring novel treatment strategies

J. Keller (Amsterdam, NL)

Recurrent *Clostridium difficile* associated diarrhoea (CDAD) is an emerging problem in hospitalised patients and in nursing homes. The pathogenesis of CDAD involves disturbance of the homeostasis of normal bacterial colonisation of the large bowel enabling the outgrowth of a pathogenic *C. difficile* strain. Recurrent CDAD may be explained by failure of antibiotics to eradicate *C. difficile* spores, which will germinate and grow out in a bowel that lacks normal colonic flora as host-defence mechanism. The absence of normal colonisation as defence mechanism is illustrated by the finding that 50% of clinical recurrences of CDAD may be caused by a different strain than the initial infecting strain (Wilcox, 1998). Furthermore, coinfection with multiple strains has been described. Patient factors associated with recurrence are older age and co-morbidity.

Hospitalised patients with CDAD require isolation precautions to prevent epidemic spread, and CDAD often prolongs hospital stay. Initial treatment with metronidazole or vancomycin during 10–14 days is effective in 70–85% of patients (Maroo, 2006). Treatment of relapse consists of a second course of antibiotics during 14 days, with a success rate of about 55% (McFarland, 2002). A subsequent relapse can be treated with 2 weeks of vancomycin followed by a tapered-pulsed vancomycin regimen lasting for several weeks. Consequently, recurrent CDAD requires long-term (and often ineffective) antibiotic treatment and costly isolation precautions in hospitalised patients and a more effective treatment strategy for recurrent CDAD is needed urgently.

In 1958, Eiseman et al described the successful treatment of 4 patients with severe antibiotic induced diarrhoea with enemas containing donor stool. Since this initial report, 81 patients treated with donor faeces for recurrent CDAD have been reported in the literature (Borody, 2004). Clinical improvement was mostly seen after 1–4 days, and there was a response in 90% of the patients without reports of recurrence. Side effects related to faecal therapy have not been reported. The efficacy of faecal therapy against CDAD may be explained by (unknown) inhibitory substances (bacteriocins) produced by the infused donor bacteria and by restoration of faecal physiology by implantation of missing flora components. Importantly, randomised controlled trials are lacking and this may be the reason that faecal therapy has not become a generally accepted treatment strategy for recurrent CDAD. The apparent lack of interest in faecal therapy may also relate to reluctance from physicians to the distasteful nature of the therapy.

We successfully treated 11 patients with recurrent CDAD with a suspension of donor faeces (protocol is outlined in Table 1). Prior to our intervention, CDAD recurred after a median duration of 70 days on antibiotics (metronidazol and vancomycine) and isolation precautions had been taken during 5–11 weeks in hospitalised patients. Faeces were delivered to the bowel by colonoscopy or nasoduodenal tube. Of note, three of these patients were infected with the PCR ribotype 027 strain that is often not responding to conventional antibiotic therapy.

In conclusion, healthy donor faecal enema administration seems to be a promising tool in the fight against recurrent CDAD not responding to conventional antibiotic therapy, also in PCR ribotype 027 strain infected patients. Recently, a randomised controlled trial to compare the efficacy of donor stool suspension with vancomycin for recurrent CDAD is initiated.

Reference(s)

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Mc Farland LV et al. Breaking the cycle: treatment strategies for 163 cases of recurrent *C. difficile* disease. Am J Gastroenterol 2002;97:1769.
Maroo S & Lamont TJ. Recurrent *Clostridium difficile*. Gastroenterology 2006; 130: 1311.
Wilcox MH et al. Recurrence of symptoms in *C. difficile* infection – relapse or reinfection? J Hosp Inf 1998; 38.

Table 1. Protocol donor faeces administration.

Donor:
- Screen for HIV, HBV, HCV, acute CMV or acute EBV infection. Test faeces for *C. difficile*, *Yersinia*, *Campylobacter*, *Shigella*, *Salmonella*, and pathogenic parasites.
- No recent use of antibiotics.
- No risk factors for transmittable diseases.
- Normal defaecation pattern.

Faeces:
- Fresh faeces (<6 hours before infusion), >100 gram.
- Suspend in 300–400 cc NaCl 0.9%.

Patient:
- 4 days vancomycin followed by bowel lavage with kleanprep.
- day 5: infusion of suspension of donor faeces in jejunum (nasoduodenal tube) or in coecum and colon ascendens (via colonoscope).
- Stop vancomycin on day of faeces infusion.

O460 The spread of *Clostridium difficile* PCR-ribotype 027 in the Netherlands since 2005 and the rise of other types

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Objectives: In 2005, outbreaks of *Clostridium difficile* associated diarrhoea (CDAD) with the hypervirulent PCR ribotype 027 / toxinotype III were first reported in The Netherlands. *C. difficile* PCR ribotype 027 was already causing considerable problems in hospitals in the United States, Canada and England. Several investigations have shown an increase in morbidity and mortality and an increased relapsing rate for this type. Studies on risk factors at individual patient level showed an association with fluoroquinolones.
Methods: The Centre for Infectious Disease Control at the National Institute for Public Health and the Environment (RIVM) set up a surveillance for CDAD in collaboration with the Leiden University Medical Center (LUMC). Hospitals are requested to send isolates or toxin positive faeces samples for typing, if they suspect type 027 based on an increased CDAD incidence or a severe clinical syndrome. From hospitals with an outbreak or transmission of type 027, information is collected, including monthly CDAD incidence.
Results: Between February 2005 until half August 2007, 1886 samples have been typed, coming from 75 healthcare institutions. In 418 cases (22.2%), PCR ribotype 027 was found. PCR ribotypes 001 (17.8%), 014 (7.2%) and 078 (7.6%) were also frequently found. In 26 of the 97 Dutch hospitals, PCR ribotype 027 was present. In one of the hospitals, a simultaneous outbreak occurred with a second ribotype (017). In 13 of the 26 hospitals, 027 occurred in isolated cases only, without further transmission in the institution or an increased incidence of CDAD. In the other 13 hospitals, the incidence was increased. PCR ribotype 027 has also been detected in 10 nursinghomes. In eight of 11 hospitals where ribotype 027 was present in 2005 or 2006 and an outbreak occurred, no ribotype 027 was found anymore since April, 2007. One hospital that had the epidemic well under control for a long time was faced with a new increase in incidence. Other PCR ribotypes appear to increase in The Netherlands and some of these types such as type 078 have the same virulence factors as type 027.
Conclusion: PCR ribotype 027 continues to spread in our country. Introduction of 027 into a hospital does not always cause outbreaks, but may remain limited to one or a few isolated cases, without increase in CDAD incidence. Ribotype 027 caused new outbreaks in hospitals that appeared to have controlled the situation.

O461 Lack of identified association between nosocomial CDAD and emergency department overcrowding and number of room changes

J. Yang, B. Brown, E. Belzile (Montreal, CA)

Introduction: *Clostridium difficile*-associated diarrhoea (CDAD) has become a common problem in Quebec hospitals, as well as in many European countries. We examine the association between crowding of patients in the emergency department and room conditions on the hospital wards and the acquisition of nosocomial *Clostridium difficile* infection. The setting is a 316-bed university-affiliated community hospital in Montreal, Quebec.
Methods: A nested case-control study design with conditional logistic regression analysis is performed using administrative data on patients aged 65 and over, admitted through the Emergency Department (ED) to a medical unit, with a recent antibiotic prescription. The relationship between three groups of variables – ED exposure measures (length of stay, level of crowding); inpatient variables (unit and room types, number of room changes); antibiotic type – and the occurrence of nosocomial CDAD is examined, while controlling for several factors previously identified as associated with CDAD transmission.

Table 1: Baseline characteristics of cases and controls (1:1 match)

Variables and descriptive statistics		Cases (n = 68)	Controls (n = 68)
Age in years	Mean (Std)	83.6 (6.8)	83.6 (6.5)
	Median [Q1–Q3]	84.0 [78.5–89.0]	84.0 [79.5–88.0]
Number of admissions	1 March to 30 June n (%)	26 (38.2)	22 (32.4)
	1 July to 30 September n (%)	10 (14.7)	13 (19.1)
	1 October to 31 December n (%)	17 (25.0)	17 (25.0)
	1 January 2004 to 31 March 2004 n (%)	15 (22.1)	16 (23.5)
ED length of stay (hours)*	Mean (Std)	21.0 (14.3)	26.3 (17.0)
	Median [Q1–Q3]	19.8 [7.8–27.7]	23.2 [16.4–32.5]
Number of ED stretchers occupied during ED stay**	Mean (Std)	19.4 (4.1)	19.7 (4.5)
	Median [Q1–Q3]	19.4 [16.0–22.9]	19.5 [16.8–23.0]
Unit of initial admission***	GAU: n (%)	8 (11.8)	n/a
	5 South Medicine: n (%)	32 (47.1)	
	8 Main Medicine: n (%)	20 (29.4)	
	ICU/CCU: n (%)	8 (11.8)	
Length of stay until *C. difficile* (days)***	Mean (Std)	12.8 (11.3)	n/a
	Median [Q1–Q3]	9.5 [5–16.5]	
Total length of stay until discharge (days)	Mean (Std)	29.9 (23.3)	24.8 (17.4)
	Median [Q1–Q3]	24.5 [13.5–36.5]	22.0 [13.0–30.0]
Number of rooms[x]	Mean (Std)	2.1 (1.2)	2.3 (1.5)
	Median [Q1–Q3]	2.0 [1.0–3.0]	2.0 [1.0–3.0]
Numer of patient exposure[o] days	Mean (Std)	14.3 (16.9)	11.2 (10.7)
	Median [Q1–Q3]	9.1 [4.9–15.3]	7.9 [3.5–15.8]
Number of deaths	n (%)	20 (29.4)	12 (17.7)
Numbers of patients transferred to LTC	n (%)	12 (17.7)	13 (19.1)
Type of antibiotic[oo]	Quinolones: n (%)	59 (86.8)	47 (69.1)
	Penicillins: n (%)	16 (23.5)	17 (25.0)
	Sulfonamides: n (%)	0 (0.0)	7 (10.3)
	Nucleosides/nucleotides: n (%)	1 (1.5)	2 (2.9)
	Cephalosporins: n (%)	20 (29.4)	8 (11.8)
	Azoles: n (%)	1 (1.5)	1. (1.5)
	Aminoglycosides: n (%)	1 (1.5)	4 (5.9)
	Miscellaneous antiprotozoals: n (%)	12 (17.7)	17 (25.0)
	Miscellaneous antibacterials: n (%)	8 (11.8)	11 (16.2)
	Miscellaneous β-lactams: n (%)	3 (4.4)	1 (1.5)

*: Time from registration in the ED to leaving for another unit.
**: Indirectly measures the level of crowding in the ED during the whole stay of cases and controls.
***: Matching criteria.
x: Calculated from admission until the date of collection of the first specimen that tested positive for *Clostridium difficile* in the infected patient for both cases and controls. If diagnosis of *Clostridium difficile* was done after discharge, then the study period for cases was from admission to discharge.
o: A private room equals zero patient encounter days; onde day in a four bedded room equals 3 patient encounter days and one day in a semi-private room equals one patient encounter day. A day in the CCU or ICU rooms 1 to 5 equals two patient encounter days and a day in ICU rooms 6 to 7 zero patient encounter days. Total exposure for cases and controls is the sum of these values for the time period from admission to the day of specimen collection for the cases of CDAD (and the calculation made for the same number of days for the controls).
oo: Antibiotics were taken from 14 days before registration at the Emergency Department until the date before occurrence of CDAD.
n/a: not applicable. Std: standard deviation.
Q1 and Q3: 1st and 3rd quartiles.

Results: Patients are 83.6 years old on average. There are 73 cases of CDAD among 824 eligible admissions. Matched analyses for 68 cases and matched controls show no significant differences between case patients and control patients for ED or inpatient variables. Multivariable analysis confirms that quinolones (OR=2.82 95%CI [1.30;6.13] and cephalosporins (OR=3.38 95% CI [1.72; 6.61] are associated with the occurrence of CDAD, and that both groups are the most administered antibiotics in the sample.
Conclusions: This study fails to identify any significant association between nosocomial CDAD and measures of ED crowding or increased patient contacts due to room changes or the use of multi-bed ward rooms. However, the validity of our methodology is supported by the association between antibiotic prescriptions and nosocomial CDAD occurrence, as already quoted in the literature. We note a temporal decrease in the reported rate of *Clostridium difficile* infection in our institution since the study period (April 1, 2003 – March 31, 2004) and suggest that the most likely reason is improving housekeeping practices over this period.

O462 *Clostridium difficile* in the paediatric population

S.N. Reddy, P. Kalima, M.H.S. Collie, D.N. Anderson, I.R. Poxton (Edinburgh, UK)

Objectives: *Clostridium difficile* Associated Disease (CDAD) cases are increasing.
There has been a shift in trends with a rising number of cases being reported in low-risk populations, e.g. children and young peri-partum women. Our aim was to review the prevalence of *C. difficile* in the in-patient paediatric population presenting to a Children's Hospital within a University Hospitals Trust from 2000–2006 inclusive, and to further assess the data set for 2006.
Methods: Patients were identified via Medical Microbiology databases. Patients ranging from greater than one-month to less than 14 years were included. Retrospective analysis of prospectively collected outcome data was then performed.
Results: There has been an increase in the number of diagnosed paediatric CDAD cases over the last year. (A detailed breakdown is shown in the table attached).
Further analysis of data for 2006 revealed 603 faecal samples were tested for *C. difficile* of which 33 samples were positive. Taking into account multiple samples testing for individual patients 22 patients were diagnosed with CDAD out of 335. Of the 22 positive patients there was no significant gender bias (Male:Female=10:12) and the median age was 3 years (range 2 months – 13 years). For this cohort 117 samples were analysed ranging from 1–31 samples per patient over a time period of 1–331days. Only 54% of patients were found to be toxin positive on their first sample. A median period of 23days (range 2–102 days) was conceded prior to detection of *C. difficile* toxin, from the time of their first negative sample being assessed to the ultimate positive sample, a median of 3 (range 2–6) samples was sent prior to detection. 23% of positive patients had CDAD re-diagnosed on subsequent samples following a negative sample. In these cases the median duration was 22 days (range 15–104days) following a median of a further 2 samples being analysed (range 2–4).

Age range (years)	2000	2001	2002	2003	2004	2005	2006
>1 month–<1	0	0	0	0	0	0	3
1–4	0	0	0	0	1	2	12
5–13	0	1	2	1	1	2	7

Conclusions: The diagnosis of *C. difficile* is increasing in this previously low-risk paediatric population and therefore is a clinical diagnosis that must be considered early. Culture in conjunction with toxin testing may have led to an earlier diagnosis in nearly 50% of patients and there is a possibility that re-infection/re-colonisation may occur in up to a quarter of Paediatric cases. Mandatory data reporting is only required for patients over 65 years resulting in this important sub-set being excluded.

