Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Dendograms in microbial phylogenetics and epidemiological typing (Symposium arranged with ESGM)

S8

Evolutionary trees in microbiology

B. Hall *(Bellingham, US)*

Phylogenetic trees are just tools for representing the relationships among organisms. The focus of this presentation is on the interpretation of phylogenetic trees, rather than on construction of those trees. Elements that will be considered are: what are branches and nodes and what do they mean? Trees are typically drawn so that they appear to depict the order of descent from common ancestors, i.e. the direction of evolution. That appearance is often misleading. Direction of evolution and order of descent are only shown by rooted trees, whereas most phylogenetic methods produce unrooted trees. What information is required to root a tree? Trees of microbial isolates often include zero-length branches. What do zero-length branches really mean? Trees are only estimates of historical relationships they are not facts. How do we determine the reliability of those estimated trees? Recombination can dramatically affect the validity of phylogenetic trees. What do we do when there is too much recombination to construct valid phylogenetic trees?

S9

Representations of strain similarities in molecular microbial typing

P. Vauterin *(Ghent, BE)*

This talk covers a variety of grouping techniques that are used to represent the relations between strains using molecular typing data. For each technique, the strong and weak points are discussed, and guidelines are given to come to a reliable interpretation of the results. The emphasis is on traditional hierarchical cluster analysis tools, and we elaborate on the limits of their applicability. In particular, the loss of information introduced by cluster analysis is discussed, as well as the problem to degenerate solutions. The particular challenges associated with the analysis and interpretation of modern large data sets potentially include thousands of organisms, which are also covered. We also discuss novel grouping techniques, such as minimum spanning trees and self-organizing maps, and show how these tools can be used complementary to traditional cluster analysis to gain further insight in the structure of the data.

Antimicrobial resistance in anaerobic bacteria. Experiences in Europe and North America (Symposium arranged with ESGARAB)

S11

Antimicrobial susceptibility patterns in different European countries

E. Nagy *(Szeged, HU)*

Antibiotic susceptibility testing of clinically relevant anaerobes in different clinical microbiological laboratories in Europe is less and less frequently carried out due to the fact, that clinicians treat many presumed anaerobic infections empirically. Even less data are available about the antibiotic resistance levels among the anaerobic normal flora isolates developed due to the use of antibiotics for infections caused by aerobic or facultative anaerobic pathogens. New antibiotics with anti-anaerobic activity may be tested on "old" isolates instead of using "recent" clinical isolates, which may create false susceptibility data and incorrect recommendations for treatment of anaerobic infections. Most systemic data in Europe are available for few genera and species of anaerobic bacteria such as *Bacteroides fragilis* and related species, *Fusobacterium* spp., *Propionibacterium acnes*, *Clostridium perfringens*, and *Clostridium difficile*. Two Europe-wide resistance studies were carried out during the past 15 years involving almost all European countries evaluating the development of resistance among *B. fragilis* and related species. A similar study was done with clinical isolates of *Propionibacterium acnes* and *Peptostreptococci*. Expressed differences were found in resistance levels between different *Bacteroides* species and between different areas of Europe for clindamycin, erythromycin, ampicillin and moxifloxacin. The same is true for *Propionibacterium acnes*. Resistance genes responsible for carbapenem and nitro-imidazole resistance can be found more frequently among clinical and normal flora isolates, than the expressed resistance. Despite of the fact that most anti-anaerobic drugs show good activity against anaerobic clinical isolates, the number of the resistant isolates is increasing parallel with the time. Accumulation of resistance markers can be observed among *Bacteroides* strains. Reported cases will be discussed where clinical failure was due to the presence of multi-resistant anaerobic pathogens.

2006 *Clinical Microbiology and Infection*, Volume 12, Supplement 4
ISSN: 1470-9465

Pathogenesis and prevention of nosocomial infections-new aspects (Symposium arranged with ESGNI)

S12

Infections associated with intravascular devices

B. Rijnders *(Rotterdam, NL)*

Notwithstanding the progress in our understanding of the pathogenesis of foreign body infection, catheter-related infection (CRI) continues to be part of the daily life in the ICU, oncology or haematology unit. The consequential morbidity and longer hospital stay brings about considerable costs. However, several interventions can reduce the incidence of CRI. 10 years ago, catheters coated or impregnated with antiseptics or antibiotics became part of our armament against CRI. However, indiscriminate prophylactic use of antibiotics increases the risk of antimicrobial resistance. Furthermore the minocyclin-rifampin impregnated catheter (the only antibiotic impregnated catheter with well documented efficacy) is not available in Europe. A catheter impregnated with chlorhexidine/silver sulfadiazine (CS) on the outer surface is available in Europe and has been shown to have the incidence of CRI. Two recent clinical studies show that an improved version of this catheter (with CS on the inner and outer surface) reduces catheter colonization with 45–72%. *In vitro* evidence about the use of antiseptic agents as a substitute for antibiotic locks for the prevention of endoluminal CRI is available. However, evidence from prospective randomized controlled clinical trials on the use of antiseptic locks is still scarce. Several clinical studies and a meta-analysis support the use of chlorhexidine as the preferred disinfectant prior to catheter insertion. Recent evidence supports the use of a chlorhexidine impregnated sponge that is placed around the insertion site of the catheter to prevent recolonization of the insertion site and therefore also exoluminal CRI of short-term central venous as well as arterial catheters. In general it is correct to state that microorganisms on the outer surface of the catheter (exoluminal) are the source of infection in most of the CRI in short-term catheters and microorganisms on the inner surface (endoluminal) become more important with longer duration of catheter use. This difference is however gradual. Therefore in most settings interventions directed against exoluminal as well as endoluminal CRI should be combined. Despite the above-mentioned new developments, the implementation of a structured teaching program on CRI (with a special emphasis on the adherence to full-sterile barrier precautions, chlorhexidine based skin disinfections and removal of the catheter as soon as possible) remains the cornerstone of CRI prevention.

S14

Ventilator-associated pneumonia

W. Krüger *(Tubingen, DE)*

Several factors contribute to the pathogenesis of VAP; therefore, several approaches have to be followed in order to prevent pathogens from invading the lung. Aspiration of contaminated oral secretions is considered to be the main mechanism. This is also true for incubated patients, where micro-aspiration occurs besides or within the folds of the inflated cuff of the endotracheal tube. Moreover, the oral cavity of severely ill patients is typically colonized with gram-negative rods, which is usually not the case in healthy subjects. Consequently, many VAP prevention measures aim at the reduction of aspiration of contaminated oral secretions. Deeply sedated patients may not be able to swallow their oral secretions. Oral hygiene and suctioning are important parts of nursing care, which also contribute to the reduction of aspiration. However, the subglottic space can usually not be emptied of secretions by oral suctioning. One approach is suctioning of subglottic secretions using a special endotracheal tube with an additional dorsal lumen right above the cuff. Whereas several researchers could not find a benefit, at least two studies showed subglottic suctioning to be effective for reducing the incidence of VAP (9, 10). However, it is definitely not indicated to replace a regular endotracheal tube by a subglottic suction tube in a patient, who is already intubated. It has clearly been shown that reintubation is a risk factor for the development of pneumonias. This is most likely due to aspiration occurring during the deflation of the endotracheal tube and during the process of intubation. This also means that thorough precautions have to be taken, when an endotracheal tube has to be changed for medical reasons: enteral feeding should be stopped several hours in advance, the patient should be placed in a semi-recumbent position, the stomach and oral cavity are suctioned as well as the subglottic space, which can be visualized and suctioned during laryngoscopy before the cuff of the endotracheal tube is to be deflated. Reduction of microbial colonization of the oral cavity is a causal approach for the prevention of VAP. It has been shown in patients undergoing cardiac surgery, that a 0.12% oral chlorhexidine solution can reduce the incidence of postoperative pneumonias, but it is still unclear, whether this should generally be recommended for surgical patients (4). Recent investigations point to the importance of bacterial colonization of teeth in the pathogenesis of VAP (5). However, antiseptic gels applied to the gingiva and teeth were so far not effective for prevention of VAP (6). Likewise iseganan, an antimicrobial peptide applied to the oral cavity was also not effective, as shown in a recent multi centre placebo-controlled double-blind trial (7). Topical application of antibiotics into the oropharynx is part of several trials using selective digestive decontamination (SDD) and the exclusive administration to the oral cavity (SOD: selective oral decontamination) was effective in reducing VAP in ICU patients (1). Meanwhile, 3 independent clinical trials have shown a survival benefit for critically ill patients treated with SDD (2, 3, 8), but the fear of increased selection pressure has hampered widespread use of SDD in critically ill patients.