O463 Emergence of reduced susceptibility to metronidazole in *Clostridium difficile*

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Objectives: Resistance to metronidazole (MET) or vancomycin in *C. difficile* (CD) has rarely been reported and generally limited to occasional strains. We have repeated previous surveillance for MET and vancomycin resistance in common *C. difficile* ribotypes.
Methods: We screened recently isolated (2005–2006) CD strains from symptomatic patients in Leeds and compared these results with those from repeat testing of historic isolates (1995–2001). Isolates of CD ribotypes 001 (n = 87), 106 (n = 81), 027 (n = 48), the most common UK types, and 10 other prevalent ribotypes (n = 57) were examined initially using a spiral gradient endpoint (SGE) method. Isolates displaying a MET MIC $\geqslant$6 mg/L, as determined by SGE, were analysed further by agar incorporation (AI) and E-test methods. AI was carried out by CLSI, and also by culture in pre-reduced Schaedler's anaerobic broth at 37ºC for 48 h, followed by multipoint inoculation of 104 cfu per spot onto Wilkins-Chalgren agar and anaerobic culture at 37oC for 48 h. Isolates were further characterised by multi-locus-variable-tandem-repeat-analysis (MLVA) to determine clonal relatedness.
Results: No reduced susceptibility to vancomycin was observed. All CD ribotype 106 or 027 isolates were fully susceptible to MET (MICs <2 mg/L). However, 21 (24%) CD ribotype 001 isolates had reduced susceptibility to MET by SGE (geometric mean MIC was 3.5 mg/L ($P < 0.001$). Results varied according to the method used, and both the agar base and broth used to prepare inocula affected the MET MIC values for CD. Neither E-test nor CLSI methods detected the CD strains with reduced susceptibility to MET, but this was confirmed by the alternative AI method. The geometric mean MICs of CD ribotype 001 isolates from 1995–2001 (n = 72) and those identified by SGE with reduced susceptibility to MET (n = 21) were 1.03 and 5.9 mg/L, respectively ($P < 0.001$). MLVA typing revealed subgroups of ribotype 001 isolates that were more closely associated with reduced susceptibility to MET than others.
Conclusion: We have detected the emergence of reduced susceptibility to MET. This phenomenon is easily missed unless appropriate methods are used. Increased vigilance is needed to identify reduced antibiotic susceptibility in CD, and to detect increased treatment failure associated with MET.

O464 Results of a phase III trial comparing tolevamer, vancomycin and metronidazole in patients with *Clostridium difficile*-associated diarrhoea

E. Bouza, M. Dryden, R. Mohammed, J. Peppe, S. Chasan-Taber, J. Donovan, D. Davidson, G. Short for the Polymer Alternative for CDAD Treatment (PACT) Investigators

Objectives: *Clostridium difficile*-associated diarrhoea (CDAD) is an increasing nosocomial problem. Treatment with vancomycin (V) or metronidazole (M) is associated with incomplete response rates, frequent recurrences, and selection for resistant bacteria. In this study, the safety and efficacy of tolevamer (T) liquid (Genzyme, Cambridge MA), a novel non-antibiotic toxin binder, was compared to V and M.
Methods: In this double-blind, multicentre (Europe, Australia, Canada) study in adult patients with acute CDAD appropriate for oral therapy, patients were randomised (2:1:1) to T 9g (3g tid, 14 days), V 500 mg (125mg qid, 10 days), or M 1500mg (375 mg qid, 10 days). In the T group, an initial 9 g loading dose was given. Patients were followed for recurrence 4 weeks after treatment cessation. The primary endpoint was non-inferiority of T vs. V for clinical success, defined as resolution of diarrhoea and absence of severe abdominal discomfort due to CDAD on Day 10.

Results: 528 patients (268 T, 125 V and 135 M) comprised the intent-to-treat population. At enrollment, CDAD was noted as mild (31%), moderate (43%), or severe (25%), first occurrence (83%) or recurrent (17%), and was similar across the treatment groups. Clinical success rates were 42% (112/268) T, 81% (101/125) V, and 73% (99/135) M, indicating similar success rates in the V and M groups ($p = 0.153$), but a lower T success rate than V or M ($p \leqslant 0.001$ for both comparators). In patients with CDAD resolution sustained through the end of active treatment, the recurrence rates were 6% for T, 18% for V, and 19% for M. Adverse events were similar across treatment arms.
Conclusion: T did not meet its primary endpoint of non-inferiority vs. V. Overall, V and M had similar responses. Recurrent CDAD occurred frequently with V and M, but was uncommon among those patients who resolved with T, suggesting that flora-sparing drugs may help reduce recurrences. These results confirm those from an identically designed parallel study performed in the US and Canada (ICAAC 2007; poster 3826).

O465 Strong effects of antibiotic stewardship and infection control measures on *Clostridium difficile*-associated diarrhoea incidence rates in northwestern Germany

H.-P. Weil, P. Gastmeier, E.J. Kuijper, U. Fischer-Brügge (Nordhorn, Hannover, DE; Leiden, NL)

Objectives: To investigate the effects of antibiotic stewardship and infection control measures on the incidence rates of healthcare-onset CDAD in 18 hospitals and of community-onset CDAD. The incidence rates of MRSA, VRE and ESBL in the hospitals were used for comparison.
Methods: Toxin-testing, cultivation, identification and PCR ribotyping of *C. difficile*, were performed according to standard procedures.
Results: In 2005, eleven hospitals (group I) in the Southern subregion had increased CDAD incidence rates (11.6 patients/10,000 patient days), whereas 7 hospitals in the Northern subregion (group II) had no elevated CDAD incidence rates (4.0 patients/10,000 PD). PCR-ribotyping of isolated *C. difficile* strains revealed that more than 80% belonged to hyperendemic multiresistant strains (001, 046) in group I hospitals, whereas no predominant PCR ribotype was detected in group II hospitals. In 2006, interventions were made in group I hospitals: antibiotic stewardship, (5/11) and infection control measures (10/11). In group II hospitals only two hospitals introduced infection control measures. In 2007 group I hospitals achieved a significant reduction of CDAD (8/10,000 PD); group II hospitals showed a constant increase of CDAD (6/10,000 PD). No significant changes regarding to MRSA, VRE and ESBL incidence rates were observed, although group I hospitals had constantly higher MRSA incidence rates. Community-onset CDAD was rarely detected in 2005 (3.8/100,000 inhabitants). A change of the diagnostic algorithm for CDAD and the information of the general practitioners about CDAD led to an increase of community-onset CDAD in 2007 to 20/100,000 inhabitants. In the Northern subregion the number CO-CDAD patients were lower (11/100,000), than in the southern subregion (26/100,000).

	2005				2006				2007			
Incidence rates*	CDAD	MRSA	VRE	ESBL	CDAD	MRSA	VRE	ESBL	CDAD	MRSA	VRE	ESBL
Group I[§]	11.6	7.1	0.6	0.5	12.2	9	0.6	1.1	8	11	0.8	1.9
Group II[&]	4	5.5	0.4	0.7	5	7.9	0.2	1.7	6	7.5	0.7	2.7

*Incidence rates: patients/10,000 patient days.
[§]11 hospitals in the southern subregion; [&]7 hospitals in the northern subregion.

Conclusions: Intervention by antibiotic stewardship t (reduction of cephalosporins and fluoroquinolones) and infection control measures has led to an overall decrease of CDAD in hospitals with increased CDAD incidence rates. There was no significant association between CDAD incidence with the incidence rate of infections due to other multiresistant bacteria, although MRSA incidence rates were also high.

O466 Comparison of disinfecting efficacy of Hydrogen peroxide-based dry mist system and hypochlorite 0.5% on *Clostridium difficile* contaminated surfaces

D. Menuet, M. Verachten, E. Girou, F. Barbut (Paris, Créteil, FR)

Background: During *Clostridium difficile*-associated diarrhoea (CDAD), a large number of spores disseminated in the patients' room. These spores may survive for weeks and are resistant to commonly used disinfectants.
Objectives: To compare the disinfecting efficacy of hypochlorite 0.5% (currently recommended in France for disinfection of CDAD patients' environment) and hydrogen peroxide-based dry mist system (Sterinis®/Sterusil®, Gloster Santé Europe).
Methods: The efficacy of both disinfectants was assessed in situ, in the rooms ($n = 31$) of patients with CDAD. A prospective study was performed in two French university hospitals from April to August 2007. At discharge, rooms were cleaned and randomised in two groups to compare the disinfection processes: the HPV group included rooms treated with 5% hydrogen peroxide-based dry mist system (1 cycle; 18 min. diffusion, 60 min. contact time) and the H group manually disinfected using 0.5% hypochlorite. Twelve environmental surfaces (100 cm2) were tested for *C. difficile* before and after disinfection, using moistened swabs that were inoculated on selective plates (taurocholate, cycloserine, cefoxitin agar) and broth.
Both disinfectants were also tested in vitro on small pieces (4 cm2) of two different materials placed in empty rooms and experimentally contaminated by 10^6 spores of 3 different strains of *C. difficile* including the 027 clone. Spores were numbered before and after disinfection and the mean reduction factor of contamination was expressed as log10 UFC.
Results: 748 surface samples were collected in the rooms of patients with CDAD. Before disinfection, the frequency of contamination was 24% and 19% in H and HPV groups, respectively ($p = 0.2$). After disinfection, a significant reduction of contamination was observed in both groups, with a relative higher level in the HPV (19% vs 2%) than the H group (24% vs 12%) ($p < 0.005$).
Results of the in vitro study showed whatever the strains and materials used, a mean reduction factor of contamination significantly higher in the HPV than the H group (4.18 ± 0.79 vs 1.8 ± 0.81, $p < 0.001$).
Conclusion: The hydrogen peroxide-based dry mist disinfection system is more effective than hypochlorite 0.5% in decreasing *C. difficile* contamination in patients' environment and might represent an appropriate alternative for the disinfection of rooms of patients with CDAD at discharge.

O467 *Clostridium difficile* is not reliably eradicated by ward bedpan washers unless alkaline detergent is used

M. Alfa, N. Olson, L. Beulow-Smith (Winnipeg, CA)

Objective: The objective of this study was to use simulated-use testing to evaluate the efficacy of *C. difficile* spore elimination by W-BPW compared to Central Processing Dept. washers (CPD-BPW).
Methods: *C. difficile* spores were suspended in sterile faeces to give ~10E6 cfu/mL and 0.1 mL of this preparation was spread over a defined surface area of the bedpan and allowed to dry 2.5 Hrs or overnight at room temperature. The inoculated bedpans were processed using the W-BPW or the CPD-BPW. Rodac plates containing CDMN media were used to detect residual *C. difficile* sporeson processed bedpans. In addition the faecal-spore suspension was placed in a sealed vial and processed through the BPWs to determine if the thermal conditions (no wash off effect) were sufficient to kill the spores. An unprocessed control was compared to the processed sample in the sealed vial to determine the Log10 reduction in spores due to thermal exposure. The ability of

thermal conditions to kill *C. difficile* spores in PBS (i.e. no organic challenge) was also evaluated using spore suspension testing at 80oC and 90oC. Two different makes of W-BPWs were evaluated.
Results: Our data on suspension testing showed that the *C. difficile* spore count was not reduced after 5 minutes at 90oC. Simulated-use testing demonstrated that the ward-BPW when used without detergent, did not effectively eliminate *C. difficile* spores from inoculated plastic or stainless steel bedpans whereas the CPD-BPW did. The thermal disinfection default cycle in the two makes of W-BPW and the CPD-BPW was 80ºC for 1 minute. The ISO15883–3 guidance document recommends these thermal conditions as adequate for reprocessing of bedpans. Exposure to these conditions resulted in ~1 Log10 reduction in spores. However, the 80oC for 1 min thermal cycle combined with the 116ºC drying cycle for 7 mins used by the CPD-BPW killed 6 Log10 *C. difficile* spores. Further testing showed that using an alkaline detergent along with the W-BPW did reliably eliminate *C. difficile* spores.
Conclusion: Despite the wide use of 80ºC for 1 minute, our data showed that these thermal conditions alone were not effective in killing *C. difficile* spores. Only when alkaline detergent was used was the W-BPW able to eliminate *C. difficile* spores from bedpans. We recommend that alkalkine detergent always be used with W-BPWs to reduce the risk of *C. difficile* spores remaining on reprocessed bedpans as this could pose a risk for nosocomial transmission.

Resisting resistance: global trends and therapeutic strategies for MRSA (Symposium organised by Forest Laboratories)

S468 European CA-MRSA: a singular situation?

F. Vandenesch (Lyon, FR)

Community-acquired meticillin-resistant *Staphylococcus aureus* (CA-MRSA) was first described in Europe at the beginning of this decade, and since 2003 an impressive worldwide spread of Panton Valentine leukocidin (PVL)-positive CA-MRSA clones has been observed. Continuous surveillance of CA-MRSA organised by the French National Reference Center for Staphylococci has revealed a number of significant trends enumerated below:

- The spread of previously continent-specific clones of CA-MRSA to other continents. Schematically in 2003, ST80 was detected in Europe, ST8 (USA300) and ST1 (USA400) in the USA, and ST30 in Oceania. In 2006, intercontinental exchanges of several clones were observed: the ST8 clone from the USA towards Europe; the ST1 clone from the USA towards Europe and Asia; the ST59 clone (USA1000) from the USA towards Asia; and the ST80 clone from Europe towards Asia.
- The spread of PVL-positive CA-MRSA from country to country. For instance, in Europe, PVL-positive CA-MRSA of ST80 was recently detected in Slovenia, Romania, and Croatia.
- New PVL-positive CA-MRSA clones emerging with different genetic backgrounds. While most of the clones described in 2003 had an agr3 background, the newly described clones are agr1 or agr2. Clone ST22 (agr1) has been found in Europe only, and clone ST377 (agr1 with a type V SCCmec) was reported simultaneously in Europe and Australia.
- PVL-positive CA-MRSA, which were initially susceptible to most anti-staphylococcal agents, have acquired new antimicrobial resistance determinants, for example, to gentamicin and ofloxacin.
- In contrast to the USA, where CA-MRSA now accounts for the majority of *S. aureus* infections in the community, the prevalence of PVL-positive CA-MRSA remains low in Northern Europe. However, Southern European countries, such as Greece, and countries located at the southern boundaries of Europe, such as Algeria, have a greater prevalence of PVL-positive CA-MRSA.
- In countries of high prevalence, PVL-positive CA-MRSA are responsible for an increasing number of hospital-acquired infections.
- Sequence variations of PVL among the different clones have been observed. The most frequently observed clone in Europe (ST80) harbours an H2 haplotype which is described as the ancestral allele that gave rise to the other variants.

These trends, although largely regionalised, must be taken into account when targeting skin infections as well as other infections, including community-acquired pneumonia. In summary, these emerging tendencies towards increased incidence of CA-MRSA and decreased susceptibility to antimicrobial agents must be considered when choosing empiric treatment options.

S469 The increasing global burden of MRSA and potential clinical implications

D.E. Low (Toronto, CA)

Meticillin-resistant *Staphylococcus aureus* (MRSA) was first described in the 1960s as a source of healthcare-associated (HA) infections. In the United States (U.S.), the National Nosocomial Infections Surveillance System reported a 12% increase in MRSA infections in 2003, compared with the prevalence noted from 1998 to 2002. Now >63% of all *S. aureus* isolates recovered from critically ill patients are MRSA and are prevalent in all regions of the U.S. Additionally, MRSA now represents a worldwide problem, with the pathogen increasingly noted in Europe and Asia. Initially, infections with this organism were seen almost exclusively in people with significant healthcare exposure. The first reports of MRSA infections among healthy persons without identifiable risk factors for HA infections were published in the 1980s. MRSA emerged as a more widespread cause of community-associated (CA) infections in the late 1990s. A comparison of CA-MRSA versus HA-MRSA reported that skin and soft tissue and otitis media infections were disproportionately due to CA-MRSA, compared with respiratory tract and urinary tract infections. However, there has been a dramatic increase in the occurrence of *S. aureus* infections in general and CA-MRSA infections in particular. In most, but not all, U.S. cities, CA-MRSA is now the most common pathogen cultured from patients with skin and soft tissue infections in emergency departments. Epidemic community-associated MRSA disease has also been reported from some rural areas. In Canada, a similar pattern of emergence is occurring with the West Coast being most affected. Of concern is the risk of greater incidence of community-acquired pneumonias caused by MRSA in the future. Although severe pneumonia caused by CA-MRSA was reported in children soon after this pathogen emerged in the U.S., reports in adults have been rare and typically associated with influenza virus infection. However, the Centers for Disease Control and Prevention reported 10 cases of severe MRSA CAP in 2006; six of the 10 patients died. Although a small sampling of patients, these cases nonetheless underscore the need for healthcare providers to be extremely vigilant for cases of severe CAP caused by MRSA.
The emergence of MRSA strains since 1961 has complicated the treatment of *S. aureus* infections, and glycopeptides (vancomycin or teicoplanin) are, in many cases, the only therapeutic alternative. Glycopeptides remain the first-line option when infection due to MRSA is suspected or diagnosed. However, vancomycin treatment failure is not uncommon, even when MRSA strains are fully susceptible to vancomycin (MIC $\leqslant 2\,\mu g/mL$). A reduction in the efficacy of vancomycin against MRSA strains with a high vancomycin MIC ($1–2\,\mu g/mL$) has been described in observational studies with low number of patients, suggesting that subtle changes in the MIC may explain clinical failures. These findings require healthcare practitioners to consider alternative treatments and look to the future, as newer, more active compounds are developed to improve the treatment armamentarium against community-acquired infections with drug resistant *S. aureus*.

S470 Prevention protocols and the rationale for new therapeutic strategies

R. Cantón (Madrid, ES)

“Search-and-destroy” is considered one of the most effective means to curtail the increased prevalence of nosocomial resistant organisms,

including meticillin-resistant *Staphylococcus aureus* (MRSA). The implementation of this strategy in certain European countries has decreased the prevalence of MRSA infection in the nosocomial setting for the first time. Nevertheless and in parallel, an increase in MRSA isolates in the community setting, including nursing homes and healthcare-associated facilities, has been observed during the past decade. As a consequence, community-acquired MRSA infections are increasingly recognised in the nosocomial setting, making it difficult, to integrate preventive strategies into standard hospital procedures. Moreover, an influx of well-dispersed community clones – most of them carrying the type IV staphylococcal cassette chromosome (SCCmec type IV) – into the nosocomial setting has been demonstrated in countries with high prevalence of MRSA in the community. The potential association of these isolates with the production of the Panton Valentine Leucocidin (PVL) is of clinical concern, as initial empirical coverage for this pathogenic organism is restricted.

Standard antimicrobial treatment protocols against both community- and hospital-acquired MRSA infections include non-β-lactam antimicrobials, among them, glycopeptides have been considered the gold standard. To date, few isolated cases of complete glycopeptide resistance in *S. aureus* (VRSA) have been reported. However, low-level resistance, or "vancomycin-intermediate *S. aureus*" (VISA), has been documented worldwide. These isolates display a thickened cell wall affecting the activity of glycopeptides. Recent data have also demonstrated the isolation of MRSA isolates with heterogenous subpopulations with decreased susceptibility to glycopeptides ("heterogeneous VISA"), and the possibility that a slight rise in modal vancomycin MIC values within the susceptible category (MIC range 1.5 to 2 μg/mL) may affect clinical success of glycopeptide treatment. This possibility has been observed in different infections, including bacteraemia. The true incidence of MRSA populations with decreased susceptibility to glycopeptides is unclear and might depend on population structure distribution. Maintaining vigilant surveillance of these resistance trends is critical to ensure appropriate coverage.

Treatment options for invasive MRSA infections currently include vancomycin, linezolid, daptomycin, tigecycline, and quinupristin/dalfopristin. Additionally, a number of compounds to combat the growing resistance problems are in development, including novel glycopeptides (dalbavancin, telavancin, and oritavancin), and next-generation cephalosporins (ceftobiprole and ceftaroline) which demonstrate excellent in vitro activity against MRSA, VISA and VRSA. The addition of these new agents to the armamentarium will be critical in managing patients with infections due to MRSA encountered both in the hospital and community settings.

ID-Tag™ respiratory viral panel (Symposium organised by Luminex)

S473 Epidemiology of respiratory virus infections using xTAG RVP test

J.B. Mahony, S. Chong, M. Smieja, A. Petrich, S. Buracond, J. Babwah (Hamilton, CA)

Background: With the discovery of five new respiratory viruses since the year 2000 and advances in molecular technology allowing the detection of 20 respiratory viruses in a single test, we studied the epidemiology of respiratory virus infections.

Methods: A total of 1,060 NP specimens collected from symptomatic patients (526 <20 yr and 534 >20 yr) were used in the study. Approximately 100 specimens (50 from each age group) were sected blindly (DFA and culture results unknown) from specimens submitted to our Regional Virology Laboratory for each month from November 1 2005 to October 31 2006. Nucleic acid was extracted using the MiniMag extractor (Biomerieux) and tested by the ID-Tag RVP Test (TmBioscience) for 20 respiratory viruses and for Bocavirus using a separate PCR. Clinical information was collected by chart review. Results were analysed by month and age.

Results: Of the 1060 specimens tested, 424 (40%) were positive for one of 16 respiratory viruses including: 205 (19.3%) Rhino/Enterovirus, 58 (5.5%) Influenza type A or B, 45 (4.1%) Parainfluenza (types 1–4), 41 (3.9%) RSV (type A or B), 41 (3.9%) Metapneumovirus, 39 (39/947, 4.1%) Bocavirus, 20 Adenovirus, 14 Coronavirus (OC43, HKU1, NL63). Twenty five of 39 Bocavirus infections were dual infections. Only Rhino/Entero, RSV and Bocavirus were more prevalent in the <20 age group ($p < 0.05$). Influenza, Metapneumovirus and Coronavirus were only present in winter/spring months while Parainfluenza type 4 was only present in summer months; all others were distributed across most months.

Discussion: This study indicated the following: 1) Rhino/Enterovirus was the most prevalent virus infection of symptomatic children and adults, 2) only RSV, Rhino/Enteroviurs, and Bocavirus showed higher infection rates in children compared to adults, 3) 90% of dual infections (mostly Boca and Rhino/Enterovirus) occured in children summer, and 4) Influenza, Metapneumovirus and Coronavirus displayed a winter seasonality. The average rate of positives diagnosed per month for <20 yr group was 63% compared to 23% for adults.

S474 Finding respiratory viruses in nasopharyngeal aspirates: comparison between direct immunofluorescence and a new multiplex PCR method

S. Jemielity, M. Barbani, T. Staub, D. Grandgirard, M. Gorgievski-Hrisoho (Berne, CH)

Respiratory viruses are among the most frequent human pathogens worldwide, leading to a significant number of hospitalisations, especially in children and in immune-compromised patients. Several new diagnostics tools for respiratory viruses have recently appeared on the market, including the xTag Respiratory Viral Panel (RVP) by Luminex Molecular Diagnostics. The goal of the present study was to compare this new multiplex PCR method with conventional direct immunofluorescence (DIF) in children's nasopharyngeal aspirates for the following viruses: respiratory sincytial virus (RSV), adenovirus (ADV), parainfluenza viruses 1–3 (PIF), influenza viruses A and B (IF), as well as human metapneumovirus (hMPV). The RVP kit permits in addition the detection of entero-/rhinoviruses, coronaviruses and parainfluenza virus 4.

In total 240 nasopharyngeal aspirates (126 DIF negative and 114 DIF positive samples) were analysed. All RSV, PIVA, IF and hMPV DIF positive samples (43, 15, 18 and 11, respectively) were confirmed as positive for the same virus by RVP. For ADV, however, only 13 out of 27 DIF positive samples (48%) were also ADV positive by RVP. The samples with discrepant results are currently being reanalysed by DIF and by a second, independent PCR.

Of note is the finding that over 64% (81/126) of all DIF negative samples were positive when analysed with RVP. This was in great part due to the additional entero-/rhinoviruses detected by RVP (75/81), but also because of additional RSV, IF, PIF, ADV and hMPV positive samples that had been missed with the DIF assays (21/81). Finally, using RVP we detected as many as 10.4% (25/240) of double and 0.5% (1/240) of triple infections, versus a single double infection detected by DIF. The implications of our findings for both diagnostics as well as clinical practices are discussed.

S475 Routine use of Tm Biosciences respiratory viral test in a hospital virology laboratory

F. Stoll-Keller (Strasbourg, FR)

One hundred thirty seven samples (swab specimens, nasopharyngeal aspirates, bronchoalveolar lavage samples) were collected from the 15th of October to the 15th of December from hospitalised patients and investigated for presence of respiratory viruses. Sixty three percent were positive for one virus and co-infections were detected among 19%. The test detected 24 RSV (22%) and 14 parinfluenza viruses (12%). Other viruses, not detected by culture and direct tests, were additionally detected: 24 Metapneumoviruses (21%), and 46 Entero/Rhino viruses

(41%). Luminex RVP was adapted to the routine work of the laboratory to give results within 36 hours to the clinicians.

S476 Sensitive and specific detection of a broad range of respiratory viruses using the Luminex RVP assay: application to routine viral diagnostics

J. Fox, K. Pabbaraju, K. Tokaryk, S. Wong, A. Wong, K. Ho (Calgary, CA)

Background and Objectives: Detection of a broad range of respiratory viruses using sensitive nucleic acid amplification tests (NATs) is invaluable for individual patient diagnosis and management of outbreaks. The wide range of potential pathogens makes such testing expensive and laborious using individual real-time NATs. The objective of this study was to compare the multiplex detection of 19 respiratory viral targets using the Luminex xTagTM Respiratory Viral Panel (RVP) with the individual real-time NATs used in ProvLab (Alberta).
Methods: Nasopharyngeal samples were pre-screened by DFA for influenza (IFV) A and B, parainfluenza (PIV) 1–3 and respiratory syncytial virus (RSV) with direct fluorescent antigen (DFA) positive samples excluded from this comparative study. Respiratory specimens were collected between December 2006 and May 2007 with nucleic acid extraction undertaken using the easyMAG® extractor and reagents (bioMérieux). Individual real-time NATs for comparison with RVP were directed against IFVA, IFVB, PIV1–4, RSV, human metapneumovirus (hMPV) and respiratory adenoviruses. A total of 1497 specimens were part of the analysis with 632 specimens analysed prospectively and 865 retrospectively by RVP. If positive results for coronaviruses or picornaviruses were obtained in the RVP assay these samples were subsequently screened by additional real-time NATs against these targets as such testing is not part of our current diagnostic routine.
Results: A total of 937 specimens were positive for one or more respiratory viruses by the individual NATs and 948 had one or more positive results by the RVP assay. The rate of detection for hMPV and IFVA was comparable by both methods (5.7% and 34.0% positive results, respectively). Detection rate for IFVB, PIV1–4 and RSV was 5.3%, 13.1%, 12.7% by the real-time assays and 4.9%, 12.6%, 10.7% correspondingly by the RVP assay. The detection of adenovirus was superior by the in-house NAT (8.2% positive compared with 4.6% by the RVP assay). However, the RVP assay identified 12.1% of samples as picornavirus positive (confirmed by sequencing), only half of which were identified by individual NATs undertaken for enteroviruses and rhinoviruses. Of the 248 IFVA positive specimens, 85.5% were sub-typed as H1 or H3 by the RVP assay. A total of 26 samples positive for coronaviruses were detected by the Luminex RVP, which were all confirmed by real-time RT-PCR assays.
Conclusion: The Luminex RVP assay meets our current standards of sensitivity and specificity and also allows for multiplex detection of 19 respiratory viral targets with considerable time and cost savings compared with alternative NATs. This method will become front line for respiratory virus testing in our laboratory.

Pregnancy and parasitic diseases: a focus on malaria

K477 Pregnancy and parasitic diseases: a focus on malaria

C. Menendez (Barcelona, ES)

Pregnant women are at increased risk for malaria infection. Although important advances had been made in the last years, the mechanisms that explain their increased susceptibility to this parasitic disease, are not yet fully understood. Malaria infection in pregnancy may be associated with maternal and fetal morbidity and mortality. The severity of this burden depends on the level of pre-pregnancy acquired immunity against malaria. Thus, the consequences of the infection are more severe in non-immune women than in those who have acquired a certain level of anti-malarial immunity. In highly endemic areas, the frequency and severity of the infection is higher in primigravidae and decreases with increasing parity. In non-immune women, the risk is similar across the parities and malaria may be an important direct cause of maternal mortality. Malaria infection during pregnancy may have important negative effects on the infant's health, through intrauterine growth retardation and prematurity or directly through congenital infection. Control of malaria during pregnancy with adequate case management and implementation of preventive tools, is a public health priority in malaria endemic areas.

Are emerging infections driven by climate change?

K478 Are emerging infections driven by climate change?

S. Randolph (Oxford, UK)

Of all the past and present (and probably also future) emergent infectious diseases, those caused by pathogens transmitted by arthropod vectors are the most sensitive to climate and most commonly assumed to have been driven by climate change. There is, however, no a priori reason to expect the rates of each system's biological and demographic processes to respond to changing patterns of temperature and moisture stress in ways that inevitably increase the risk of human (or livestock) infection. A prerequisite of reliable early warnings about the future is a system-specific explanation of the past. Tick-borne encephalitis (TBE), one of the two most widespread, prevalent and medically significant vector-borne diseases in Europe, will be presented as a case study. Epidemiological data at fine temporal and spatial resolution record major upsurges in incidence over recent decades. The timing, degree and abruptness of these upsurges differ markedly between countries, and even between districts within countries. A significant discontinuity in temperature conditions in 1989 across Europe could have created a generally more permissive environment for TBE virus transmission by improving the circumstances necessary for larval and nymphal stages of the tick to co-feed on rodents. The pattern of climate change, however, is too uniform within and between countries to provide the sole, or even the most important, explanation for the extreme spatio-temporal heterogeneity in TBE epidemiology. Instead, a nexus of interacting factors affecting both the risk of infection and exposure of humans to that risk, and each differing in force in space and time, is a more powerful model. Analysis of the situation in Central and Eastern Europe has established that many of these factors were driven by the socio-economic changes associated with the end of Soviet rule, and include climate, land cover, land use, wildlife, agricultural practices, industrial activities, (un)employment and income. Western and Nordic Europe must be incorporated to achieve a coherent pan-European explanation. The same principles may apply to the periodic epidemics of Crimea-Congo haemorrhagic fever.

Varicella zoster infection

S483 Varicella zoster virus latency: implications for new vaccines

J. Cohen (Bethesda, US)

Varicella-zoster (VZV) causes chickenpox and the virus establishes latency in cranial nerve and dorsal root ganglia. VZV DNA was detected in 4% of neurons from human trigeminal ganglia at autopsy with a median of 7 viral genomes per positive neuron. Latent VZV DNA was rarely, if ever, detected in nonneuronal cells. The virus can reactivate from latently infected ganglia to cause shingles. The varicella vaccine virus may also reactivate and cause shingles, especially in immunocompromised patients. We have used PCR to detect VZV DNA in the blood of immunocompromised patients who present with abdominal pain in the absence of a rash, to allow early therapy prior to development of the rash of shingles. At least six VZV genes (genes 4, 21, 29, 62, 63, and 66) are expressed in latently infected ganglia in humans. We have constructed VZV mutants lacking five of these genes

and found that three of the genes are essential for replication in cell culture, one is important for efficient replication, and one is dispensable for replication. Mutations in the coding region of one of the latency genes (gene 63) resulted in impairment in replication in vitro and a marked reduction in latency in rodents. Replacement of the native promoter for one of the latency genes (gene 29), with the major immediate-early cytomegalovirus promoter, resulted in a virus that replicated to levels comparable to wild-type virus, but the virus was impaired for latency in rodents. These studies suggest that changes in the coding or promoter region of latency genes in VZV might serve as useful vaccine candidates. Such vaccines might be less likely to establish latency and reactivate in humans, and may be especially useful in immunocompromised patients.