References:

1. Bergmans DCJJ, Bonten MJM, Gaillard CA, Paling JC, van der Geest S, van Thiel FH, Beysens AJ, de Leeuw PW, Stobberingh EE. Prevention of ventilator-associated pneumonia by oral decontamination. Am. J. Respir. Crit. Care Med. 2001; 164:382–388.
2. de Jonge E, Schultz MJ, Spanjaard L, Bossuyt PPM, Vroom MB, Dankert J, Kesecio-glu J. Effects of selective decontamination of digestive tract on mortality and acquisition of resistant bacteria in intensive care: a randomized controlled trial. Lancet 2003; 362:1011–1016.
3. de la Cal MA, Cerda E, Garcia-Hierro P, van Saene HK, Gomez-Santos D, Negro E. Survival benefit in critically ill burned patients receiving selective decontamination of the digestive tract: a randomized, placebo-controlled, double-blind trial. Ann. Surg. 2005; 241:424–430.
4. DeRiso AJ, Ladowski JS, Dillon TA, Justice JW, Peterson AC. Chlorhexidine gluco-nate 0.12% oral rinse reduces the incidence of total nosocomial respiratory infection and

nonprophylactic systemic antibiotic use in patients undergoing heart surgery. Chest 1996; 109:1556–1561.
5. El-Solh AA, Pietrantoni C, Bhat A, Okada M, Zambon J, Aquilina A, Berbary E. Colonization of dental plaques. A reservoir of respiratory pathogens for Hospital-Acquired Pneumonia in institutionalised elders. Chest 2004; 126:1575–1582.
6. Fourrier F, Dubois D, Pronnier P, Herbecq P, Leroy O, Desmettre T, al. e. Effect of gingival and dental plaque antiseptic decontamination on nosocomial infections acquired in the intensive care unit: a double-blind placebo-controlled multi centre study. Crit. Care Med. 2005; 33:1728–1735.
7. Kollef M, Pittet D, Sanchez Garcia M, Chastre J, Fagon JY, Bonten M, Hyzy R, Fle-ming TR, Fuchs H, Bellm L, Mercat A, Manez R, Martinez A, Eggimann P, Daguerre M. A randomized double-blind trial of iseganan in prevention of ventilator-associated pneumonia. Am. J. Respir. Crit. Care Med. 2006; 173:91–97.
8. Krueger WA, Lenhart F-P, Neeser G, Ruckdeschel G, Schreckhase H, Eissner H-J, Forst H, Eckart J, Peter K, Unertl KE. Influence of combined intravenous and topical antibiotic prophylaxis on the incidence of infections, organ dysfunctions, and mortality in critically ill surgical patients. A prospective, stratified, randomized, doubleblind, placebo-controlled clinical trial. Am. J. Respir. Crit. Care Med. 2002; 166:1029–1037.
9. Smulders K, van der Hoeven H, Weers-Pothoff I, Vandenbroucke-Grauls C. A rando-mized clinical trial of intermittent subglottic secretion drainage in patients receiving mechanical ventilation. Chest 2002; 121:858–862.
10. Valles J, Artigas A, Rello J, Bonsoms N, Fontanals D, Blanch L, Fernandez R, Bai-gorri F, Mestre J. Continuous aspiration of subglottic secretions in preventing ventilator-associated pneumonia. Ann. Intern. Med. 1995; 122:179–186.

News in the field of toxoplasmosis (Symposium arranged with ESGT)

S15

Past, present and future drug treatment of *Toxoplasma gondii*

F. Derouin *(Paris, FR)*

An ideal anti-Toxoplasma drug would be preferentially parasiticidal against the different parasitic stages, prevent stage differentiation from tachyzoite to bradyzoite, would penetrate into cysts and be well distributed in the main sites of infection. Based on *in vitro* and *in vivo* data recorded for the last 35 years, it appears that no such a drug exists today and that no marketed new compound can be expected for several years, despite the recent identification of new potential drug targets (apicoplast, parasite penetration). Among drugs that can be used safely for treatment of congenital or cerebral toxoplasmosis, no drug or drug regimen is as effective as the combination of pyrimethamine + sulfadiazine. Among the ''new'' macrolides, azithromycin seems to be the most efficient *in vivo* and synergizes with pyrimethamine. Some new fluoroquinolones are active *in vivo* and *in vitro* but their potential toxicity is a concern for treatment congenital toxoplasmosis. Atovaquone remains of interest for reduction of parasite burden in a long-term treatment strategy and a second line treatment in case of intolerance to sulfonamides or pyrimethamine. Cotrimoxazole is effective *in vitro* and *in vivo* against tachyzoites and remains the first line drug combination for primary prophylaxis of toxoplasmosis in immunocompromised patients. Interestingly, anti-HIV protease inhibitors are inhibitory for *T. gondii in vitro* at a concentration that can be achieved in human. It should be noted that for most drugs, experimental data are dramatically lacking for treatment of ocular toxoplasmosis and on the efficacy of long-term treatment of chronic infection on brain cyst burden. Indeed, significant progresses can be noted on the *in vitro* and *in vivo* methods of investigation of drugs, including the use of transformed parasite expressing enzyme activity or bioluminescence, the development or QSAR methods and high throughput screening for the activity small molecules against parasitic enzymes or *T. gondii* penetration into cells. One can expect this will result in identification of new drug strategies for the future. This review also points out major lacks of information on the susceptibility of *T. gondii* strains according to their genotypes. Marked differences of susceptibility have been occasionally reported but need to be confirmed, on the basis of the new knowledge on strain genotyping. Similarly, the possible existence of natural or drug selected resistance need also to be investigated.

S16

Detection and genotyping of *Toxoplasma gondii* by real-time PCR and pyrosequencing

B. Edvinsson, M. Lappalainen, B. Evengård for the European Study Group on Toxoplasmosis *(ESGT)*

Objectives: *Toxoplasma gondii* is a protozoan parasite with worldwide distribution. Primary infections in humans are usually asymptomatic or characterized by non-specific symptoms. The infection is, however, severe or fatal in patients with a suppressed immune system, and is here most often caused by chronic infections that become reactivated. Rapid and sensitive diagnosis by PCR may be of importance for the survival of these patients. PCR techniques used to detect the parasite in patient samples are not yet standardized. We have compared the performance of two real-time PCR assays targeting two different repetitive DNA elements of *T. gondii*. We also aimed to develop a pyrosequencing assay to discriminate between the three main genotypes of *T. gondii*; i.e. type I, II and III.
Methods: The sensitivities for detecting *T. gondii* by real-time PCR when targeting the 35-fold repeated B1 gene or a 200–300 fold repeated 529 bp elements were compared. The impact on the diagnostic sensitivity when using two different types of internal amplification controls, either amplified by the primers used for detection of *T. gondii* or by a different set of primers, was tested. For genotyping, real-time PCR and pyrosequencing were used to analyse a fragment of the GRA6 gene in 21 *T. gondii* isolates.
Results: The sensitivity was higher when the 200–300 fold repeated 529 bp element was used. The diagnostic performance of the real-time PCR targeting the 529 bp elements was not affected by inclusion of an internal amplification control. The Pyrosequencing assay discriminated between type I, II and III *T. gondii*.

Conclusions: Real-time PCR, including an internal amplification control, can be used to detect low amounts of *T. gondii* in a sample by amplification of a part of the 529 bp elements. This may be of importance if the aim is a sensitive and rapid diagnosis of toxoplasmosis in immunocompromised patients. Pyrosequencing can be used for rapid and high throughput genotyping of the parasite in clinical findings, and should help to further elucidate if there are differences in the clinical manifestations of toxoplasmosis caused by type I, II or III *T. gondii*.

How to prevent microbial biofilm formation on medical devices (Symposium arranged with ESGB)

S18

Drug releasing polyurethanes able to inhibit microbial biofilm growth on medical devices

G. Donelli *(Rome, IT)*

In recent years, a number of different strategies have been developed to prevent medical device-associated infections, including device coating with antimicrobial agents able to inhibit bacterial colonization and biofilm formation. However, the possible emergence of multi-drug-resistant microorganisms, as consequence of the sub-inhibitory antibiotic concentrations released from the implanted device, is regarded as a significant risk. On the other hand, the largely increased antibiotic resistance is a well established feature of microbial species growing in sessile mode to form a biofilm, such as *Staphylococcus*, *Pseudomonas* and *Candida* spp. which are the most frequently implicated bugs in device colonization. In order to inhibit microbial colonization of medical devices and to minimize multi-drug resistance, we designed experimental models based on the adsorption on functionalized polyurethanes of: (i) a water-insoluble antibiotic not used for systemic therapy; (ii) two water-soluble antibiotics, belonging to different classes, both commonly used in systemic therapy; (iii) ions of transition metals known for their broad spectrum of antimicrobial activity as possible adjuvants of antibiotics; (iv) an antifungal drug highly effective for treatment of *Candida* infections and (v) pore-former molecules able to improve the antibiotic loading and control its release. In particular, we developed new drug-releasing polymers loaded with: (i) usnic acid as a water-insoluble antibiotic molecule able to exert its activity at the device level; (ii) rifampin and cefamandole nafate as an association of two water-soluble antibiotics exhibiting two different modes of action; (iii) silver ions and ciprofloxacin exhibiting a synergistic role; (iv) fluconazole as a wide-spectrum antifungal drug and (v) albumin and polyethylenglycol as pore formers promoting the release of rifampin, cefamandole nafate and fluconazole from polyurethanes. The results of these *in vitro* studies suggest that the combined entrapping of antibiotic or antifungal drugs and pore-formers in these functionalized polyurethanes seem to be promising both in preventing bacterial colonisation and biofilm formation and controlling the emergence of microbial resistance.