The role of RNAs in the regulation of virulence and antibiotic resistance gene expression

S484 Small non-coding RNAs controlling pathogenesis

K. Xavier (Oeiras, PT)

Many bacteria use a form of cell-cell signaling termed quorum sensing to coordinate gene expression as a function of the population density. Quorum sensing enables bacteria to coordinate population-wide responses that play a crucial role in virulence. Complex quorum sensing networks enable bacteria to detect signals that provide information regarding the number of individuals in the population, the different species of bacteria present in the community, and additional inputs, such as environmental and metabolic signals. Recently, it has become clear that small non-coding RNAs (sRNA) are often the central regulators modulating gene expression at central steps of quorum sensing cascades and therefore coordinate the complex networks that control virulence in bacteria. Importantly, multiple functionally redundant sRNAs act together at the same key step of these regulatory cascades. These different sRNAs are differentially expressed and respond to different signals and thus provide a mechanism of integrating different environmental stimuli and translating the combined information into the appropriate output. We study a quorum sensing signal named autoinducer (AI-2), which unlike most quorum sensing signals, it is not species-specific. AI-2 is produced and detected by a wide variety of bacteria and allows interspecies communication. By studying interspecies signalling in consortia we found that *Escherichia coli* can manipulate AI-2 signalling in organisms such as the human pathogen *Vibrio cholerae* and interfere with other species' ability to regulate virulence. Here we show that in this enteric bacterium the AI-2 synthase, LuxS, is regulated by a sRNA.

New antimicrobials

O486 Mechanism of action of iclaprim in *Staphylococcus aureus*

C. Oefner, M. Bandera, G.E. Dale, A. Haldimann, S. Mukhija, S. Parisi, S. Lociuro (Reinach, CH)

Objectives: Iclaprim (ICL) belongs to the diaminopyrimidines class of antimicrobial dihydrofolate reductase (DFHR) inhibitors for which trimethoprim (TMP) is the most well known representative. ICL exhibits an expanded spectrum of activity and is notably very potent against important Gram-positive pathogens, including meticillin-resistant *S. aureus* (MRSA). The purpose of this study was to substantiate previous studies on the mode of action of ICL in *S. aureus*.
Methods: One TMP-susceptible (wt-DHFR, ATCC 25923) and two TMP-resistant DHFRs (one carrying a specific F98Y point mutation and one from a multidrug resistant strain 101) were used in this study. The DHFR's were overexpressed and purified and used for the determination of IC50's, Ki's, binding by isothermal titration calorimetry (ITC) and co-crystallisation with ICL and TMP.
Results: Both ICL and TMP exhibited competitive binding with all three enzymes. ICL IC50's and Ki's were 10-, 44- and 32-fold and 20-, 17- and 29-fold lower than those of TMP, for wt-, F98Y and 101-DHFR, respectively. In ITC, both ICL and TMP produced exothermic binding with negative changes in enthalpy. The association constant (Ka) for the F98Y enzyme for ICL was 13-fold stronger than for TMP and the calculated deltaG was −1.88 kcal/mol. Co-crystallisation data showed that the diaminopyrimidine moiety of ICL and TMP binds similarly in both wt- and F98Y enzymes and that the lipophilic part of both molecules is situated in the hydrophobic channel of the enzymes formed by the side-chains of residues L20, L28, V31, I50 and L54. The larger size of the ICL lipophilic residue resulted in an enlarged hydrophobic contact surface area with the protein of about 35 $Å^2$.
Conclusions: ICL and TMP bind to and inhibit *S. aureus* enzymes in a similar manner. The increased potency of ICL towards TMP-R DHFR enzymes is due to a larger binding contact area which is in agreement with the enhanced activity of ICL against both TMP-S and TMP-R *S. aureus* isolates.

O487 The activity of ME1036 against community-acquired pneumonia bloodstream isolates

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Objectives: ME1036 is a novel parenteral carbapenem with enhanced activity against Gram-positive pathogens, including multiple drug resistant staphylococci, streptococci and *Enterococcus faecalis*. This study evaluated the activity of ME1036 against isolates from hospitalised CAP patient blood samples in the UK during 2000 to 2005.
Methods: The study investigated 1337 recent isolates, including *H. influenzae*: β-lactamase (BL)-positive and -negative; *M. catarrhalis*; *S. aureus*: meticillin-susceptible (MS) and -resistant (MR); *S. pneumoniae*: penicillin-susceptible (PenS), -intermediate (PenI) and -resistant (PenR); and *S. pyogenes*. Susceptibility tests with ME1036 and 8 comparators were performed according to CLSI broth microdilution methods.
Results: MICs required to inhibit 90% of each species (MIC90) are shown in the Table for ME1036 and other selected comparators. ME1036 was highly active against all CAP bacteraemia isolates and more potent than all of comparators tested, including meropenem, especially against *H. influenzae*, MRSA, MSSA and PenIR *S. pneumoniae*. All isolates tested were universally susceptible to ME1036 with the highest MICs at 2 mg/L. ME1036 was active against isolates resistant to other antimicrobial agents, including meropenem non-susceptible *S. pneumoniae*.

Pathogen (# isolates)	MIC_{90} (mg/L)			
	ME1036	Amoxicillin-Clavulanate	Meropenem	Levofloxacin
H. influenzae – BL neg (94)	0.015	1	0.12	0.03
H. influenzae – BL pos (25)	0.015	1	0.12	0.015
M. catharrhalis (9)	0.03	2	0.06	0.25
S. aureus – MS (136)	0.015	1	0.25	0.5
S. aureus – MR (28)	2	⩾32	128	⩾32
S. pneumoniae – PenS (762)	⩽0.008	0.03	⩽0.015	1
S. pneumoniae – PenI (97)	0.015	1	0.25	1
S. pneumoniae – PenR (148)	0.06	4	1	1
S. pyogenes (38)	⩽0.008	⩽0.015	⩽0.015	1

Conclusion: ME1036 is a broad-spectrum carbapenem with exquisitely potent activity against Gram-positive pathogens and other respiratory pathogens such as *H. influenzae* and *M. catarrhalis*. ME1036 has the potential to be a useful new agent for the treatment of serious community- and hospital-acquired respiratory tract infections.

O488 In vivo bactericidal effect of a new proline-rich peptide A3-APO on an ESBL-producing *Escherichia coli* strain

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Objectives: The aim of this study was to examine the in vivo efficacy of a new proline-rich antibacterial peptide A3-APO previously proved to be in vitro bactericidal on some enteric bacterial strains.
Methods: CD-1 female mice of 15–20 g were pretreated with 18 mg/kg cisplatin for 3 days to impair kidney clearance similar to that of human. Then they were challenged intraperitoneally (ip) with 10^8 CFU per g mouse of an extended-spectrum β-lactamase producing *E. coli* strain. Four, 8 and 12 hours after challenge 40 mg/kg imipenem, 10, 20 and 40 mg/kg A3-APO were administered to 10 mice of each concentration of the antimicrobials. Prior to drug administration, blood was taken from the tail vein of 3–5 mice either the infected and untreated or the infected and treated groups for determining blood bacterial counts.
Results: Blood bacterial counts amounted to 10^5 CFU/ml by the 4th hour of challenge and exceeded 10^7 CFU/ml by the 12th hour in the untreated mice. In contrast, each concentration of A3-APO decreased the blood bacterial level by two log10 units four hours after the 1st administration, e.g. from 10^5 to 10^3 CFU/ml similar to that after imipenem treatment. Continuous dosing of either the peptide or imipenem retained the blood bacterial counts at the lowest detectable levels.
Conclusion: To our knowledge, this is the first antibacterial peptide that is effective in mortality models of Gram-negative systemic infection and exerts bactericidal activity in vivo in doses comparable to traditional antibiotics without notable toxic side effects.
Supported by the Sbarro Health Research Organization and Hungarian OTKA T46186.

O489 A new approach towards the prevention and treatment of osteomyelitis: synthesis and in vitro studies of bisphosphonated rifamycin prodrugs

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Objectives: Osteomyelitis is an infection of bone primarily caused by staphylococci. Its treatment often requires a combination of surgical intervention and prolonged antibiotic therapy, generally relying on outpatient parenteral antibacterial therapies for weeks. Here we report the development of a new class of prodrugs where rifamycins are tethered to a bisphosphonate moiety possessing high affinity for osseous tissues. These compounds deliver the antibiotics directly to the infection site where they will be concentrated and released over time to exert their therapeutic activity.
Methods: Compounds were synthesised by tethering rifabutin, rifalazil or adequately designed rifamycins to a bisphosphonate moiety via cleavable covalent linkers. The affinity of these compounds towards bone powder was determined individually by measuring the amount of unbound prodrug in the supernatant by bioassay against the total amount of material following the exposure to bovine bone powder in phosphate-buffered saline (PBS) for 1h at 37ºC and centrifugation. The rate of cleavage was determined by resuspending the obtained bone pellet in either PBS or 50% serum in PBS overnight at 37ºC, and measuring by bioassay the drug content in the supernatant after centrifugation.
Results: A total of 14 prodrugs were synthesised using different chemically and/or enzymatically cleavable linkers on rifamycin derivatives. Most prodrugs displayed near quantitative bone binding, validating the selection of a bisphosphonate as the bone-seeking moiety. Following incubation of the bone-bound prodrugs at 37ºC for 24h, the levels of regenerated parent antibiotics were in the range of 0 to 2.65 mol% in PBS and 0 to 2.68 mol% in 50% rat serum.
Conclusions: The ability of bisphosphonated rifamycin class prodrugs to bind efficiently to bone powder and to release their parent antibacterial agents over time was established in vitro. This suggests they could be used to deliver their antibacterial rifamycins to the bone after systemic administration. This augurs well for the potential of this approach towards the prevention and treatment of osteomyelitis.

O490 Comparative study of the efficacy of low- and high-molecular inhibitors of influenza virus haemagglutinin

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Background: Human infections with influenza A and B viruses are initiated by specific interactions of the viral glycoprotein hemagglutinin with carbohydrate chains of cell surface receptors, terminating predominantly in Sia2–6Gal disaccharides. Synthetic analogs of the cellular receptors, which are able to compete with natural cellular receptors, have potential for development of anti-influenza therapeutics. Synthesis of low-molecular inhibitors of influenza virus is more preferable for the development of therapeutics by reason of possible toxicity, immunogenicity and incomplete biodegradation of polymeric compounds. The objectives of this study were to compare the anti-influenza activity and to clarify the mechanisms of action for low-molecular and high-molecular (polymeric) compounds, containing Sia2–6Gal disaccharides.
Methods: We have conducted the comparative study of the antiviral effect of low- and high-molecular hemagglutinin inhibitors on influenza A (H1N1, H2N2, H3N2) and B viruses in the inhibition assay of infectious focus forming in MDCK cells. To characterise efficacy of inhibitors in vivo we have investigated a mouse model, based on measuring the value of fifty percent respiratory infectious dose (RID50) for mice. To elucidate mechanism of action for hemagglutinin inhibitors we have examined influenza virion morphology by the negative contrast techniques.
Results: The values of fifty percent inhibiting concentration (IC50) obtained in MDCK were 0.03 (±0.005) microM for low-molecular hemagglutinin inhibitor and 0.1 (±0.01) microM for high-molecular hemagglutinin inhibitor. Intranasal administration of 0.25 mg/kg low-molecular inhibitor or 1.25 mg/kg polymeric inhibitor completely protected mice from influenza virus A/Aichi/2/68 (H3N2) infection. Electron microscope study of the virion morphology showed direct damage of influenza virus particles by low- and high-molecular compounds, and allowed us to propose mechanism of virucidal action of hemagglutinin inhibitors.
Conclusion: These data show significant antiviral effect of low- and high-molecular hemagglutinin inhibitors on influenza viruses at low micromolar concentration. Although we do not consider high-molecular hemagglutinin inhibitor as candidate anti-influenza drug due to the probable toxicity and limited biocompatibility of polymer, low-molecular hemagglutinin inhibitor has potential for the prevention of influenza virus infection as specific virucidal therapeutic.

Ticked off: lyme borrelliosis

O491 Detection of *Borrelia bissettii* in the South Bohemia region of the Czech Republic: cases of single and multiple infections in humans

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Twelve serum samples from patients with symptomatic borreliosis and 1 sample (cardiac valve tissue) from patient with endocarditis and cardial stenosis associated with chronic borrelial infection were collected in the hospital of Ceske Budejovice (CZ). Serological tests gave the negative results in all cases. The goal of our project was to analyse the samples of the human origin with molecular techniques. The serums and a cardiac valve tissue were used for direct DNA purification. The flagellin gene was chosen as a target for analysis. The PCR primer set for amplification of fla gene from *Borrelia burgdorferi* sensu lato (Bb SL) complex was used. The direct sequence of amplicon indicated the

presence of different sequences. The cloning step allowed the separation of them. Ninety six recombinants from each sample were sequenced. In silico RFLP analysis with 5 restriction endonucleases was conducted. "Virtual" hybridisation with the probes designed for the detection of Bb SL and specie-specific probes were used. The Maximum Parsimony heuristic search was performed in PAUP by implementing the tree-bisection-reconnection algorithm. Alignment was 488 characters long, 53 of them were parsimony informative. All 13 recognised species of Bb SL complex were used as controls. As results, 4 cases were confirmed to carry *B. burgdorferi* ss, 4 – *B. bissettii*, 1 – *B. garinii*, and 4 – multiple spirochete specie. From the 4 co-infected samples 3 were defined as double infection with *B. burgdorferi* ss and *B. bissettii* and 1 as a triple infection with *B. bissettii*, *B. burgdorferi* ss, and *B. garinii*. The identity of each species was confirmed by similarity search using the GenBank, RFLP pattern, virtual hybridisation and PAUP. Until recently it was thought that only 3 genospecies, firmly established and well accepted, might cause Lyme disease (LD), i.e. *B. burgdorferi* sensu stricto, *B. afzelii*, and *B. garinii*. However, few other genospecies have also been connected to LD or were isolated from humans with LD symptoms. They are *B. bissettii* and *B. spielmanii*. After the first isolation of *B. bissettii* from the 9 samples of human origin in Slovenia, our results are the second evidence of involvement of *B. bissettii* in LD in Europe. The presence of *B. bissettii* as a single strain in humans with symptomatic borreliosis or chronic borrelial infection is the strong support of the fact that *B. bissettii* is not only the member of the LD complex, but is a causative agent of the disease.

O492 *Borrelia burgdorferi* in ticks and patients on the Dutch North Sea island of Ameland

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Objectives: The Dutch island Ameland is described previously as a hot spot for *Borrelia burgdorferi* in ticks. We investigated the percentage infected ticks and the risk of subsequent development of clinical symptoms of Lyme disease in persons with one or more tick bites consulting a family practice on the island.
Methods: From January 2004 to December 2006, 214 ticks were collected from 169 patients and tested for the presence of *Borrelia* DNA by PCR. Six to 18 months after the removal, the patients were questioned about erythema migrans and other symptoms possibly related to *B. burgdorferi* infection. Blood samples were taken from a subset of patients to investigate for *B. burgdorferi* antibodies.
Results: In 44 (20.6%) ticks *B. burgdorferi* DNA was detected. Forty patients were bitten by at least one positive tick. Follow up information was available from 144 persons, 36 (25%) with a positive tick and 108 (75%) with a negative tick. In 124 of 144 persons the ticks were removed within 24 hours, including 33 of the 36 persons with positive ticks. None of the 144 patients reported having an erythema migrans. Twelve persons reported a non-specific red discoloration of the skin, not fulfilling critera for EM, on the site of the tick bite during the first weeks after the tick bite. Of these, four were bitten by a positive tick and eight by a negative tick. Surprisingly the four patients with a positive tick all consulted their own family doctor and were treated with doxycyclin, whereas none of the eight with a negative tick did so. One patient, bitten by a positive tick that had been on the skin for more than 24 hours, reported systemic symptoms compatible with Lyme disease. She reported no redness on the site of the tick bite. Lyme serology was performed on 27 persons with a positive tick, two were positive, including the patient with systemic symptoms. Of serology performed on seven persons with a negative tick, one was positive in IgG.
Conclusions: In this cohort of patients with tick bites the chance to develop Lyme disease was low, even with 20.6% of the ticks positive with *B. burgdorferi*. This is probably related to the fact that the majority of ticks were removed within 24 hours. Our findings support the policy described in the Dutch CBO-guideline on Lyme borreliosis to preserve antibiotic therapy for patients developing symptoms of Lyme disease.