Antimicrobial utilisation in Europe: future directions (Symposium arranged with ESGAP)

S20

Mapping antibiotic consumption and resistance in the community

M. Heginbothomfor the Welsh Antibiotic Study Group

Surveillance is critical to the understanding of antibiotic resistance and the ability to control its spread. It facilitates the monitoring of trends, informs clinical practice, guides intervention, allows assessment of the effects of change and the detection of new and untoward events. Timely feedback of consumption and resistance data to stakeholders creates ownership and aims to improve prudent prescribing. However, the impact of any surveillance information can be greatly influenced by its presentation. Geographical Information System (GIS) is mapping and analysis technology that allows large quantities of information to be visualized and analysed within a geographic context. GIS mapping provides a powerful medium for the dissemination of surveillance data. Various surveillance groups use GIS technology to map resistance data at country and regional level e.g. European Antimicrobial Resistance Surveillance System; NPHS Wales have used GIS technology to map resistance, consumption, deprivation and practice demography data at Local Health Board and General Practice level. European surveillance data mapped by country and region will be reviewed. Welsh surveillance data mapped by LHB and general practices will be presented, and the factors and determinants of antibiotic consumption and resistance discussed. The merits of using GIS for surveillance purposes will be open for debate.

S22

Point-prevalence surveys of anti-infective use

B. Dean Franklin *(London, UK)*

Surveys of anti-infective use have been carried out twice a year. For the last six years at Hammersmith Hospitals NHS Trust, using point prevalence methodology. A team of clinical pharmacists collect data on all inpatients scheduled to receive one or more systemic anti-infective on the day of the study, as well as the total number of patients seen. Data collection takes place over a four-day period; one quarter of the wards are surveyed on each day. Systemic anti-fungal and anti-viral drugs are included. The information obtained includes the number of patients scheduled to receive one or more anti-infective on the day of the study, number of anti-infective per patient, percentage of anti-infective are given intravenously, as well as details of the combinations and durations prescribed. Use of anti-infective on the Trust's reserved list is examined in more detail. The trust's antibiotic steering group uses the results obtained to highlight areas for intervention. These have included targeting

areas for intravenous to oral switch, agreeing local policies where there was no standard practice, and identifying clinical areas where inappropriate combinations were being used. The results are fed back to clinical directorates as part of their performance management information. More recently, ten local hospitals have collected equivalent data as part of a benchmarking exercise. This presentation will illustrate the information obtained, and discuss the advantages and disadvantages of this approach to study anti-infective use.

Nosocomial mould infections (Symposium arranged with EFSIG)

S24

Source of moulds in the hospital setting

J.-P. Gangneux *(Rennes, FR)*

Moulds are ubiquitous in nature and reproduce by making spores able to travel through air. They can cause illness, in particular the thermotolerant *Aspergillus* species that produce numerous conidia 2–4 m in diameter. Invasive aspergillosis (IA) is a major opportunistic infection among patients with severe and prolonged neutropenia. Spore inhalation is the usual route of infection, but other routes may exist. The nosocomial origin of aspergillosis has been demonstrated in epidemic situations with airborne spore transmission via unfiltered air, and massive environmental contamination during construction and renovation work both inside and outside the hospital. Air control measures are currently the more effective way of significantly reducing the incidence of nosocomial aspergillosis. Patient rooms should have adequate capacity to minimize accumulation of fungal spores using high-efficiency particulate air filtration, laminar air flow systems, high rates of room-air changes, positive pressure, and well-sealed rooms. Innovative systems producing purified air may represent an interesting alternative to the air filtration. Monitoring of environmental fungal contamination is strongly recommended to detect increases in conidia density and to assess air filtration efficiency. The follow-up of the air and surface fungal loads is highly recommended in hospital units, which benefit from air control measures, in addition to specific investigations in case of *Aspergillus* infection. It requires efficient biocollectors, adequate surface sampling methods and suitable culture conditions that must be evaluated before. Progress in terms of air filtration leaded to decrease of IA in neutropenic patients during the first few weeks after bone marrow transplantation, but delayed IA still occur. The sources and routes of spore transmission remain unclear, and alternative sources of contamination should be more investigated and controlled. Opportunistic fungal pathogens have been recovered from several hospital water distribution systems in the USA and authors suggested that aspergillosis is waterborne. However, the environmental risk of IA linked to water should be interpreted according to the local situation, because differences in water collection and treatment exist. Besides, several foods can be colonized by moulds and can lead to spore absorption or aerosolization and subsequent systemic infection. It has been previously recommended that immunocompromised patients should avoid such contaminated foods but one alternative is to disinfect potentially contaminated foods, especially with regard to *A. fumigatus*. Lastly, the clothing of visitors and medical staff, and carriage by personal and medical materials are potential vectors of spores. Preventive measures might then be appropriate.

S25

Post-operative aspergillosis

A. Pasqualotto *(Manchester, UK)*

Aspergillus spp. is ubiquitous, aerobic fungi that occur in soil, water, and decaying vegetation; the organism also survives well in air, dust, and moisture present in health-care facilities. Similar to other filamentous fungi, *Aspergillus* species are usually acquired from an inanimate reservoir, more commonly by the inhalation of small airborne spores. While invasive aspergillosis typically affects severely immunocompromised patients, cases of surgical site infections have been reported in immunocompetent individuals. The aim of this presentation is to summarize the world literature regarding this topic. This will be illustrated by the presentation of three cases, originated from personal knowledge of the authors, and not part of any outbreak. The first of these cases was a man who underwent elective aortic valve replacement and died of *Aspergillus endocarditis*. The second was a teenager who underwent elective neurosurgery for Chiari I malformation, received 4 weeks of postoperative dexamethasone and died despite amphotericin B therapy. The third was a woman who developed a cerebral abscess due to *Aspergillus fumigatus* after neurosurgery augmented with dexamethasone and recovered following voriconazole treatment. Medline, LILACS and EMBASE databases were searched and references from relevant articles were reviewed. Conference abstracts were reviewed as well. After Frank and Alton firstly published their study of postoperative aspergillosis in 1933, more than 500 cases have been reported. Cardiac and ophthalmologic surgery, and surgical dental procedures provided the most commonly reported cases. Postoperative cases of endocarditis and aortitis affected mostly immunocompetent male patients submitted to aortic valve replacement. Median time from the surgery to the diagnosis was 2.7 months. An antemortem diagnosis occurred in only 43.1%, and blood cultures are rarely positive (6.4%). Mortality for these conditions was 92.7%. Vascular graft aspergillosis occurred after a median time of 8 months after surgery. *Aspergillus* graft infections usually occur on the suture line of a previous aortotomy, and definitive diagnostic procedures for these patients were generally culture of the excised aortic graft or the peripheral embolus and biopsy of the contiguously affected vertebral disk space. Most of the patients with postoperative aspergillosis following neurosurgery had received treatment with corticosteroids, and mortality for this condition was 68.0% surprisingly low if we consider the mortality rates for immunosuppressed patients with disseminated aspergillosis involving the central nervous system. *Aspergillus* wound infections occurred after a median time of 17 days after surgery, and many of these patients were immunosuppressed. *Aspergillus flavus* was the aetiology of 36.8% of these infections. Successful treatment for postoperative aspergillosis requires rapid diagnosis, surgical debridement and antifungal therapy, probably voriconazole. In order to improve the outcome, better diagnostic methods are needed, particularly for cases of endocarditis and aortitis. In most patients, the source was presumed to be airborne infection during the surgical procedure. Prevention of these infections requires special care with the ventilation system in the operating room, proper disinfection techniques, and appropriate storage of surgical materials. A single proven postoperative case of aspergillosis is sufficient to initiate epidemiological investigations.

ISSN: 1470-9465

S26

Nosocomial infections due to emerging moulds

M. Cuenca-Estrella *(Madrid, ES)*

Opportunistic molds have emerged during the past decade as important causes of morbidity and mortality in some group of patients. At present *Aspergillus* spp. is one the most common cause of infection in bone marrow/stem cell transplant recipients and mucorales are increasingly reported as causing lethal infections, despite aggressive medical and surgical interventions. In addition, rare emerging molds are becoming a more common cause a deep and invasive fungal infections. Both hyaline hyphomycetes and black fungus species are increasingly reported as causing nosocomial mycoses (pneumonia, CNS infections and disseminated infections) refractory to conventional therapy. *Fusarium* spp. has been implicated in nosocomial outbreaks of respiratory and disseminated mycosis. *Scedosporium* spp. cause disseminated and localized infections in hospitalized patients. *Paecilomyces lilacinus* and *Scopulariopsis brevicaulis* are rare emerging hyphomycete that can cause nosocomial invasive infections in immunocompromised patients, and *Alternaria* spp. has been described in cases of deep infection in solid organ transplant recipients. Several factors have been signalled as causes of the emergence of rare moulds at hospital. Environmental changes, antimicrobial pressure, an expanding population of immunocompromised hosts and an increasing of capabilities to identify rare fungi are the most commonly argued. It should be noted that emergent fungal infections would continue to increase in these settings. Efforts should be made to identify nosocomial emerging molds at species level and to know susceptibility profile of these organisms. Correct characterization of these species can be significant at therapeutic level in view of their distinct antifungal susceptibility profile. DNA amplification-base methods could be used for characterization of emerging pathogens. Early diagnosis procedures and new therapeutic approaches can be also needed.