O493 A new (14th) member of *Borrelia burgdorferi* sensu lato complex

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Recently, we studied 118 *Borrelia* isolates, cultured from samples collected from a variety of rodents, birds and ticks in 9 different localities of the southern region of the United States. In addition to a large group of highly diverse strains related to *Borrelia bissettii*, and another, rather homogenous group of strains that represent *Borrelia burgdorferi* sensu stricto, a group of 16 isolates with unusual characteristics was found. All 16 isolates were cultured from ear biopsies of the rodents Peromyscus gossypinus and Neotoma floridana, the main reservoir hosts of *Borrelia* in the southern US. The rodents were trapped in 5 different localities of South Carolina from September 1994 till August 1997. The new methodology of multilocus sequence analysis (MLSA) that involved the study of rrf-rrl intergenic spacer region, rrs (16S rRNA) gene, flagellin gene, ospA gene and p66 gene, was used to clarify the taxonomic status of this new highly homogenous group of South Carolina isolates. All 13 recognised species of *B. burgdorferi* sl complex were used as controls. The association of this species with the Lyme disease group was confirmed by multiple results of MLSA. The virtual hybridisation of the rrf-rrl spacer region, flagellin gene, ospA gene with the probes previously described, and experimentally used among these loci for identification of *B. burgdorferi* sl species showed 100% identity. Unique RFLP patterns were discovered in the rrf-rrl intergenic spacer region and flagellin gene of these South Carolina isolates. Unique signature nucleotides were allocated in the 16S rRNA gene. The Maximum Parsimony heuristic search was performed in PAUP by implementing the tree-bisection-reconnection algorithm for all 5 loci. Phylogenetic analysis shows that each of the main species of the *B. burgdorferi* sl complex forms a coherent cluster. All sequences from the 16 South Carolina isolates were clustered together and separately from the other species in the *B. burgdorferi* sl complex. Contrary to dogma, *B. burgdorferi* sl is present and widely distributed in the southern US. It occurs in many parts where it was not previously thought to occur. A substantial amount of additional results highly support the designation of a new *B. burgdorferi* sl species from the southern United States. Further analysis of the 16 cultured *Borrelia* strains from South Carolina is still in progress. The new species will be described formally in another article.

O494 Three different approaches for detection of *Borrelia burgdorferi* sensu lato antibodies in routine laboratory diagnostics

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Objectives: The most common microbiological approach for confirmation of borrelial infection is detection of antibodies to *Borrelia burgdorferi* sensu lato in body fluids with serological methods. The methods differ in relation to several characteristics including sensitivity and specificity. No ideal serological test has been available. The aim of the study was to analyse and compare detection of immune response by three different serological tests.
Materials and Methods: In the present study we tested sera of 249 patients with suspected Lyme borreliosis, collected from routine work, with three serological methods targeting different borrelial antigens: i) in immunofluorescence assay (IFA) a local isolate of *B. afzelii* (strain SA/91), the most frequently isolated *Borrelia* species in Slovenia, was used as an antigen; ii) in Lyme Borreliosis ELISA kit (DakoCytomation, Denmark) the antigen was purified native *B. burgdorferi* flagellum, and iii) in chemiluminiscence immunoassay LIAISON® (Diasorin, Saluggia, Italy) antigen for IgM antibodies detection is OspC while for IgG antibodies Vls E antigen is used.
Results: The presence of borrelial IgM antibodies in patients sera was ascertained in 1/249 (0.4%), 15/249 (6%), and 9/249 (3.6%) with IFA, ELISA and LIAISON®, respectively. The presence of IgG antibodies was established in 31/249 (12.4%), 64/249 (25.7%), and 82/249 (32.9%) with IFA, ELISA, and LIAISON®, respectively.

All three tests gave concordant results in 221/249 (88.8%) and 143/249 (57.4%) for IgM and IgG antibodies, respectively.
Conclusion: For detection of borrelial IgM antibodies ELISA was found to be more sensitive than the other two tests, while LIAISON® was found to be more sensitive test for detection of IgG antibodies. Differences in sensitivities of the three tests could be the result of different antigens and/or distinct method utilised for antibody detection. Discrepancies in findings obtained on identical sera strongly indicate the need for standardisation of the serological methods for detection of *Borrelia burgdorferi* sensu lato infection.

O495 Serological indicators of good outcome after antibiotic treatment in Lyme borreliosis

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Objectives: We have previously reported the results of an antibiotic treatment study of disseminated Lyme borreliosis (mainly neuroborreliosis (LNB) and arthritis (LA)) where we analysed whether the treatment with i.v. ceftriaxone should be followed with a course of oral amoxicillin (Oksi et al; Eur J Clin Microbiol Infect Dis 2007). No difference in the clinical outcome, as evaluated with the visual analogue scale, was observed between the two groups. In addition, borrelia serology using an in-house whole bacterium ELISA and a commercial assay based on borrelial flagella as the antigen (IDEIA, Dako) was carried out on patient samples. No statistically significant difference in the antibody response was found between patients who received amoxicillin or placebo. Importantly, changes in antibody levels were not associated with clinical outcome indicating that the above tests can not be used to predict the outcome.
The objective of the present study was to examine the development of the borrelia specific antibodies in these patients using an array of borrelial antigens. Specifically, we wanted to look for serological markers for good response to antibiotic treatment.
Methods: Samples drawn from the patients at the initiation of the antibiotic treatment and about 12 months after the treatment were analysed using an in house recombinant IgG and IgM line immunoblot (19 IgG antigens, 18 IgM antigens; Goettner et al; J Clin Microbiol 2005) and recomBlot *Borrelia* IgG immunoblot (11 antigens; Mikrogen). All patients independent of the treatment were included in the analysis and paired samples were available from 110 patients. The association of outcome with changes in antibodies directed to individual antigens was statistically tested using Pearson's chi-squared test. P-values less than 0.05 were considered statistically significant.
Results:

i. Decline in IgG antibodies against VlsE was observed more often in patients with good outcome than in patients with poor outcome. The decline was observed more often in patients with LNB than in patients with LA.
ii. Decline in IgG antibodies against OspC was observed more often in patients with good outcome compared with patients with poor outcome. As with VlsE, decline in OspC IgG antibodies was observed more often in patients with LNB than with LA.

Conclusion: The results suggest that decline in antibodies directed to VlsE and OspC proteins may be used as an indicator of good outcome after antibiotic treatment in Lyme borreliosis.

Community-acquired lower respiratory tract infection

S496 It takes two to tango – bacterial-viral interactions in the pathogenesis of respiratory tract infections

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Over the last decade, influenza and pneumonia have ranked as the 7th leading cause of death in the United States. In the developing world, the problem is even more acute, as respiratory tract infections are the leading cause of death in children outside of the neonatal period. *Streptococcus pneumoniae* is the leading bacterial cause of pneumonia, sepsis, otitis media, and meningitis and accounts for a significant portion of this mortality. Influenza is also a major contributor, both as a primary infection and by interacting with bacterial pathogens to increase the incidence and severity of pneumonia. While the pneumococcus is the most common secondary pathogen following influenza in typical inter-pandemic years, affecting mainly the young and the frail elderly, *Staphylococcus aureus* is seen more commonly when highly virulent viral strains circulate, and has been emerging in recent years as a cause of necrotising pneumonia as a co-pathogen with influenza in healthy children and young adults. Our laboratory studies the mechanisms that underlie the interactions between respiratory viruses such as influenza virus and bacterial pathogens such as *S. pneumoniae* and *S. aureus*. The approach we have taken is to isolate specific virulence factors in the virus or bacterium and use genetic approaches to modify or delete them, allowing assessment of their impact in relevant animal models. Certain themes of cooperation between pathogens have emerged from this analysis, giving us insight into specific pathogenic mechanisms and revealing how these differ for different viral-bacterial pairs, or between different strains of one of the pathogens. These findings in the laboratory can then be related back to the epidemiology of co-infections in humans, helping define the challenges we face in moderating morbidity and mortality from these pathogens. The ultimate goal of this work is to apply this knowledge to facilitate treatment and prevention of these common diseases by targeting the cooperative interactions that make co-infections so deadly.

S497 Implications of bacterial-viral interactions to present day pandemic preparedness – the Spanish flu revisited

K.P. Klugman (Atlanta, US)

It was recognised during the so-called Spanish influenza pandemic in 1918 that the majority of deaths due to the flu occurred more than 7 days after the onset of symptoms. In the overwhelming majority of cases of pandemic influenza, signs and symptoms resolved by day 6, but in a subgroup with significant mortality, illness worsened in the second week of illness. There is abundant evidence that most of the mortality during the pandemic was due to the combination of influenza plus bacterial super-infection. The most common bacterial cause of death was the pneumococcus, but nosocomial spread of group A streptococci was often lethal, as were staphylcocccal infections. Up to 50% of young soldiers' deaths due to influenza in 1918 were complicated by bacteraemia. While antibiotics were not available in 1918, it is naive to believe that antibiotics will reliably save individuals suffering from the cytokine storm of pandemic influenza and simultaneous bacteraemia. Prophylactic antibiotics should be considered, but as progression to pneumonia may be impossible to predict, the amount of antibiotic needed to be given will be considerable (to all those infected with influenza). An additional approach to reduce mortality is vaccination – vaccination against the pandemic influenza strain will be the first priority if such a vaccine is available. Vaccines are currently however available to prevent pneumococcal infection. Data from a randomised trial of pneumococcal conjugate vaccine (PCV) show that children immunised with PCV have 45% less hospitalisation for pneumonia due to endemic influenza. The 23 valent pneumococcal vaccine (23v) is highly protective against bacteraemia when given to healthy young adults. Widespread PCV administration to children including a booster dose in the second year of life is essential to ensure their protection plus the best chance of herd immunity to the PCV types in the older population. Healthcare workers, military personel and other first responders should receive the 23v vaccine when the pandemic threat level reaches level 5. Attempts should be made at that time to also strengthen existing recommendations to immunise at risk and elderly persons with the 23v vaccine. Pandemic influenza plans at present fail to recognise the potentially essential role of pneumococcal vaccine in the prevention of mortality from pandemic influenza.

Improving the speed of diagnosing fungal infections

S500 To culture or not to culture? That is the question

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The number of immunocompromised patients continues to rise globally promoting increase in the incidence and variety of life-threatening fungal infections. In addition, the epidemiology of invasive fungal infections (IFI) is changing; IFI such as aspergilloses, are increasingly reported in non-neutropenic paediatric and adult critically ill patients. As a result, there is pressing need to improve the accuracy and speed of diagnosis to proficiently support clinical decisions.

Despite the recognised problematical nature of culture methods for the identification of fungi in clinical specimens, which rely on the isolate's phenotypic differences in morphology and physiology, and when possible on its ability of mating, the clinical value of culture-based diagnosis remains indisputable. Positive culture establishes the diagnosis of IFI and influences therapeutic options either through susceptibility testing or via accurate characterisation of mould isolates with inherent resistance to amphotericin B-eg. Scedosporium spp., *A. terreus* or A. nidulans.

Microscopy of histological specimens, using fungal-specific stains, like Gomori's methenamine silver (GMS) complemented with periodic acid-Schiff (PAS) can reveal fungal elements and tissue-fungus cellular interactions. Also, direct microscopy of wet potassium hydroxide mounts of biological fluids, and fluorescent microscopy with Calcofluor white, Uvitex 2B or Blankophor P, of either fresh clinical specimens, such as BAL and corneal specimens or paraffin-embedded tissue, provide rapid results on presence/absence of fungal elements. Although positive histology and direct microscopy are non-specific tests, since sporogenous structures are typically absent in human material, they nonetheless give a fast diagnostic clue to the presence of regularly or irregularly, septate, aseptate or rarely septate hyphae, dictate use of selective media for culture and justify administration of antifungal therapy. Furthermore, culture-based identification by morphological and physiological criteria is complex. In many taxa definitive phenotypic features are difficult to observe or are highly variable. Besides, most clinical isolates lack a sexual cycle and for many pathogens the concept of a species is still poorly defined. Consequently, there is compelling need for rapid, precise and reproducible molecular methods to identify clinical fungi.

To effectively exploit the tremendous variation in the DNA of fungi in order to (a) determine the source of a fungal infection, (b) to resolve the status of certain established and emerging pathogenic species, (c) to track the transmission of strains in the hospital, (d) to recognise strains of specific virulence or resistance to antifungals, (e) to elucidate diversity and population genetics of fungal pathogens, and (d) to identify genotypes for use in drug research and diagnostics, the question "to culture or not to culture", even in the ". . . omics" era, is purely rhetorical.

Epidemiology of MRSA and VRE

O508 High prevalence of "livestock-associated" meticillin-resistant *Staphylococcus aureus* ST398 in swine and pig farmers in Belgium

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Backgrounds: Meticillin-resistant *Staphylococcus aureus* (MRSA) is a major pathogen in hospitals and in the community. Recently, a novel MRSA genotype has been reported among livestock animals, farmers and veterinarians in the Netherlands and neighboring countries. These animal associated strains are resistant to SmaI macrorestriction and belong to sequence type (ST) 398 by MLST. The objectives of this study were to determine the prevalence and risk factors for MRSA carriage in farmers and their household contacts in Belgian swine farms and characterise these stains genotypically and phenotypically.

Methods: Farmers and household contacts residing on 49 swine farms were screened for MRSA carriage by culturing nasal swabs onto selective agars. MRSA identification were confirmed by PCR and genotyped by pulsed field gel electrophoresis (PFGE) after SmaI macrorestriction, staphylococcal cassette chromosome mec (SCCmec) typing, spa sequence typing and MLST. Susceptibility to 18 antimicrobials was determined by the disk diffusion method. Demographic data, animal exposure and medical history were recorded to determine risk factors of MRSA carrier status in this population.

Results: Of 127 farmers screened, 48 (38%) were positive for MRSA. Carriers were found in 25 farms from seven provinces of Flanders and Wallonia. The prevalence was independently associated with porcine MRSA carriage and the frequent contact with pigs, horses and dogs. MRSA carriage was associated with higher levels of personal protection and hygiene. By molecular typing, all isolates were resistant to SmaI digestion and belonged to ST398 with spa types t011 or t034 with SCCmec type IV or type V. MRSA strains were resistant to tetracycline (100%), trimethoprim (100%), MLS (>50%), aminoglycosides (>40%) and ciprofloxacin (32%). The MRSA strains from human shared the same characteristics as those found in pigs.

Conclusions: A high prevalence of MRSA carriage was found (38%) in swine farmers and family members. Risk factors for MRSA colonisation included frequent contact with pigs and other farm animals and higher levels of protection and hygiene. MRSA strains from farmers and pigs belonged to the ST398 "animal MRSA clone" which has been reported in livestock animals, farmers and veterinarians in Europe.

O509 Emergence and dissemination of USA300 MRSA in Australia

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Objective: To report the emergence of ST8-MRSA-IVa (USA300 MRSA) in Australia.

Background: A state-wide MRSA management programme has prevented healthcare associated MRSA clones from becoming endemic in Western Australian (WA) hospitals. The programme that commenced in 1982 is similar to the "Search and Destroy" policy used in northern Europe and involves the notification of all MRSA positive patients and healthcare workers with referral of isolates to the Gram Positive Bacteria Typing and Research Unit for epidemiological typing.

Although multiple clones of community associated MRSA (CA-MRSA) have been characterised in WA (WA CA-MRSA), they have rarely caused healthcare associated outbreaks. Several non WA CA-MRSA community clones have recently been isolated in WA including the "European CA-MRSA" (ST80-MRSA-IV), the "Taiwan CA-MRSA" (ST59-MRSA-VT), the "Western Samoan CA-MRSA" (ST30-MRSA-IV) and the "Queensland CA-MRSA" (ST93-MRSA-IV). Unlike WA CA-MRSA, these clones are generally Panton-Valentine leucocidin (PVL) positive and are commonly associated with skin and soft tissue (SST) infections. Although found in some hospitalised patients, nosocomial transmission has not been documented.

Methods: Phenotypic and genotypic characterisation of MRSA.

Results: Using MLST/SCCmec typing 38 "ST8-MRSA-IVa" isolated in WA were found to be PVL positive and had the same pulsed-field gel pattern and spa type (t008) as the USA300 MRSA clone. The majority of these isolates were from SST infections predominantly with abscess formation. Over 66% of patients were younger than 40 years. Thirty one of the strains were erythromycin resistant (EmR), with 87% clindamycin susceptible (inducible resistance not detected – unlike EmR WA CA-MRSA which are generally inducibly resistant to clindamycin). From January 2004 to October 2007 the number of isolates increased several-fold (from 4 to 17 cases per year) prompting public health concern.

Conclusions: In the United States USA300 MRSA is not only the predominant cause of community acquired infection but is also rapidly emerging as a major cause of healthcare acquired infection. It is apparent that USA300 MRSA has entered the WA community. As a consequence the WA Health Department has recently commenced a "Search and Destroy" policy aimed at both this and other PVL positive imported

strains of CA-MRSA In addition, in some hospitals, screening has been extended to high risk and surgical unit admissions.

O510 Prevalence of ST398 and other genotypes of meticillin-resistant *Staphylococcus aureus* in Dutch hospitals

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Objectives: To determine prevalence of ST398 MRSA (associated with professional exposure to animals) and other MRSA isolates within Dutch hospitals.
Methods: A prevalence survey in 51 Dutch hospitals from July 2006 to January 2007 (3 months retrospectively and 3 months prospectively), monitoring all patients and healthcare workers (HCWs; only in post-exposure surveys) screened for and identified with ST398 or other MRSA strains (including several clinical characteristics) was carried out. Participating hospitals were divided into tertiles based on pig-density in the catchment area.
Results: During 306 months of observation 7802 patients were screened; 12% (642/5543) because of a risk factor for ST398 (79% out-patient department). Of the 498 new MRSA patients identified (65% with screening; 35% unexpected carriers) 31% carried ST398 MRSA (92% detected through screening). The overall prevalence of MRSA in the screened population was 3.6% (95%-BI: 3.2–4.0). Prevalence were 2.5% (95%-BI: 1.9–3.1), 3.8% (95%-BI: 3.1–4.5; $p = 0.007$) and 4.6% (95%-BI: 3.7–5.5; $p = 0.000$), respectively, in low, moderate and high pig density region hospitals. Prevalence of ST398 MRSA was 12.4% in patients screened in hospitals in regions with a high or moderate pig density and 0% in hospitals in regions with a low pig density. The prevalence of other MRSA isolates in patients screened in these hospitals was between 1.1–2.9%. In post-exposure screenings, unrelated ST398 MRSA carriers (index case had different genotype) were found in 0.06% (3/4794) of HCWs and none of the patients (0/1951). Other MRSA isolates (genotype different from index case and excluding ST398) were detected in 0.1% (8/5758) of HCWs and 0.5% (10/2134) of patients.
Conclusion: In this first large survey of ST398 MRSA in Dutch hospitals we found that MRSA is more prevalent in regions with a high or moderate pig density, solely because of an increase of ST398 MRSA. Almost 80% of new carriers are detected in out-patient departments and 92% because of active surveillance. ST398 MRSA colonisation is rarely encountered in HCWs and patients during post-exposure screenings of index cases with different genotypes.

O511 Enterotoxic meticillin-resistant *Staphylococcus aureus*: prevention and control

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Objectives: Superantigenic exotoxins such as toxic shock toxin 1 appear to be major virulence factors in hospital meticillin-resistant *Staphylococcus aureus* clones (HA-MRSA) in Japan, and staphylococcal enterotoxin may be involved in the septic shock and MRSA enterocolitis. To control enterotoxic MRSA infections, we examined the homeostasis of intestinal bacteria.
Methods:

1. MRSA proliferation according to the control of intestinal bacteria:
   A. Four GAM broth tubes were prepared, and tubes were inoculated 10^5 cfu/ml of viable MRSA328GTS, *Escherichia coli* ATCC25922, *Enterococcus faecalis* ATCC29212, and *Bacteroides fragilis* GM7000, and cultivated for 24 hours (= independent cultivation). Next, a GAM broth tube was prepared, and inoculated mixed up with above 4 strains (= mixed cultivation).
   B. After managed under eight days of total parental nutrition (TPN) and continuous administration of antacid, viable bacterial counts in large bowel of male Wistar rats (body weight 250g) were counted after an inoculation of 10^9 cfu/rat MRSA238 solution by gastric tube on day four.
2. Control of intestinal flora and MRSA proliferation:
   A. Viable MRSA counts were compared among mixed cultivation tubes added moxalactam (MOX), metronidazole (MTN), and MOX+MTN.
   B. (1) Chemoprophylaxis (CP) group: rats were inoculated MRSA solution after three days of oral antibiotic, kanamycin and MTN. (2) MOX group: rats were administered MOX for three days after an inoculation of MRSA solution. (3) Probiotics group: Probiotics, bio-three(R) 1g/day, were orally administered every day after inoculation of MRSA solution.

Results:

1. (A) Independent cultivations were at 11, 9, 9, and 10, and mixed cultivations were at 5.2, 7.6, 7.5, 9.5 (logCFU/ml).
   (B) Viable bacterial counts of the *S. aureus* in TPN was 3.6, and the MRSA in TPN+MRSA was 3.0 (not significant), and was able to consider the MRSA multiplication control by intestinal bacteria.
2. (A) the counts of MRSA were 3.7, 4.5, and 7.5, respectively. MRSA were proliferated under suppression of two or more major intestinal bacteria.
   (B) The counts of MRSA were elevated to 6.8 in CP+MRSA+MOX, and diarrhoea was observed. The counts were decreased to 1.8 after treatment by probiotics, and frequency of the diarrhoea was decreased from 60% to 25%.

Conclusion: Disturbances on intestinal flora were suggested to be a major cause of enterotoxic MRSA infections, and a control of intestinal flora will be important to prevent enterotoxic MRSA infections.

O512 Epidemiological surveillance of MRSA using Raman spectroscopy

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Objectives: Meticillin resistant *Staphylococcus aureus* (MRSA) is a leading cause of hospital acquired infections. Efficient infection control and monitoring of this microorganism depends on microbial typing. Genetic techniques such as Pulsed Field Gel Electrophoresis (PFGE) provide high discriminating power, but are time consuming and costly. In the recent years we have transformed Raman spectroscopy into a rapid identification and typing tool in clinical microbiology. This technique provides information about the overall molecular composition of intact bacterial cells and is highly specific. Over 100 isolates were used to validate Raman spectroscopy as a typing method for MRSA, according to recently published guidelines.
Methods: All isolates were cultured for 20 hr on Trypticase Soy agar plates. Biomass was suspended in 10 microliter of sterile distilled water, transferred onto a quartz slide and allowed to dry. Spectroscopic fingerprints were obtained using a dedicated Raman spectrometer, requiring 10 to 60 seconds per sample. Cluster analysis on these fingerprints was performed using the pair wise correlations as a distance measure in combination with Ward's cluster algorithm.
Results: The reproducibility of the Raman procedure is high. Multiple measurements of the same set of isolates resulted in isolate-specific clusters. Using a well-characterised strain collection, we found that the discriminatory power (D=0.98) is comparable to that of the gold standard, PFGE (D=0.99–1).
Conclusion: Using Raman spectroscopy as a typing method, a significant decrease of turn-around time can be achieved, allowing interventions to limit transmission to be taken earlier. The typing information gathered with this technique is comparable to information obtained by genotyping methods. Therefore we conclude that Raman spectroscopy is an easy-to-use and rapid alternative in the battle against MRSA.

O513 German *Enterococcus faecium* bacteraemia isolates from 1991 to 2007 show a complex clonal structure and multiple acquisitions of virulence genes and vancomycin resistance gene clusters

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Objectives: To investigate the characteristics and molecular dynamics of *Enterococcus faecium* bacteraemia isolates from Germany between 1991–2007 in the background of rising VRE rates in Germany.
Methods: Altogether 124 isolates from 35 German cities were included. The isolates were taken from our strain collection from the last 16 years. Copy isolates were excluded. They were typed by MLST and MLVA. The presence of virulence marker genes esp and hyl and the van genotype (vanA/B) were identified using a multiplex PCR. A PCR for the enterococcal IS element IS16 enriched in hospital-adapted strains was also performed. Antibiotic susceptibilities were determined by microbroth dilution according to the German DIN standard.
Results: Only two isolates were not typeable by MLVA. MLST was more discriminatory than MLVA (discriminatory index 0.897 vs. 0.824) giving altogether 31 different MLST vs. 26 MLVA types. Almost all isolates (n= 119/124) were ampicillin resistant. Based on MLST more than 95% of the *E. faecium* isolates belonged to the clonal complex CC17 of hospital adapted, epidemic strains. 63 *E. faecium* were vancomycin resistant (51%; 58 vanA, 5 vanB). About half the isolates possessed the esp gene (n= 64/124; 52%) and 52 (42%) the hyl gene. IS16-PCR was positive in 98 *E. faecium* (79%). *E. faecium* isolated before 2000 seldom possess esp or hyl, among isolates from 2004 on both became more frequent. Some clonal types like ST17 and ST18 were isolated over a range of 10–12 years. Possession of virulence genes esp and hyl varied suggesting acquisition or loss of those markers at multiple times. Other clonal types like ST192 mainly appeared between 2004–2007 and showed a fixed virulence gene pattern: 21/24 ST192 isolates possessed both esp and hyl, each one possessed either hyl or esp and one ST192 from 1998 lacks both determinants.
Conclusions: Only few *E. faecium* from the early 1990ies were included and those mostly did not belong to CC17. They were from single infections and thus not part of a cluster or outbreak. Nowadays the hospital-adapted *E. faecium* may represent a different pathogenic quality. Our data show association of those isolates mainly with clusters or outbreaks and enrichment of virulence-associated genes (esp, hyl) among different clonal types over time. Even in settings where VRE are still rare, hospital-adapted types are widely distributed awaiting acquisition of vancomycin resistance determinants as a next step.

O514 Environmental contamination: a risk factor for acquisition of CC17 ampicillin-resistant *Enterococcus faecium*

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Objectives: The last decade colonisation and infections with ampicillin-resistant *Enterococcus faecium* (ARE) increased in our hospital, a trend seen worldwide. Based upon MLST, epidemic and most invasive ampicillin resistant isolates cluster in clonal complex 17 (CC17). Patient-to-patient transfer via hands of healthcare workers (HCW)is considered an important route in the spread of ARE. In addition, environmental (env.) contamination may contribute to ARE acquisition. In this study we quantified ARE colonisation and env. contamination rates on a haematology ward where patient colonisation is endemic in order to better understand the role of env. contamination in ARE epidemiology.
Methods: Between March 5th and May 25th '07, all admissions (adm) on a 20-beds haematology ward were screened for rectal ARE-carriage <24 hrs after admission and <48 hrs before discharge. Subsequently, the environment of ARE-carriers was screened for ARE at 8 predetermined sites (blood pressure cuff, over-bed table, television remote control, bed rails, inside handle bathroom door, soap dispenser, toilet seat, control panel infusion pump) once weekly and after discharge until all swabs were negative. Swabs, enriched in Enterococcal Broth, were cultured on Enterococcosel agar plates with ampicillin (16 μg/ml). All ARE isolates were typed with MLVA.
Results: Of 72 adm, 64 (89%) were screened for ARE on adm, of which 14 (22%) were colonised. Of 35 ARE negative adm that were screened before discharge, 9 acquired ARE: acquisition rate of 26%. The mean colonisation pressure was 38% (range: 14–69%). From 18 colonised adm 412 env. swabs were taken of which 98 (24%) were ARE positive. Sites most often contaminated were the toilet seat (43%), over-bed table (38%) and television remote control (31%). Genotyping revealed presence of 3 circulating CC17 strains (MT1, MT159 and vancomycin-resistant MT287). In 96% of adm the rectal strain was concordant with the strains isolated from the environment. MT159 was found predominantly: in 16 (70%) colonised patients and 73% of the positive env. cultures.
Conclusion: Endemicity of ARE colonisation on our haematology ward is characterised by high admission, high acquisition and high env. contamination rates of CC17 ARE. The frequently occurring env. contamination may act as an additional source for cross-transmission, propagating ARE directly or via contaminated HCW. Infection prevention measures for ARE (or VRE) should not only target on direct patient-to-patient transmission, but also on env. hygiene.

O515 First report of clinical and epidemiologic characterisation of vancomycin-resistant enterococci from mainland China

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Objectives: Although infections caused by vancomycin-resistant enterococci (VRE) have been reported increasingly worldwide, there have been rarely reported in mainland China. We investigated the clinical and epidemiological characteristics of VRE nosocomial infections in Beijng Chaoyang Hospital, a 1100-bed tertiary-care teaching hospital in Beijing, China.
Methods: A matched case-control study was conducted to identify the individual risk factors for VRE infection/colonisation, and a retrospective cohort study to examine the prognostic factors of VRE infection. van genes were detected by multiplex PCR. Pulsed-field gel electrophoresis was used for molecular typing.
Results: The rates of VRE isolation increased from 2.6% for 2003 to 6.8% for 2006. A total of 38 vancomycin-resistant single-patient isolates of VRE were recovered between June 2003 and March 2007. The multivariate analysis revealed two significant independent risk factors for VRE versus vancomycin-susceptible enterococci (VSE): previous use of vancomycin (OR 18.22; 95% CI, 4.57–72.6) and inclusion in a dialysis programme (OR 8.69; 95% CI, 1.94–38.84). Having a malignant disease remained protective in the multivariate model (OR 0.26, 95%CI, 0.07–0.97). Crude mortality rate differences were not statistically significant (VRE 53.6% vs VSE 38.6%; OR, 1.066; $p = 0.915$). The only epidemiologic risk factor for associated mortality by multivariate analysis was increasing severity of illness, measured by APACHE II score (VRE 17.49± 6.83 vs VSE 11.81±4.85; $p = 0.001$). The total hospital stay was longer in VRE-infected patients than in those with VSE infections (VRE 55.2±27.2 ds vs VSE 39.3±30.7 ds; $p = 0.022$). van B gene was detected in 14 *E. faecalis* isolates, all of which were identified as a single clone that was prominent before the year 2005. vanA gene was positive in 21 *E. faecium* isolates, 10 of which showed resistant to vancomycin (MIC $\geqslant$ 256mg/L), but sensitive to teicoplanin (MICs 2–12 mg/L). Twenty-one isolates of *E. faecium* belonged to 9 different clones, 90.4% of which were isolated in year 2006 and 2007.
Conclusion: There has been a significant increase in the numbers of VRE infections over 3 years in our institute. Previous vancomycin therapy and involvement in dialysis programme were risk factors for development of VRE infections. Surveillance for VRE, prudent use of vancomycin and strict adherence to infection control measures are required to prevent further emergence and spread of VRE.