Guidelines for *Helicobacter pylori* infections revisited. The Maastricht-3 2005 consensus report (Symposium arranged with EHSG)

S27

Indications for *H. pylori* eradication revisited

E. Kuipers *(Rotterdam, NL)*

The Nobel prize of Medicine 2005 has been awarded for the discovery of *H. pylori* as an important human pathogen. In the past 25 years since this initial discovery, much research has been performed to find optimal therapies for this bacterial infection, and to determine the benefits of *H. pylori* eradication in patients with various upper gastrointestinal and other conditions. Over the past decade, repeated international consensus meetings have on the basis of this literature gradually developed and adapted guidelines for the management of *H. pylori* infection. The Maastricht guidelines developed under the authority of the European *H. pylori* Study Group have over the years in particular had an impact on the clinical approach to the *H. pylori*-positive patient. These Maastricht guidelines have recently been revised (1) and focus on indications for therapy, diagnostic approaches, and treatment issues. Conditions that had been accepted in previous Maastricht guidelines as strong, definite indications for *H. pylori* treatment were again accepted as such. These included in particular peptic ulcer disease and gastric B-cell MALT-lymphoma, but also atrophic gastritis and 1st degree relatives of gastric cancer patients. Furthermore, *H. pylori* eradication was accepted as an appropriate option for *H. pylori*-positive patients with investigated non-ulcer dyspepsia, whereas *H. pylori* test and treat was accepted as an appropriate option for patients with uninvestigated dyspepsia. Routine testing for *Helicobacter pylori* was not recommended in GERD, but the consensus committee advised to consider *H pylori* testing in patients on long-term maintenance therapy with PPIs. *H. pylori* eradication was considered to be of value in chronic NSAID users but insufficient to completely prevent NSAID-related ulcer disease *H. pylori* infection should be sought for and treated in patients with either unexplained iron deficiency anaemia, or ITP. These indications and their background will be discussed during the meeting.

References

1. Malfertheiner P, Megraud F, O'Morain C, F. Bazzoli, El-Omar E, D. Graham, R. Hunt, T Rokkas, N. Vakil, Kuipers, E.J., The European *Helicobacter* Study Group *et al*. Current concepts in the management of *Helicobacter pylori* infection – The Maastricht III Florence Consensus Report 2005. Submitted.

S28

Diagnosis and treatment of resistant *H. pylori* infection

F. Mégraud *(Bordeaux, FR)*

The management of *H. pylori* infection has been well established during the last 10 years. Recommendations were made in the Maastricht Conference in 1996, and were updated in 2000. Most of them have been used in other consensus conferences worldwide. Nevertheless, some points have emerged these past 4 years, which led to questions and discussions at the Maastricht-3 Conference.

Diagnosis pre treatment: With regard to diagnostic tests, the discussion focused on the value of non-invasive tests other than the urea breath test (UBT). A first statement concluded that serology could be considered as a diagnostic test in some situations, e.g. bleeding ulcers, gastric atrophy, MALT lymphoma and current use of PPI or antibiotics. Indeed, PPI are a source of false negative results for all diagnostic tests except serology, and should be stopped at least 2 weeks before performing the test. In contrast, it was stated that neither the doctor tests (near-patient tests) nor the detection of *H. pylori* antibodies in urine and saliva, had any current role in the management of *H. pylori* infection. The situation is different for the stool test, which was considered acceptable, on the same grounds as UBT for *H. pylori* diagnosis, especially in the case of implementation of the test and treat strategy. The importance of performing culture for clarithromycin susceptibility testing, before using clarithromycin-based treatment as a first line treatment, was hardly debated. Culture was recommended if primary resistance to this antibiotic was higher than 15–20% in the respective geographical area or population, as well as after 2 treatment failures. The importance of monitoring the primary

antibiotic resistance in reference laboratories in different areas was also stressed. In the event that clarithromycin susceptibility testing under such circumstances is impossible, this antibiotic should not be used. In contrast, it was agreed that testing metronidazole susceptibility is not routinely necessary in the management of *H. pylori* infection. Metronidazole susceptibility testing needs further standardization before being recommended as a first line treatment.

How to treat? The recommended first line therapy therefore remains PPI-clarithromycin-amoxicillin or metronidazole if the primary resistance to clarithromycin in the area is lower than 15–20%. There is a small advantage to using metronidazole instead of amoxicillin and therefore, this combination was found to be preferable in areas where the prevalence of metronidazole resistance is lower that 40%. The consensus was also that a 14-day rather than 7-day treatment duration had a slight advantage in terms of treatment success. However, a 14-day treatment is not cost effective in most countries. Another addition to the Maastricht-2 Consensus is that bismuth-based quadruple therapies, when available, are acceptable as alternative first line therapies. With regard to second line therapies, bismuth-based quadruple therapies remain the best option. If unavailable, PPI-amoxicillin or tetracycline and metronidazole are recommended. As previously proposed, the rescue therapy after failure of 2 courses of different therapies should be based on antimicrobial susceptibility testing. For follow-up after *H. pylori* eradication, UBT remains the preferred test. If unavailable, a laboratory-based stool test preferably using monoclonal antibodies, could be used. The timing of this follow-up should be at least 4 weeks after the end of eradication treatment.

S29

Prevention of gastric cancer by *H. pylori* eradication

P. Malfertheiner *(Magdeburg, DE)*

In the light of the most recent clinical developments in the management of *H. pylori* infection, the European *Helicobacter* Study Group (EHSG) organized the Maastricht III Consensus Report that involved 50 participants from 26 countries around the world. This abstract provides a brief summary of some novel and major aspects relevant for clinician routine. In the new edition of the Maastricht guidelines three areas have been addressed: (a) Indications/contraindications for *H. pylori* eradication, with special focus on dyspepsia, patients exposed to NSAIDs or Aspirin, and the relationship with GERD; (b) Diagnostic procedures-Standard and rescue therapy of *H. pylori* infection and (c) Prevention of gastric cancer and other complications related to *H. pylori.* Selected recommendations and statements are listed as follows: (a) Indications/contraindications for *H. pylori* eradication and relevant statements.

Uninvestigated dyspepsia: *H. pylori* test and treat is an appropriate option for patients with uninvestigated dyspepsia.

NSAIDs: (i) In patients who are naïve NSAIDs users, *H. pylori* eradication may partially prevent peptic ulcers and/or bleeding; (ii) In patients on long term NSAIDs and peptic ulcer and/or ulcer bleeding, PPI maintenance therapy is superior to *H. pylori* eradication in preventing ulcer recurrence and/or bleeding.

Aspirin: Patients who are on long-term aspirin with ulcer disease and significant clinical bleeding should be tested for *H. pylori* and if positive receive eradication therapy.

GERD: *H. pylori* eradication does not cause GERD.

Atrophic gastritis: *H. pylori* eradication halts the extension of atrophic gastritis and may lead to regression of atrophy. The effect on intestinal metaplasia is uncertain.

Iron Deficiency Anaemia: *H. pylori* infection should be sought for and treated in patients with unexplained iron deficiency anaemia.(b) Diagnosis of *H. pylori* infection:(i) The non-invasive tests that can be used for the test and treat strategy are UBT and the stool antigen tests. Certain kits for serology with high accuracy can also be applied; (ii) PPI is a source of false negative diagnostic tests except serology, PPI should be stopped for at least 2 weeks before to perform diagnostic test and (iii) it is recommended to follow up patients after *H. pylori* eradication with UBT, if available. If this diagnostic procedure is not available, a laboratory-based stool test, preferably using monoclonal antibodies, could be used

(c) Prevention of gastric cancer: (i) *H. pylori* eradication prevents development of preneoplastic changes (intestinal metaplasia, atrophic gastritis) of the gastric mucosa; (ii) eradication of *H. pylori* has the potential to reduce the risk of gastric cancer development and (iii) the optimal time to eradicate *H. pylori* is before preneoplastic conditions (atrophy, intestinal metaplasia) are present

Treatment strategy: First line therapy for *H. pylori* eradication: PPI-clarithromycin - amoxicillin or metronidazole therapy remains the recommended first line therapy in populations with less than 15–20% clarithromycin resistance prevalence in population in less than 40% metronidazole resistance prevalence PPI-clarithromycin - metronidazole is preferable. Quadruple therapies are alternative first line therapies.

In case of failure – second line therapy: Bismuth-based quadruple therapies remain the best second line therapy, if available, if not, PPI-Amoxicillin or tetracycline and metronidazole are recommended.

Subsequent failures – rescue therapy: The rescue therapy should be based on antimicrobial susceptibility testing

New diagnostic techniques for the microbiological laboratory and general practitioner

S36

Breakthroughs in bacterial detection, identification and typing

M. Struelens *(Brussels, BE)*

In 2005, a number of novel methods applicable in the clinical microbiology laboratory have been reported for the detection, identification to species level and sub-typing of bacterial pathogens. Breakthroughs have been made in rapid detection of bloodstream infections in neonates and adult patients with sepsis by use of broad range bacterial DNA detection and identification. Successful methods include universal gene analysis by real-time PCR, PCR-DNA micro-array hybridization, or PCR-pyrosequencing testing of blood specimens. Also, several highly accurate sequence-based methods were developed and validated for identification of clinical cultures of *staphylococci*, *mycobacteria*, *nocardia*, and *Burkholderia* species. Progress has also been achieved in rapid detection of carriers of multi-resistant bacteria, especially methicillin-resistant *S. aureus* (MRSA) from superficial swabs. Several chromogenic selective culture media

have shown sensitivity of 80–90% with excellent specificity for MRSA detection within 20–24 hours of incubation. Real-time PCR based on the *orf*X-SCC*mec* junction has shown similar accuracy for MRSA detection within 2 hours after specimen collection. For tuberculosis, advances were made in rapid detection, identification to species level and detection of rifampin and isoniazid resistance directly in clinical specimens by molecular methods, including PCR-pyrosequencing. Epidemiological typing has benefited greatly from the extension and validation of sequence-based systems and databases, including novel MLST schemes for *Acinetobacter* and *B. cepacia* complex. Single nucleotide polymorphism analysed by rapid methods like real-time PCR with melt curve analysis or allele-specific PCR have been developed for *E.coli* and *Campylobacter*. VNTR-MLVA schemes have also been validated for rapid typing of several human pathogens. In general, most publications focus on the ''proof of concept'' of these rapid and highly informative molecular assays at the level of clinical accuracy. There is however, too few evaluation of the clinical utility/superiority of these assays in routine application in terms of cost-effectiveness of medical/public health interventions based on test results. Thus, the need for funding this kind of intervention research should urgently be addressed by health technology assessment authorities.

S37

Progress in detection of bacteria responsible for infections in challenging clinical situations and of antimicrobial resistance

R. Leclercq *(Caen, FR)*

Identification by conventional techniques of bacteria responsible for infections remains challenging in situations when these bacteria are rare in the clinical sample or difficult to grow or when antibiotic treatment has been administered early. Bone and joint infections, endocarditis and meningitis are examples of these types of situation. Another challenging issue is the rapid detection of bacteria in case of severe infection or for prevention of neonatal infection by group *B. streptococci*. Several molecular homemade or commercially available techniques can now be used which provide reliable results. Detection of antimicrobial resistance might be useful when resistance is poorly expressed in conventional tests or for epidemiologic purposes. In particular, detection of methicillin resistance in *staphylococci* has been the subject of extensive works. Several methods are now available which allow the detection of PLP2a expression or of the *mec*A gene. Several commercial kits are available for the detection of the *mec*A gene that is generally based on DNA amplification followed by hybridization. These kits generally allow species identification in the same experiment. Similarly, identification of vancomycin resistance genes in *enterococci* can be performed using either homemade multiplex PCR or commercial kits. The cost of these techniques is decreasing but remains to be balanced with the benefits that are expected from their use.

S38

Molecular diagnostics in virology, overcoming the hurdles

H.G.M. Niesters *(Rotterdam, NL)*

The advantages of molecular diagnostics in virology has become clear for a growing number of clinically important questions, and has left from the research state to become integrated in the routine diagnostic setting. The repertoire of assays available was mostly focussing on the detection of the blood-borne viruses (HBV, HCV and HIV-1), but due to the availability of new and easy-to-use technologies (mostly real-time amplification based) the detection of every target is possible. Targets like the herpesviruses and a large panel of viruses infecting the respiratory tract, are already routinely detected in larger (university) hospitals. This is also pushed by the "urgent" need to be prepared for the detection of new and emerging viruses, like SARS and influenza virus (whether H7N7 or H5N1), but also due to the possibilities to develop assays within a relative short period of time for the newly detected viruses, like the coronaviruses HKU and NL-63, and BOCA virus, which has increased our insight in the clinical implications of these viruses in respiratory tract infections. More knowledge with easy-to-use real time technologies, the improved isolation and detection platforms, the availability of better controlled proficiency panels for an increasing number of targets, and the introduction of universal internal controls, have all resulted in a better and more standardized assay performance. This resulted not only in a discrepancy of the number of commercially available assays used for virological targets compared to in-house develop assays, but also in the acceptability of molecular diagnostics, simply because clinical decisions can now be better made due to the information provided. The last two hurdles, costs and time-to-result are very close to be solved. The developments of molecular technologies and assays during the last years, as well as the detection of new pathogens which in most cases cannot be detected by other technologies like virus culture or serology, and the improved quality control possibilities, has generated from virology a dynamic discipline with an impact in good clinical practice. Although improvements are always possible, like multiplexing, small volumes, very fast turn-around-time, DNA chip technology, the finish is just around the corner.

S40

Mycology

E. Roilides *(Athens, GR)*

Invasive fungal infections (IFIs) have become a major cause of increased morbidity and mortality in immunocompromised patients. While a number of new antifungals have been lately introduced in the fight against opportunistic fungi, patients with these generally devastating diseases have had very dismal prognosis partly due to the late diagnosis of IFIs. Historically, microscopic examinations of body fluids or tissue biopsies as well as culture of the material on appropriate culture media have been the principal modalities for diagnosis of IFIs. High resolution CT scans serially performed have been proven to be sensitive tool of diagnosis of fungal pneumonias in haematological patients. Lung densities with halo sign and central cavitation of small nodules with air crescent formation can be found in adult patients and they are very useful signs of invasive aspergillosis (IA). Recently, novel diagnostic methods both serological and molecular have been developed and evaluated as well as improved culturing techniques. Identification now is performed more accurately to the species level with the use of automated methods and genetic sequencing. Evaluation of susceptibility of clinical fungal isolates to antifungal drugs is also more widespread and more standardized. Correlation between *in vitro* and *in vivo* effectiveness has been achieved at least in the case of *Candida* infections. Among serological methods, galactomannan assay has become very helpful in the early diagnosis of IA and its sequential performance in high-risk patients has been shown to help. Its use is still problematic in young pediatric patients in

whom the assay has given low specificity with a high number of false positive results as well as low sensitivity in certain cases such as chronic granulomatous disease. Evaluation of galactomannan in body fluids other than blood is also feasible and provides important information; it needs, however, further standardization. β-glucan is another metabolite that is increased in serum of patients with fungal infections and specifically with invasive candidiasis. It is being under intense study. Detection of nucleic acids of fungi by the use of polymerase chain reaction (PCR) is a powerful method for rapid identification of fungi; this method, however, needs further development and standardization before it is widely applied in the clinical microbiology lab. These techniques coupled with enhanced radiology are expected to enable the physician to diagnose serious fungal infections earlier in their course increasing the chance for an improved outcome.

Neurological sequelae of bacterial and viral infections

S41

Campylobacter-induced Guillain-Barré syndrome

H.P. Endtz *(Rotterdam, NL)*

Almost 150 years after its first description by Gustave Landry, the Guillain-Barré syndromes (GBS) are the leading cause of acute autoimmune neuromuscular paralysis worldwide. Major advances have been made in recent years unravelling the pathophysiological mechanisms of GBS. Overall, approx. 60% of the cases was preceded by infections, mostly flu-like infections or gastroenteritis caused by *Campylobacter jejuni*. The discovery of ganglioside mimics on microbial glycans has been quintessential and human as well as animal studies now support an important role for molecular mimicry in the pathogenesis of GBS. Evidence is accumulating that genetic polymorphism of *C. jejuni* determines the anti-ganglioside antibody reactivity and the clinical presentation of GBS. Genes involved in the synthesis and transfer of sialic acid appear to play a crucial role. In mice, injection of wild type GBS strains induces anti-ganglioside antibodies whereas Cst-II knockout mutants do not. Acute motor axonal neuropathy (AMAN) and Fisher's syndrome in particular, have been associated with anti-GM1/GD1a and anti-GQ1b antibodies, respectively. B-cell tolerance may explain the fact that most *C.jejuni* infections are not followed by GBS, although the mechanism is still poorly understood. Being carbohydrates, human gangliosides elicit a T-cell-independent humoral response. However, for reasons not specified here, T-cells also are likely to play an important role in the pathogenesis of GBS but their role is not clear yet. Recent attempts to identify human immunosusceptibility factors have been unsuccessful. Further research is clearly needed in order to discover new treatment modalities. Recent developments in the biosynthesis surface-bound ganglioside derivatives may open new avenues for novel and specific diagnostic and therapeutic approaches.

S42

The involvement of *Chlamydia pneumoniae* in multiple sclerosis

E. Fainardi *(Ferrara, IT)*

Multiple Sclerosis (MS) is a presumed autoimmune chronic inflammatory demyelinating disease of the Central Nervous System (CNS) of unknown aetiology. Epidemiological observations indicate that exposure to an environmental factors, such as a virus, in combination with genetic predisposition could be implicated in MS pathogenesis. However, direct evidences for an infectious aetiology in MS are still lacking. Recently, a potential role of a causative agent of MS has been suggested for *Chlamydia pneumoniae*, a Gram-negative, obligate intracellular bacterium that can induce a chronic infection and presents a high seroprevalence in adults. After the initial high rates reported for molecular and culture demonstration of *C. pneumoniae* in cerebrospinal fluid (CSF) of MS patients, the association between *C. pneumoniae* and MS was intensively investigated. Epidemiological studies reported that seropositivity for *C. pneumoniae* was strongly related to the risk of clinical exacerbation and progressive disease, but only moderately linked with the risk of developing MS. Conflicting results were obtained with the use of different techniques. In polymerase chain reaction (PCR) and isolation studies, CSF evidence of *C. pneumoniae* in MS was either less frequent than previously described, with equivalent amounts in MS and other neurological diseases, or absent. Evidence of *C. pneumoniae* infection was not observed in MS brain lesions. An intrathecal production of anti-*C. pneumoniae* antibodies within the CNS was found in a variable proportion of patients with MS and other neurological disorders or undetectable. *C. pneumoniae* was able to induce an experimental autoimmune encephalomyelitis (EAE), the animal model of MS, in rats. The presence of *C. pneumoniae* in brain tissue and CSF was recently demonstrated in a subgroup of MS patients at immunohistochemical, molecular and ultrastructural levels. This growing body of data argues against a central role for *C. pneumoniae* as a candidate in MS pathogenesis and suggests that an association between *C. pneumoniae* and MS is not selectively restricted to MS, but is shared by other neurological conditions. These findings also raise the possibility that *C. pneumoniae* is present in a subset of patients with MS in which *C. pneumoniae* could cause a brain chronic persistent infection acting as a cofactor in the development of the disease. Nevertheless, the actual involvement of *C. pneumoniae* in MS still remains matter of debate.