O516 Increase of resistance to glycopeptides and epidemiological changes among nosocomial enterococci detected during 2007 in a Portuguese hospital

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Objectives: To monitor the prevalence and epidemiology of glycopeptide resistant (GR) and high-level gentamicin resistant (HLGR) *Enterococcus faecalis* (Efl) and *E. faecium* (Efm) in a Lisbon hospital during the first semester of 2007. To compare the results with data obtained during year 2006.
Methods: Microbial identification and antimicrobial susceptibility were performed with the VITEK 2 system. GR and HLGR Efl and Efm were confirmed by PCR detection of vanA/B and aac(6′)-aph(2″) genes. Other aminoglycoside resistance genes (aph(2″)-Ib, aph(2″)-Ic, aph(2″)-Id) and virulence genes (cylA, asaI, gelE, hyl) were detected by Multiplex-PCR. Clonal relationships were assigned by PFGE and virulence profiles. Multilocus sequence typing was performed among GR isolates.
Results: A total of 138 enterococci were collected in the first semester of 2007: 73% Efl, 26% Efm, and 1% E. casseliflavus. 6% of Efl and 14% of Efm were GR and approx. 46% were HLGR. Most (>80%) Efl were resistant to erythromycin, quinupristin-dalfopristin-Q/D and tetracycline-TE, 50% to ciprofloxacin-CIP, and 0% to ampicillin-AMP. One and 11 Efl were resistant and intermediate to linezolid, respectively. Comparing with Efl, Efm were resistant to AMP/CIP (100%) and resistance to TE (33%) and Q/D (5%) was lower. Only the aac(6′)-aph(2″) genes were detected (56 out of 63 HLGR). GR-Efl (n = 6) and GR-Efm (n = 5) were vanA-genotype. HLGR/GR-Efl (n = 45) were of 9 PFGE patterns, 6 of them not identified in 2006. PFGE-AO was prevalent (36 isolates) associated with genotypes cylA-asaI-gelE-esp (33%), cylA-asaI-gelE (28%), asaI-gelE (17%) and asaI-gelE-esp (11%). GR-Efl belong to the lineage ST6 (PFGE AO/cylA-asaI-gelE-esp or cylA-asaI-gelE). HLGR/GR Efm (n = 18) were of 10 PFGE patterns of which 5 were co-dominant (11 isolates). Distribution of virulence genes by proportion of PFGE patterns was: esp-45%; 22%-hyl/esp; 11%-hyl and 22% without virulence genes detected. GR-Efm belong to lineages ST17 (PFGE-x/hyl-esp), ST18 (PFGE-d/hyl-esp) and ST125 (PFGE-d/hyl).
Conclusions: Enterococcal infections increased in 2007 (138 isolates in 6 months), comparing with 2006 (171 isolates in 12 months) and GR increased from 3% to 9%. PFGE AO remains prevalent but carriage of the esp gene decreased from 90% to 40%. New Efm clones with different genetic backgrounds emerged in 2007 indicating the need for active surveillance in this particular hospital as well as in other Portuguese hospitals where a similar trend may be observed.

O517 Ampicillin-resistant *Enterococcus faecium* clonal complex 17 is widespread in healthy dogs: anthropozoonosis or zooanthroponosis?

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Objectives: An increase in nosocomial infections caused by ampicillin-resistant *Enterococcus faecium* (AREfm) has been recently observed in some European countries. Based on multilocus sequence typing (MLST), most hospital AREfm isolates belong to one distinct genogroup, clonal complex 17 (CC17). In this study, we investigated the occurrence of AREfm CC17 in faecal samples collected from healthy dogs in Denmark and in England.
Methods: 210 healthy dogs were screened for the occurrence of AREfm using a selective isolation procedure, i.e. plating on Slanetz Bartley agar containing 32 μg/ml ampicillin. Presumptive AREfm were confirmed by a species-specific PCR and their resistance patterns were determined according to CLSI guidelines. The purK gene was sequenced in all isolates and a subset of 15 isolates was further analysed by MLST analysis.
Results: AREfm was detected in 59 (28%) dogs. Based on MLST or identification of the CC17-specific purK 1 allele, at least 44 (75%) of the isolates belonged to CC17. Four sequence types were observed: ST78 (n = 8), ST19 (n = 3), ST192 (n = 3) and ST266 (n = 1). All these genotypes have been previously isolated from hospitalised patients. In particular, ST78 and its single-locus variant ST192 are among the most common STs in European hospitals. ST78 was isolated from a dog and 10-year old boy living in the same household, suggesting possible transmission between dogs and humans living in close contact. Resistance to erythromycin (97%), ciprofloxacin (95%), tetracycline (83%) or rifampicin (56%) was frequent. Only few isolates were resistant to gentamicin (5%), linezolid (14%) and quinopristin/dalfopristin (15%) and all were susceptible to vancomycin.
Conclusion: This is the first report describing the occurrence of AREfm CC17 in dogs. The results suggest that dogs may contribute to the spread of this AREfm genetic lineage in the human population. The unexpected and widespread occurrence of hospital-adapted clones in dogs raises an important question concerning the evolution of this clonal complex: does CC17 originate from humans (anthropozoonosis) or from dogs (zooanthroponosis)? Canine AREfm are currently screened for putative virulence genes (esp, acm, hyl, orf903, orf905, orf907, orf2351 and orf2430) and the results of this screening will be used for discussing the evolutionary relationship between human and canine strains in the conference presentation.

HIV/AIDS: clinical science and therapy

O518 Compliance with screening for hepatitis B virus and hepatitis C virus infection prior to initiation of antiretroviral therapy among HIV-1 infected patients in a resource-limited setting

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Objective: To assess the compliance of laboratory screening for hepatitis B virus (HBV) and hepatitis C virus (HCV) infection prior to initiation of antiretroviral therapy (ART) among HIV-1 infected patients in a resource-limited setting.
Methods: This observational study was conducted by including HIV-1 infected patients from 3 cohorts of ART initiation in a tertiary-care HIV clinic in Bangkok, Thailand, between January 2004 and October 2007. The medical records were reviewed; demographic data, baseline CD4 and HIV-1 RNA, ART regimen, and date and test results of HBV surface antigen for HBV infection and Anti-HCV antibody for HCV infection were retrieved from the records.
Results: A total of 638 patients were included; mean (SD) age was of 38.4 (8.4) years and 53% were male. Median (IQR) baseline CD4 cell count and HIV-1 RNA were 246 (77–459) cells/mm^3 and 143,000 (45,825–445,000) copies/mL, respectively. Prior to initiation of ART, HBV infection and HCV infection were screened in 371 (58%) patients and 273 (43%) patients, respectively. All patients who were screened for HCV infection were also screened for HBV infection. There were no differences of demographics or baseline characteristics between patients who were screened for HBV or HCV infection and those who had never been screened for HBV or HCV infection ($p > 0.05$). Among 371 patients who were screened for HBV infection, 36 (9.7%) had HBV infection. HCV infection was found in 24 from 273 (8.8%) patients who were screened for HCV infection. Infection of both HBV and HCV was observed only one (0.4%) patient. NNRTI-based ART regimens were initiated in 573 (90%) patients; the rest received PI-based regimens. Of 638 patients, 625 (98%) received 3TC in the regimens. There was no difference of ART regimen or 3TC use between patients who had and did not have HBV infection ($p > 0.05$). After availability of tenofovir in Thailand in December 2006, patients who were found to have HBV infection from screening received tenofovir with lamivudine in ART regimen.
Conclusion: In resource-limited setting, only approximately half of HIV-1 infected patients get screening for HBV infection and HCV infection prior to initiation of ART. Lack of screening is observed in generalised population of HIV-1 infected patients and is not associated with any factor. Since the prevalence of HBV infection is relatively high, screening

of HBV infection prior to initiation of ART should not be omitted in resource-limited settings.

O519 Non-AIDS defining non-HAART-related severe clinical events have a high incidence in HIV-patients on HAART and are associated with virological failure: a 7-years follow-up cohort study (ANRS CO8)

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Objectives: To determine the incidence and risk factors of non-AIDS defining and non-HAART-related (NANHR) severe clinical events in a large cohort of HIV-infected patients in France.
Methods: The APROCO/COPILOTE (ANRS CO8) cohort enrolled 1281 patients, in 1997–1999, at the initiation of a protease inhibitor-containing antiretroviral regimen. All severe clinical events (grade 3 or 4 events according the ANRS classification, hospitalisation, extension of hospitalisation or death) were reviewed by a validation committee. Risk factors for NANHR severe clinical events were analysed using multivariate Cox models with CD4 and plasma HIV RNA viral load (pVL) as time-dependent covariates.
Results: After a median follow-up of 88 months (7664 patient-years), 713 NANHR severe clinical events were recorded in 385 patients. Incidence of NANHR severe clinical events was higher than AIDS-related events one, 9.3/100 patient-years and 2.0/100 patient-years, respectively. Most frequent NANHR severe clinical events were bacterial infections (n = 196, 27%), cancer-related events (n = 68, 9.5%), cardiovascular (n = 68, 9.5%) and psychiatric events (n = 61, 8.5%). In the adjusted multivariate analysis, occurrence of a first NANHR severe clinical event was significantly more frequent in patients older than 60 years (hazard ratio [HR] 2.1; 95% confidence interval [CI] 1.3–3.2), co-infected with HCV (HR 1.7; 95% CI 1.4–2.1), a CD4 <100 cells/mm3 at the time of the event (HR 2.5; 95% CI 1.8–3.6) and a pVL > 4 log10 copies/mL at the time of the event (HR 1.9; 95% CI 1.5–2.5). Factors associated with bacterial infections were similar to those associated with all types of NANHR severe clinical events, and the risk (HR) increased with the level of virological failure: 2.48 (95% CI 1.48–4.17) for pVL >4 log10 and <5 log10 copies/mL and 4.08 (95% CI, 2.28–7.36) for pVL > 5 log10 copies/ml. The only factor associated with non-AIDS cancers was age (HR 2; 95% CI 1.6–2.6) but a CD4 >500 cells/mm3 at the time of the event tended to have a protective effect (HR 0.5; 95% CI 0.3–1; p = 0.06).
Conclusions: We recorded a high incidence of NANHR severe clinical events (4 fold than that of HIV-defining events) in our patient population. Virological failure may favour the occurrence of NANHR severe clinical events and especially bacterial infections. These results give further arguments against interruptions of antiretroviral treatment.

O520 Natural polymorphisms associated with integrase inhibitor drug resistance in Estonian HIV-1 CRF06$_{cpx}$ strains

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Objectives: We aimed to characterise the genetic diversity in integrase (IN) region of HIV-1 circulating in Estonia and to compare their genetic diversity with that of B-subtype viruses in ARV treatment naive (ARV-TN) and experienced (TE) patients.
Methods: A total of 104 ARV-TN (median age 26 y; 57 male; 59 IDU) and 10 TE but none treated with integrase inhibitors (INI) were analysed. All viruses in the latter population had at least one primary RT DRM with the average number of 2.8. The most commonly seen mutations were K103N (n = 9), M184V (n = 7) and V179E (n = 5). A direct sequencing of plasma viral RNA for both populations was performed in IN region (aa 1–289). Subtyping was carried out using phylogenetic analysis (neighbor-joining). The functional positions and the prevalence of reported INI resistance mutations (A38K, H51Y, T66I, V72I, L74IMA, V75I, E92QD, T97A, F121NY, T125KY, A128T, E138K, G140AS, Y143HCR, Q146KR, S147G, Q148H, V151I, S153AY, M154I, N155HS, K156N, E157Q, K160D, G163KR, V165I, V201I, I203M, T206S, S230NR, V249I, R263K and C280Y) were analysed.
Results: The phylogenetic analysis in IN region revealed that a total of 101/114 viruses formed a highly homogenic cluster with CRF06$_{cpx}$ reference sequences, one with CRF02$_{AG}$, subtype A1 and subtype B each, and 10 remained unclassified.
Mean pair-wise genetic distances in TN and TE populations in IN region of CRF06$_{cpx}$ viruses were similar, 0.014 and 0.016, respectively. In TN population 43 integrase inhibitor DRMs were described, of which the following ones occurred with the highest frequency: V72I in 92%, L74I in 100%, V201I in 97% and T206S in 96% of cases. The amino acid distributions in CRF06$_{cpx}$ viruses at any position of IN region in treated and naive patients were similar. The DDE and HHCC site residues were absolutely conserved in all populations. Compared with the subtype B the presence of IN DRMs V72I, V201I and T206S was more common in CRF06$_{cpx}$ viruses and was seen with the frequency of 17% vs 92%; 11% vs 97% and 9% vs 96%, respectively.
Conclusions: The DRMs in RT region were not associated with the induction of any compensatory mutations in IN region. Compared with subtype B in CRF06$_{cpx}$ viruses the presence of naturally occurring secondary DRMs in IN region was more frequent. The importance of this during the ARV treatment with INI remains to be identified in future clinical trials.

O521 A single planned interruption of treatment in perinatally-HIV-infected children: effect on HAART-related toxicities

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Objective: HAART-related long-term toxicities are being increasingly reported in perinatally-HIV-infected children, including hyperlactataemia, dyslipaemias, and hepatic and bone marrow toxicity. Planned interruptions of HAART (PIH) arise with the potential to reduce drug exposure and toxicity.
Methods: We present a case series of 14 perinatally-HIV-infected paediatric patients (10 girls, median age 7.7 years at PIH) with optimal long-term response to a first-line HAART regimen who underwent a single PIH. Hemoglobin, total neutrophil counts, lactate levels, triglycerides, total, LDL and HDL cholesterol, alanine aminotransferase and amylase plasmatic levels were assessed at PIH and 12 months later, while off therapy. Non-parametric tests were used as appropriate.
Results: At PIH, patients had remained a median time of 4.8 and 4.5 years on therapy (5 out of 14 on a protease inhibitor-based regimen) and with complete suppression of viral replication, respectively. One month after treatment interruption, a blip in HIV plasmatic viral load up to a median value of 4.6 log copies/mL (range 2.9–5.6) was observed in all cases; HIV viral load stabilised thereafter. No clinical progression occured, despite a progressive decrease in CD4 cell percentages/counts was observed in most cases. None of the patients had to reinitiate therapy during the 12-month follow-up. One year after PIH, decreases in total cholesterol (median values, from 163 to 134 mg/dL; Wilcoxon rank test, p = 0.03), LDL cholesterol (from 90 to 77 mg/dL, p = 0.064), HDL cholesterol (from 60 to 43 mg/dL, p = 0.002) and lactate levels (from 1.3 to 0.9 mmol/L, p = 0.026) were observed. No changes were observed in the rest of studied parameters. All variables remained within normal/acceptables values at both time-points. None of the patients showed symptoms consistent with hyperlactataemia, hepatitis or pancreatitis, neither while on HAART nor during PIH.
Conclusions: In this series, a 12-month PIH lead to a significant decrease in total and HDL cholesterol, and lactate levels. However, all parameters remained within normal values at baseline and after PIH, and no clinical symptoms were observed. The long-term clinical consequences of HAART-related toxicities in perinatally-HIV-infected paediatric patients remain unknown, as well as the potential benefit of HAART-sparing strategies.

O522 SIV-specific CD8+ T-cells mediated protection from uncontrolled viral replication after vaginal challenge in live-attenuated immunised rhesus macaques

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Objectives: Live-attenuated lentivirus immunisation protects rhesus monkeys from uncontrolled viral replication after vaginal challenge with pathogenic SIVmac239. Local polyfunctional Gag-specific CD4+ and CD8+T cell responses are present in the genital tract of SHIV89.6-immunised rhesus macaques at the time of SIV challenge. To further assess the role of CD8+ lymphocytes in vaccine-induced protection, a group of SHIV-vaccinated monkeys was depleted of CD8+ lymphocytes on the day of challenge with SIVmac239.
Methods: SHIV89.6-immunised (n = 12) and non-immunised (n = 9) animals where necropsied at 14 days post-challenge (PC). Anti-CD8 (cM T807; 50mg/kg) was administered to an additional group of immunised monkeys (n = 6) on the day of the intravaginal challenge with SIVmac239. Lymphocytes from freshly digested cervicovaginal tissues, lymph nodes and peripheral blood where analysed by polychromatic flow cytometry for Gag specific responses (intracellular cytokines, degranulation and cell death/survival signals).
Results: Viral RNA (vRNA) levels were high in all tissues of non-immunised animals (GI tract ≫ systemic lymph nodes > genital tract). In immunised animals, virus replication was controlled in all tissues and SIV dissemination beyond the genital lymph nodes was limited. Only 2 immunised animals had moderate levels of vRNA in the GI tract and systemic tissues, with a similar distribution to the control animals. Strikingly, CD8+ lymphocyte depletion eliminated the beneficial effect of the SHIV immunisation, and by 7 days PC, this group had the highest plasma vRNA levels of all groups. Interestingly, the distribution of viral replication was different, and the highest levels of vRNA were found in the genital tract. Moreover, the only Mamu-A01*monkey in this group, which was the only animal that partially controlled the virus in the different tissues, was the only monkey with detectable specific CD8+T cell response in the genital lymph nodes.
Conclusion: In summary, the establishment of a memory SIV-specific polyfunctional T cell response in the genital tract induced by live-attenuated immunisation may account for protection from intravaginal SIV challenge. Further, depletion of CD8+T cells eliminates the live-attenuated lentivirus mediated protection.