S43

Neurological complications of reactivated Varicella Zoster virus infection

T. Bergström *(Göteborg, SE)*

Varicella Zoster virus (VZV) infections of the central nervous system (CNS) constitute a major part of the burden of disease of this virus. The clinical scope includes encephalitis, brain stem encephalitis, myelitis, cerebellitis, cerebral vasculitis, ventriculitis, facial palsy, radiculitis and meningitis. Recently, improved diagnostics has revealed that VZV is a major CNS virus in several European countries. We here present clinical and laboratory data on 76 cases, collected during 1992–2005 in the Göteborg area, in whom VZV DNA was detected by PCR in the cerebrospinal fluid (CSF). During the last three-year period, the yearly incidence of VZV CNS infections rose sharply to become the most frequently detected virus in CSF at our laboratory. This shift coincided with the introduction of a novel TaqMan PCR system based on the gene coding for glycoprotein B. The age-related incidence was surprisingly evenly distributed with the exception of a peak at 70–80 years. In line with studies by others, a large proportion of the cases had no skin lesions, i.e. neither

shingles nor zoster. These two findings are compatible with that VZV might recur more often during life than previously thought and that manifestations in ''CNS only'' might be a clinically important part of the VZV disease spectrum. Viral DNA quantities in the CSF were found to be high as compared with similar data from CNS infections caused by herpes simplex virus 1 and 2. We suggest that VZV should be sought for in all cases of CNS infections of suspected viral origin that recommendations regarding doses and duration of antiviral therapy of such VZV infections should be thoroughly discussed, and that long-term follow-up studies of neurological outcome should be performed.

S44

Possible viral aetiology of multiple sclerosis

P.G.E. Kennedy *(Glasgow, UK)*

The demyelinating disease Multiple Sclerosis (MS) is the most important cause of neurological disability in young adults in Northern temperate regions. While the disease is probably multifactorial in aetiology, several lines of evidence indicate that there is a strong environmental element in its causation. A possible viral component to MS pathogenesis has been considered a possibility for many years, and while there is a long list of viruses that have been reported to be present in MS brain tissues using various techniques, previous reports of virus isolation have generally not been confirmed. This whole subject has therefore remained highly controversial. More recently, many groups have used sophisticated molecular virological techniques in attempts to address this issue. It is also possible that a virus may only be involved in the initial stages of MS-the so-called 'hit and run' scenario- in which case evidence of active viral infection in MS brain tissue would not be expected. Also, if MS is truly a heterogeneous disease, then a virus or indeed any other aetiological component may be involved in some cases but not others. This talk will give a broad overview of past and current attempts to identify viruses, viral nucleic acids and viral antigens in MS tissue. It will also focus on recent work that has implicated a possible role of Human Herpes Virus-6 (HHV-6) in MS, primarily studies that have suggested that viral DNA and RNA is increased in MS lesions compared with normal tissues, although the data in that area too remains controversial as the possible role of human endogenous retroviruses (HERV) in MS which will also be mentioned. There has also been recent interest in abnormal immune responses to EBV in MS patients. The latter indicates that a viral infection and altered immunity in MS are not necessarily inconsistent.

Evolution of β-lactamases

S46

CTX-M β-lactamases: the molecular story of a clinical success in community and hospital

R. Canton *(Madrid, ES)*

CTX-M extended-spectrum related β-lactamases were first detected in human clinical isolates nearly simultaneously in Germany and Argentina in 1989. Since then a dramatic increase in prevalence has been observed all over the world both in the nosocomial and community settings. Factors fuelling this situation include genetic aspects related to efficient *bla*CTX-M gene dissemination and enzymatic expression, bacterial host-specific features and frequent co-resistance to other antimicrobials. *bla*CTX-M genes are linked to apparently simple genetic structures including IS sequences (ISEcp1-like insertion sequences) and integrons (In60-like integrons) and to a lesser extent to phage-related constructions. These genetic supports are inserted in higher-order genetic complex structures such us transposable elements (Tn21-like transposons) and plasmids, which undoubtedly facilitate the mobility of *bla*CTX-M genes. The allodemic population structure, commonly associated to CTX-M producing isolates, illustrates this situation. Nevertheless, successful epidemic CTX-M producing clones have been also identified not only in the nosocomial setting but also in the community. *bla*CTX-M gene environments and surrounding structures might have also facilitated the acquisition of other resistance cassettes or their integration in structures previously harbouring these resistance cassettes. Co-resistance, including aminoglycosides, fluoroquinolones, trimethoprim and sulfonamides facilitates successful maintenance of CTX-M producing isolates and co-selection within bacterial communities. Intestinal colonization with CTX-M producing *E. coli* isolates has been increasingly recognized suggesting a potential source for clinical isolation. Nevertheless, CTX-M producing isolates have been associated with phylogenetic group D, commonly associated with extra intestinal pathogenic isolates but also with fluoroquinolone resistant isolates. On the other hand, enzymatic expression of early CTX-M ESBL yields higher resistance to cefotaxime than ceftazidime. However, CTX-M producing isolates have compensated this partial resistance phenotype with amino acid substitutions that expand the hydrolytic properties of CTX-M enzymes to ceftazidime and/or with the acquisition of other ESBL, mainly from SHV or TEM families, which generally affect both expanded spectrum cephalosporins. Continuous evolution of CTX-M enzymes and successful persistence and mobilization of *bla*CTX-M genes allow maintenance and increasing isolation of CTX-M producing isolates in clinical setting.

S47

The role of integrons in the spread of β-lactamases

G.M. Rossolini *(Siena, IT)*

Objectives: Mobile β-lactamase genes are carried on a broad repertoire of genetic elements, including transposons, composite transposons, integron-borne gene cassettes and integron-associated CR1 structures. The objective of this presentation will be to review and discuss the role of the integron system in the dissemination of clinically-relevant β-lactamase genes.
Methods: A comprehensive review of the scientific literature, of sequence databases, and of experimental results derived from the molecular characterization of β-lactam resistant clinical isolates was carried out to generate an updated compilation of β-lactamases encoded by integron-associated genes. Comparative analysis of integron structures containing β-lactamase genes was used to deduce information on their phylogeny.
Results: Genes encoding β-lactamases of all known molecular classes (A through D) can be found as integron-associated. A number of class A, class B and class D β-lactamase genes are carried on mobile gene cassettes inserted into integrons, while some additional class A and some class C β-lactamase genes are found in integron-associated CR1 structures. From the clinical

standpoint, the most important β-lactamase genes carried on gene cassettes are those encoding the metallo-β-lactamases of the IMP-, VIM-, GIM- and SIM-types, and those encoding some extended-spectrum serine β-lactamases (ESBLs) of molecular classes A (e.g. the VEB- and GES-type enzymes) and D (e.g. some members of the OXA-2 and OXA-10 lineages). The other integron-associated β-lactamase genes of clinical importance are those encoding class A ESBLs of some CTX-M-type lineages (CTX-M-2 and CTX-M-9) and those encoding AmpC-like enzymes of the DHA and CMY/MOX lineages, carried CR1 structures.

Conclusions: Integrons play an important role in the dissemination and expression of several emerging clinically relevant β-lactamase genes among major gram-negative pathogens including members of the family *Enterobacteriaceae*, *Pseudomonas aeruginosa* and *Acinetobacter* spp. The considerable structural diversity of integrons carrying the same β-lactamase-encoding cassettes underscores the role of cassette mobility in the dissemination of these resistance determinants. The presence of additional integron-associated recombination mechanisms enhances the potential of these elements in the dissemination of β-lactamase genes in the clinical setting.

Quinolone resistance and efflux

O50

Type II topoisomerase mutations in *Enterobacter cloacae* clinical isolates harbouring QNR and implication for quinolone resistance

E. Cambau, C. Lascols, J. Robert, V. Cattoir, V. Jarlier, C.J. Soussy *(Creteil, Paris, FR)*

Objectives: QNR is a new mechanism of resistance to quinolones due to the protection of DNA gyrase by the QNR protein. First described in *Escherichia coli* and *Klebsiella pneumoniae*, recent studies showed that in France it is disseminated mainly in *Enterobacter cloacae*. Although qnr was known to confer a low level of resistance to quinolones, most of the clinical *E. cloacae* qnr-positive strains harboured a high level of fluoroquinolone resistance suggesting associated mechanisms of quinolone resistance.
Methods: Detection of mutation in the quinolone resistance-determining region (QRDR) of the DNA gyrase (*gyr*A, *gyr*B) and topoisomerase IV (*par*C and *par*E) genes was performed for 22 qnr-positive strains of *E. cloacae* isolated in 3 hospitals in Paris. MICs of nine quinolones were determined by the E-test method.
Results: Two mutations, one in *gyr*A (S83I or S83F or S83Y) associated to one in *par*C (S80I), were observed in 11 qnr-positive strains. One mutation, in *gyr*A only (S83I or S83F or S83Y), was observed in 8 of the qnr-positive strains. The 3 remaining strains showed wild-type sequences of QRDR in *gyr*A, *gyr*B, *par*C and *par*E. All qnr-positive strains were intermediate or resistant to nalidixic acid, pefloxacin, norfloxacin, ofloxacin, and moxifloxacin but 3 strains were susceptible to ciprofloxacin (CIP) and 2 to levofloxacin (LVX) based on EUCAST-CASFM breakpoints. Geometric mean CIP MICs were 23 mg/l (range 4–> 32 mg/l) for qnr-positive strains with 2 topoisomerase mutations, 1.9 mg/l (range 0.12–8 mg/l) for qnr-positive strains with 1 topoisomerase mutation and 0.53 mg/l for strains devoid of topoisomerase mutations (range 0.38–0.75 mg/l). When transferred by conjugation from *E. cloacae* to *E. coli* BM13, qnr was shown to confer a low level of quinolone resistance with CIP MIC of 0.1 mg/l and LVX MIC of 0.25 mg/l but a 10- to 24-fold increase in MICs of all quinolones.
Conclusion: 19/22 (86 %) of the *E. cloacae* clinical strains that harbour qnr also harboured topoisomerase mutations that explain their high level of quinolone resistance. None of the 9 quinolones tested can easily detect for the presence of qnr except in *E. cloacae* strains devoid of topoisomerase mutations where a low level of quinolone resistance may indicate the presence of qnr.

O51

Emergence of plasmid-mediated quinolone resistance QnrS determinant in *enterobacterial* isolates in Europe

L. Poirel, C. Leviandier, P. Nordmann *(Le Kremlin Bicetre, FR)*

Objectives: The plasmid-mediated quinolone-resistance determinant QnrA has been identified previously in *Enterobacteriaceae* from USA, Asia and Europe and QnrS has been found in a *Shigella flexneri* isolate in Japan. Our goal was to look for QnrA and QnrS determinants in a collection of 185 nalidixic-acid resistant *enterobacterial* isolates recovered in Bicêtre hospital, France, during a six-month period in 2005 and in 187 extended-spectrum β-lactamase (ESBL)-positive *enterobacterial* isolates recovered in a three-year period (June 2002–June 2005). A previous study performed in our hospital from a collection of 297 nalidixic acid resistant *Escherichia coli* isolates recovered in 2003 identified QnrA in a single isolate whereas presence of *qnr*S gene had not been tested in that study or in any other study so far.
Methods: PCR were performed using specific primers for the *qnr*A and *qnr*S genes. Cloning and PCR experiments were performed to identify the upstream- and downstream-located regions of both genes. Pulsed-field gel electrophoresis (PFGE) was performed to compare the genotypes of the strains identified as Qnr positive.
Results: Among the 185 nalidixic acid-resistant but ESBL negative isolates recovered in 2005, one QnrA-positive *Klebsiella pneumoniae* isolate, one QnrS-positive *Serratia marcescens* isolate and four QnrS-positive *Enterobacter cloacae* isolates (corresponding to two different clones by PFGE) were identified. Most of these determinants were found to be transferable by conjugation or transformation to *E. coli*. Among the ESBL-positive isolates, no Qnr-positive isolate was identified in 2002. In 2003 and 2004, only a single QnrA-positive *Escherichia coli* isolate (VEB-1 positive) and a single QnrA-positive *E. cloacae* isolate (SHV-12 positive) were identified, respectively. In 2005 (six months), one QnrA-positive *Enterobacter aerogenes* isolate (CTX-M-1 and SHV-12-positive) and three QnrS-positive isolates (one CTX-M-1-positive *E. coli*, one TEM-52-positive *E. cloacae* and one SHV-12-positive *E. cloacae*) were identified. All these Qnr-positive isolates were resistant to nalidixic acid.
Conclusion: This works demonstrates further that Qnr determinants are emerging in Europe, and that the QnrS determinant might be more prevalent that QnrA. This is the

second identification of QnrS after that from Japan. Noteworthy, QnrS-positive isolates are not always ESBL producers.

O52

Detection of *qnr*S in clinical isolates of *Enterobacter cloacae* in Spain

M.E. Cano, J.M. Rodríguez-Martínez, J. Agüero, A. Pascual, J.M. García-Lobo, C. Velasco, L. Martínez-Martínez *(Santander, Seville, ES)*

Objective: To determine the presence of the *qnr*A and *qnr*S genes in clinical isolates of *Enterobacter* species in two hospitals, located in Santander (North of Spain) and Seville (South of Spain).
Methods: We evaluated 202 clinical isolates of *E. cloacae* (n = 155) and *E. aerogenes* (n = 47), collected at the Univ. Hosp. Marqués de Valdecilla (Santander) and the Univ. Hosp.Virgen Macarena (Seville) between January 2004 and October 2005. Identification and susceptibility testing were performed by the MicroScan WalkAway 96 System (Dade Behring) in Santander and by the Vitek 2 System (bioMerieux) in Seville. The isolates had different resistant phenotypes, including AmpC hyperproduction, ESBL production, resistance or decreased susceptibility to quinolones, and/or resistance to aminoglycosides. All 202 isolates were screened with a double PCR assay using specific primers: a multiplex PCR for *int*I1, *orf*513 and *qnr*A and another PCR for *qnr*S. qnr-positive results were confirmed by DNA sequence analysis. REP-PCR typing was performed in *orf*513 and *qnr* containing isolates. In *qnr*-positive isolates intermediate or susceptible to CIP by the automated system, the MICs of ciprofloxacin (CIP) and nalidixic acid (NA) were additionally determined by E-test.
Results: *qnr*S was detected in 22 *E. cloacae* among the 118 isolates of this species from Santander (18.6%). A BLAST analysis of the nucleotide sequences revealed 100% identity with the previously published *qnr*S sequence. Neither *qnr*A nor *qnr*S genes were detected in *E. clocae* from Seville nor in *E. aerogenes* from both locations. Five different REP-PCR profiles were obtained for the *qnr*S-positive *E. cloacae*, containing 14, 4, 2, 1 and 1 isolates respectively. All *qnr*S-positive isolates were resistant to CIP (MIC > 2 mg/l), except 4 clonally related isolates, which were susceptible to both CIP (MIC: 0.38 mg/l), NA (MIC 6–8 mg/l), 1 strain CIP-intermediate (MIC: 1.5 mg/l) and NA-resistant (MIC: 48 mg/l). Although 31 isolates of *E. cloacae* carried *orf*513, none of the studied organisms contained *qnr*A. In contrast, *qnr*S was found in 2 clonally related isolates bearing *orf*513.
Conclusions: *E. cloacae* containing the *qnr*S gene has been detected in Northern Spain. Some of these isolates were intermediate or susceptible to ciprofloxacin.

O53

Determination of the QNR–DNA gyrase interaction site by a genetic approach

A. Cesaro, V. Cattoir, C. Lascols, C.J. Soussy, E. Cambau *(Creteil, FR)*

Objectives: QNR is a new mechanism of quinolone resistance due to the protection of gyrase by the QNR protein. Since quinolone inhibition of gyrase is hampered by Qnr, we hypothesized that the Qnr-gyrase interaction occurs at a site closed to that of interaction of quinolones, i.e. the quinolone resistance-determining region (QRDR) in the GyrA and GyrB subunits.
Methods: First, *in vitro* selection of quinolone resistant mutants was performed comparatively with a wild-type (WT) reference *Escherichia coli* strain and with an *E. coli* transconjugant harbouring the *qnr*A gene. Selection was carried out using nalidixic acid (NAL), ciprofloxacin (CIP) and moxifloxacin (MOX). Mutants were chosen on the basis of quinolone MICs and mutations in the *gyr*A and *gyr*B QRDRs were determined. Second, *qnr*A was introduced by conjugation into *E. coli* strains referenced for *gyr*A mutations (S83L, D87Y, D87G, D87N), *gyr*B mutations (D426N, K447E), *gyr*A + *par*C (S83L + S80R), and *gyr*A + *par*E (S83L + L445H).
Results: For the WT *E. coli*, *gyr*A mutation was observed in 10/10 mutants selected by NAL, 7/7 mutants selected by CIP and in 5/12 mutants selected by MOX. For the *E. coli qnr+*, *gyr*A mutation was observed in 18/22 mutants selected by NAL, 1/11 mutants selected by CIP and 0/18 mutants selected by MOX. Analysis of the *gyr*A mutations showed that whereas 68% (15/22) of the mutations occurred at the codon 87 (D87G, D87H, D87N or D87Y) in mutants selected from the WT *E. coli*, only 1/19 harboured a D87Y mutation in mutants selected from *E. coli qnr+*. Conversely, 95 % (18/19) of the mutations observed in mutants selected from the *E. coli qnr+* occurred at the codon 83 (all S83L) but 7/22 for WT *E. coli* mutants. In all 18 one step-mutants and in all 8-second-step mutants selected by MOX from *E. coli qnr+*, no mutation was observed either in the QRDRs in *gyr*A, *gyr*B, *par*C and *par*E, or in the entire *gyr*A and *gyr*B genes, whereas selection by MOX from the *E. coli* wild-type resulted in 5 *gyr*A mutants (D87N [n = 3] or D87G [n = 2]). After introduction of *qnr* in the strains referenced for topoisomerase mutations, MICs of CIP and MOX increased 2- to 16-fold. NAL MICs did not increase whatever the topoisomerase mutation harboured by the recipient strain.
Conclusion: From the results of the two approaches, D87 seems to be one of the amino acid involved in QNR-gyrase interaction, and S83 seems to preferentially interact with NAL and CIP in presence of QNR but not with MOX.