O523 Long-term efficacy of nevirapine-based anti-retroviral therapy among HIV-1 infected patients with/without previous rifampicin, and treatment outcomes of tuberculosis: a 144-week prospective study

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Objectives: To evaluate the long-term efficacy of nevirapine (NVP)-based antiretroviral therapy (ART) among HIV-1 infected patients who previously received this regimen with rifampicin (RIF) and to assess the long-term outcomes of tuberculosis (TB) in a resource-limited setting.
Methods: HIV-1/TB co-infected patients receiving RIF (group A) and HIV-1 mono-infection not receiving RIF (group B) were enrolled to receive NVP 400 mg/day with stavudine and lamivudine in a prospective study. Plasma HIV-1 RNA and CD4 cell counts were studied every 12 weeks through 96 weeks and then every 24 weeks until 144 weeks. Re-evaluation of clinical TB and chest X-ray were performed at week 144. Genotypic resistance testing was conducted in patients who had HIV-1 RNA >1,000 copies/mL.
Results: Of 140 patients (70/group), 68% were male and median (IQR) CD4 was 29 cells/mm^3. Of 70 patients in group A, 31 (44%), 20 (29%), 14 (20%), 3 (4%), 2 (3%) patients were diagnosed with pulmonary TB, disseminated TB, cervical TB lymphadenitis, gastrointestinal TB and TB meningitis, respectively. By intend-to-treat analysis, 61% (43/70) in group A and 57% (40/70) in group B maintained plasma HIV-1 RNA <50 copies/mL at 144 weeks of ART (P = 0.731, OR=1.194, 95%CI=0.608–2.346). At week 144, median (IQR) CD4 was 367 (260–541) cells/mm^3 and 393 (286–501) cells/mm^3 in the corresponding groups (P = 0.646). Of 70 patients in each group, 10% (7/70) and 9% (6/70) patients in the corresponding groups developed HIV-1 RNA >1,000 copies/ml (P=1.000). For NRTI-resistance associated mutations, M184V was observed 71% (5/7) in group A and 83% (5/6) in group B (P=1.000). TAMs, K65R, and Q151M were observed 0% (0/7), 29% (2/7), and 0% (0/0) in group A and 17% (1/6), 0% (0/6), and 0% (0/6) in group B, respectively (P > 0.05). For NNRTI-resistance associated mutations, Y181C/I was the most common mutation and found 71% (5/7) in group A and 50% (3/6) in group B (P = 0.592). For 70 outcomes of TB in group A, 55 (79%) were cure/completed treatment; 7 (10%), 5 (7%), 2 (3%) and 1 (1%) were lost to follow-up, died, transferred care and recurrent TB, respectively.
Conclusions: There is no difference of the 144-week efficacy between HIV-1/TB co-infected patients receiving RIF and HIV-1 mono-infection not receiving RIF. Long-term TB outcomes are favourable. In resource-limited settings, NVP 400 mg/day-based ART is an appropriate option for HIV-1 infected patients who receive RIF.

O524 Efficacy and safety of once-daily atazanavir/ritonavir compared to twice-daily lopinavir/ritonavir, each in combination with tenofovir and emtricitabine, in antiretroviral naive HIV-1 infected subjects. The CASTLE Study (AI424–138) 48-week results

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Objectives: Atazanavir/Ritonavir (ATV/r) is as effective as Lopinavir/Ritonavir (LPV/r) with more favourable lipid and GI profiles in treatment-experienced HIV-infected patients. Comparative data in antiretroviral (ARV)-naive patients are needed.
Methods: CASTLE is a randomised, open-label, multicentre, ongoing 96 week study to assess non-inferiority (10% margin) of ATV/r 300 mg/100 mg once-daily (QD) versus LPV/r 400 mg/100mg twice-daily, both in combination with fixed-dose tenofovir (TDF) 300mg/emtricitabine (FTC) 200 mg QD, in treatment-naive patients. The primary endpoint was the proportion of patients with HIV RNA <50 c/mL at week 48; planned secondary assessments included percent with HIV RNA <400 c/mL, CD4 cell count change, and safety.
Results: 883 patients randomised, 878 treated. Baseline (BL) demographics and characteristics were well balanced. Median CD4 205 cells/mm^3; median plasma HIV RNA 4.98 log10 c/mL. At week 48, mean CD4 increases from BL for ATV/r and LPV/r were 203 and 219 cells/mm^3, respectively. Fewer patients on ATV/r (2%) than LPV/r (8%) initiated lipid lowering therapy. The proportion of patients with a TC: HDL ratio >5 at week 48 was 12% and 20% on ATV/r and LPV/r, respectively. Patients on ATV/r had a lower incidence of Grade 2–4 treatment-related diarrhoea (2% vs 11%) and nausea (4% vs 8%) than LPV/r. Grade 3–4 ALT/AST elevations were low (⩽ 2%) on both arms. Discontinuations prior to week 48 were: ATV/r, 9%; LPV/r, 13%. AE-related discontinuations were 2% and 3% on ATV/r and LPV/r, respectively. Three patients (<1%) discontinued ATV/r due to jaundice/hyperbilirubinaemia.
Conclusions: In treatment-naive patients, ATV/r demonstrated similar efficacy, a lower incidence of GI-related AEs, and a significantly better lipid profile (TC, TG, non-HDL) compared to LPV/r. In combination with TDF and FTC, both ATV/r and LPV/r were well tolerated with few discontinuations through 48 weeks.
These data have been accepted for oral presentation at the 15th Conference on Retroviruses and Opportunistic Infections, February, 2008. ECCMID would be the first European presentation of these important data.

	ATV/r	LPV/r	Difference Estimate (95% CI) (ATV/r – LPV/r)
CVR[a]	n = 440	n = 443	
% <50 c/mL	78	76	1.7 (–3.8, 7.1)
% <400 c/mL	86	82	3.3 (–1.5, 8.1)
CVR[a], Baseline CD4 <50 cells/mm^3	n = 58	n = 48	
% <50 c/mL	78	63	
Fasting Lipid mean % Δ from BL at 48 weeks[b]	n = 421	n = 415	
Total-C (TC)	12	24	–9.5 (–11.8, –7.0)[c]
LDL	12	15	–2.9 (–7.1, 1.5)
HDL	27	32	–3.8 (–7.8, 0.3)
Non-HDL	7	21	–11.6 (–14.5, –8.7)[c]
TG	13	51	–25.2 (–29.8, –20.2)[c]

[a]Confirmed Virologic Response (ITT), Non-Completers = Failure.
[b]Last Observation Carried Forward.
[c]$p < 0.0001$.

O525 Safety reporting in randomised clinical trials of HAART: systematic review

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Background: Selection of highly active antiretroviral therapy (HAART) is currently based on the relative efficacy and toxicity of the different regimens. While reporting of efficacy is standardised in most studies, the reporting of adverse events (AE) is highly variable. The CONSORT statement provides guidance on reporting of adverse events. In this study we assessed compliance with the CONSORT guidelines and the influence of sponsorship on AE reporting.
Methods: PubMed and CENTRAL were systematically searched for all published randomised controlled trials assessing HAART for naive adult HIV-infected individuals. Quality of AE reporting was assessed according to CONSORT guidelines. AE severity grading was based on the NIAID definitions.
Results: Forty two articles including 16045 patients met the inclusion criteria. AE definition was specified in 26 of the studies and discontinuation rules were specified in 10. AE collection mode was indicated in two studies only (questionnaire for lipodystropy development). In 40 studies no information regarding AEs assessment mode was given. Ten studies presented all harms, 23 presented only AEs attributed to study drugs and nine studies did not specify any information regarding AE attribution. Clinical AEs were reported with the following severity grades: 14 studies reported severity grades of 1–4, 16 studies 2–4, 10 studies 3–4 and two studies reported only AEs that led to discontinuation. Twenty seven studies reported AE that occurred only above a threshold. Eight studies reported AEs that occurred in more than 5% of the patients, 4 above 10%, 2 above 15% and one above 20%. Moreover, three studies reported selected AE without details of how or why the selection was done. Of the 42 studies, 14 were academic and 28 were sponsored by a pharmaceutical company. In 13 of the 14 academically sponsored studies all AE were reported (no threshold set), whereas only one of the 28 studies sponsored by the industry reported all AE ($p < 0.001$).
Conclusions: Substantial variability in AE compilation and reporting was found. Variability was influenced by sponsor identity (whether academic or pharmaceutical). These facts cast doubt on result completeness and on our ability to choose therapies based on current published data.

O526 Incidence and predictors of nevirapine-associated rash in experienced HIV patients who switched from other antiretroviral regimens to nevirapine-based regimen

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Objectives: Nevirapine (NVP)-related rash is well recognised adverse effect. NVP-related rash and hypersensitivity commonly occur in patients with high CD4. In naive patients, NVP should be avoided in male with baseline CD4 ⩾400 or female with CD4 ⩾ 250. However, NVP is alternative choice in some patients who have side effects from other regimens. Fast CD4 recovery, gender and ethnicity contribute to development of rash. In HIV-naive Thai patients, risk of rash from NVP is 30%, which is higher than other reports. NVP-related rash in experienced HIV patients who switched to NVP-based regimen has limited data.
Methods: We reviewed data of patients who switched antitretroviral regimen (ARV) to contain 200 mg bid of NVP in new regimen. Demographic data, previous ARV regimen, incidence and type of rash and outcome were analysed. The incidence of rash was compare with the results from 2NN study. Clinical data of patients with and without rash were compared. Severe rash is defined as having urticaria or rash with constitutional symptoms or serum sickness or Stevens Johnson Syndrome or toxic epidermal necrolysis.
Results: A total of 174 patients switched ARV regimen to NVP-based regimen and 162 patients had available data for further analysis. Mean and median CD4 of enrolled patients were 499 and 471 respectively. Most (95%) of patients had virologic control before switching to NVP. Most patients had no significant changes of CD4 before and after switching. Prevalence of rash was 21% and 9% of patients had severe rash. Using cut-off levels of CD4 at ⩾400 in male and ⩾250 in female, we found no significant difference between incidence of rash between the groups. Severe rash occurred in male and female patients with mean and median CD4 cells were 430,467 in male and 413, 342 in female respectively. In female who had rash and CD4 ⩾400, significantly had severe rash than female with CD4 <400 ($p < 0.05$).

Characteristics	Rash (35)	No rash (127)
Sex (M:F)	1:1.1	1:1.1
Mean body weight (BW) (kg)	55.6	56.6
BW in male patients (mean, median)	59.3, 61	61, 50
BW in female patients (mean, median)	52.8, 52	54.0, 53
CD4 (cell/mm^3)		
Mean	481	504
Median	474	466
CD4 in male patients		
Mean	508	514
Median	474	457
Range	203–841	62–1,251
CD4 in female patients		
Mean	463	488
Median	399	473
Range	203–1,023	59–1,233
Mean and median CD4 in male with severe rash	430, 467	
Mean and median CD4 in female with severe rash	413, 342	
Male patients with CD4 ⩾ 400	12/43 (27%)	31/43 (72%)
with CD4 < 400	2/25 (8%)	23/25 (92%)
p-value	p = 0.05	
Female patients with CD4 ⩾ 250	17/68 (25%)	51/68 (75%)
with CD4 < 250	4/14 (28%)	10/14 (72%)
p-value	p = 0.78	

Severe rash is defined as having urticaria or rash with constitutional symptoms or serum sickness or Stevens Johnson Syndrome or toxic epidermal necrolysis.

Conclusion: Incidence of NVP-related rash in experienced HIV patients with high CD4, is lower than naive patients (21% VS 30%). No rapid rising of CD4 levels after switching to NVP, may explain lower incidence of rash in our patients. Most occurrences of rash are mild. Cut-off levels

of CD4 could not predict the risk for rash development. However, female have more chance to develop severe rash from nevirapine compare to male who have same level of CD4. Switching ARV to NVP-based regimen should be cautioned and carefully monitored, especially in female with CD4 $\geqslant$ 400.

O527 CXCR4-using HIV-1 in antiretroviral-naive patients with primary or chronic infection

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Objectives: To characterise correlates of CXCR4-using (X4) or CCR5-using (R5) HIV-1 strains in a cross-sectional analysis of therapy-naive patients (pts).
Methods: Plasma from 26 pts with acute-recent-infection (<1 year, AR), 22 with early-chronic- (>300 cd4/ul, ECh) and 24 with advanced-chronic-infection (<300 cd4/ul, AdvCh), all harbouring HIV-1-B-subtype, were evaluated by sequencing a 35-amino-acids region from env gene, and by interpretation with PSSM-genotype prediction. In a subset of pts, peripheral blood mononuclear cells (PBMC) and cerebrospinal fluid (CSF) were tested.
Results: The cd4 median was 606 (range 230–1143), 395 (304–1145) and 153 (2–291) cells/ul for AR, ECh- and AdvCh-infected, cd4 percentage was 25 (8–44.5), 29.5 (15–36.6), and 14 (2.4–24.2), HIV-RNA plasma viraemia was 150,600 (145–450,294), 78,566 (45–262,900) and 65,403 (2725–665,993) copies/ml, and HIV-DNA proviral loads was 864 (93–18,005), 1279 (55–6357) and 3237 (45–17,308) copies/10^6 PBMC, respectively. At sequence evaluation R5-strains were found in all pts, with the exception of five X4-strains among AR (19.2%) and four X4-strains in AdvCh (16.6%) (p = not significant in any possible comparison by Fisher exact test). Among AR pts, those with X4-strains had similar cd4 (578 vs 633), lower cd4 percentage (24.5 vs 28.6), similar HIV-RNA plasma levels (150,600 vs 167,616) and higher DNA-load (2441 vs 696) with respect to R5-strain-harbouring subjects.
At PBMC-strain analysis, 4 AdvCh-pts with plasma-X4-strains revealed a concordant X4-strain in 3 cases, and an R5-strain in 1. 8 AdvCh-pts with plasma-R5-strains revealed a concordant R5 strain in 7 cases, and an X4-strain in 1.
Among 5 plasma-X4 AR-pts, 1 R5-strain was found in PBMC. 7 plasma-R5 acute-pts had an R5-strain in PBMC. Among few CSF studied, an X4 was found, from a AR-pt harbouring R5-strains in plasma and PBMC.
Conclusion: The presence of X4 variants is described to be more frequent in the late stages of the disease, but frequently can be transmitted as a X4/R5 mixture and X4-strains can be demonstrated without clonal analysis in the early phase of infection. In AdvCh-pts discordant pictures in different compartments can be found. These evidences may have implications for therapy, suggesting a co-receptor-use analysis of both plasma- and PBMC-strains as soon as possible after diagnosis. Cautions have to be maintained before using CCR5-inhibitors in the very early stages of disease, monitoring for an early selection of X4 variants.