O54

Putative implication of YceE (transporter protein) and *omp*N (porin) in the resistance to quinolones in *Escherichia coli*

A. Fabrega, J. Sanchez-Cespedes, M.M. Tavio, M. Ruiz, J. Vila *(Barcelona, Las Palmas, ES)*

Objectives: The interplay between decreased permeability and increased efflux may play an important role in the acquisition of quinolone-resistance in *Escherichia coli*. The main objective of this study was to investigate the expression of outer membrane protein(s) and efflux pump(s) as a mechanism of resistance to quinolones in two isogenic quinolone-susceptible and resistant *E. coli* strains following a genomic and proteomic approach.
Methods: A quinolone-resistant mutant was selected with norfloxacin from an *E. coli* clinical isolate. Norfloxacin accumulation in both wild-type and mutant strains was determined by a fluorescent assay. DNA microarrays containing the whole genome of *E.coli* were used to analyse the differential expression of genes. RT-PCR was used to confirm DNA microarrays results. 2D-gel electrophoresis was used to perform a protein analysis in both strains.
Results: Accumulation assay demonstrated that the quinolone-resistant *E.coli* mutant strain presented a lower intracellular norfloxacin accumulation than the wild-type strain. DNA microarrays showed that 27 genes were over expressed in the mutant, among which *acr*A, *acr*B, *yce*E and *omp*N were found; whereas only 8 genes were down-regulated, among which *omp*F

was found. RT-PCR analysis confirmed the increased expression of *acr*A, *acr*B, *yce*E and *omp*N, and the decreased expression of *omp*F. 2D-gel electrophoresis confirmed the lower expression of *omp*F and showed the overexpression of TolC in the mutant strain.
Conclusions: It is known that AcrAB/TolC plays an important role in quinolone-resistance acquisition in *E.coli*. However, the overexpression of *yce*E and *Omp*N may also play a role in quinolone resistance acquisition in this microorganism.

O55

Potential of fluoroquinolones to induce the expression of the *acr*AB efflux pump and the global regulator *mar*A in *Salmonella hadar*

S. Schmidt, P. Heisig *(Hamburg, DE)*

Objectives: Fluoroquinolones (FQs) are often the antibiotics of choice to combat gastrointestinal, respiratory and urinary tract infections as well as infections of the skin and soft tissue (SST). However, bacterial resistance is becoming an increasing problem. Resistance to FQs is acquired by alterations of the target (mutations in type II topoisomerase genes *gyr*AB, *par*C) as well as by decreased uptake and increased efflux. One important multidrug resistance (mdr) efflux pump in *Salmonella* is AcrAB-TolC, consisting of an RND-type transporter (AcrB), a membrane fusion protein (AcrA) and an outer membrane channel (TolC). Enhanced expression of the efflux system can be due to e.g. mutations in the gene *acr*R for the local regulator AcrR or an altered expression of the global regulator system *mar*RAB. The question addressed in the present study is, if in addition FQs are capable of inducing the expression of the AcrAB efflux pump and/or global regulator systems like MarRAB.
Methods: *Salmonella Hadar* was cultivated in the presence of the respective FQ (0.5 × MIC, which corresponds to approximately 1% of the concentration achieved in the tissue during therapy, as well as 50 × MIC for ciprofloxacin) and harvested at OD600 nm 0.7. After isolation of total RNA (Qiagen RNeasy Mini Kit) and reverse transcription using gene specific primers, expression changes of *acr*A and *mar*A genes were determined by real-time PCR using SYBR Green.
Results: After induction with norfloxacin a 6.2-fold upregulation in *acr*A gene expression was determined, whilst *mar*A gene expression was upregulated 3.8-fold. The presence of moxifloxacin during cultivation led to a 1.8-fold upregulation in *acr*A gene expression, however no expression change in *mar*A was observed. Exposition neither to ciprofloxacin (0.5 and 50 × MIC) nor to levofloxacin, ofloxacin, marbofloxacin, pradofloxacin, garenoxacin nor BayY3118 (0.5 × MIC) resulted in any expression changes in *acr*A or *mar*A, respectively.
Conclusions: From the assortment of structurally diverse FQs only norfloxacin and to a lesser extend moxifloxacin are able to effect an increased *acr*A and *mar*A expression. Regarding the resistance development it is reassuring to observe that the induction of mdr efflux by FQs is not of clinical relevance in *Salmonella*.

O56

Horizontal gene transfer contributes to fluoroquinolone resistance in *Streptococcus pyogenes*

C.B. Duesberg, S. Malhotra-Kumar, T. Welte, H. Goossens, M.W.R. Pletz *(Hannover, DE; Antwerp, BE)*

Objectives: To date, reports on *S. pyogenes* resistant to fluoroquinolones have been rare, but a recent Belgian surveillance study found a prevalence of 5.4% among nearly 2800 patients with tonsillopharyngitis due to *S. pyogenes* (Malhotra-Kumar *et al*. Clonal spread of fluoroquinolone non-susceptible *Streptococcus pyogenes*. J Antimicrob Chemother 2005; 55:320–325). Fluoroquinolone resistance in *S. pyogenes* arises due to mutations in the topoisomerase type II enzymes. These mutations can arise spontaneously, or they can be acquired by horizontal gene transfer. Acquisition of fluoroquinolone resistance by interspecies recombination has already been described in *Pneumococci*, but not in *S. pyogenes*. The aim of the present investigation was to elucidate the contribution of horizontal gene transfer to the acquisition of fluoroquinolone resistance in *S. pyogenes*.
Methods: Fluoroquinolone-resistant *S. pyogenes* previously characterized for resistance mechanisms during the surveillance study cited above were studied. Presence of the D91N substitution in *par*C, which is not a feature of wild type *S. pyogenes*, was used as a marker for selecting isolates. Of the 34 isolates studied, 21 were non-susceptible (ciprofloxacin MIC≥ 2 mg/l) and 13 were susceptible to fluoroquinolones. The sequences of the fluoroquinolone resistance determining region of *par*C were used to perform a BLAST search. The first 26 hits were chosen for further analysis. Alignments were conducted by ClustalX, and phylogeny was constructed by the Bayesian method. Recombination was statistically confirmed using the maximum chi-squared test.
Results: Phylogenetic analysis revealed evidence of horizontal gene transfer in 13 isolates (38.2%) belonging to different emm-types and pulsed field gel electrophoresis clusters. In all 13 isolates recombination with the bovine pathogen *Streptococcus dysgalactiae* subsp. dysgalactiae was confirmed by a maximum chi-squared test at a statistically significant ($p < 0.05$) or highly significant level ($p < 0.001$).
Conclusions: Our results prove that horizontal gene transfer from other streptococcal species contributes to fluoroquinolone resistance in *S. pyogenes*. *S. pyogenes* exhibiting evidence of horizontal transfer were more likely to be fluoroquinolone-resistant. Thus, fluoroquinolone use in agriculture might also indirectly impact resistance in the human pathogen *S. pyogenes*.

O57

New mutation at the position 81 in GyrA in clinical strains of *Mycobacterium tuberculosis* resistant to quinolone: report of two clinical cases and functional analysis of DNA gyrase mutant enzymes

S. Matrat, N. Veziris, V. Jarlier, E. Cambau, C. Truffot-Pernot, J. Camuset, E. Bouvet, A. Aubry *(Paris, Créteil, Argenteuil, FR)*

Objectives: Fluoroquinolone resistance is emerging in *M. tuberculosis* (Mtb) with increasing drug use. DNA gyrase is the sole target of quinolone in Mtb (lack of topoisomerase IV). Mutations in the heterotetramer GyrA2GyrB2 are associated with resistance to quinolones. We report two cases of infection with multidrug resistant tuberculosis (MDR-TB) strains carrying a new G81A mutation in GyrA. We also investigated the role of the amino acid at this position in the resistance to quinolones in Mtb.
Methods: Two mutant genes were produced by site-directed mutagenesis of the wild type *gyr*A gene from Mtb strain H37Rv: *gyr*A g263c (as found in our clinical strains, see results) and *gyr*A g262t (as found by Takiff in an *in vitro* mutant selection, AAC, 1994). GyrA and GyrB subunits (WT and mutants GyrA G81A and GyrA G81C) were overexpressed in *Escherichia coli*, purified and used to reconstitute highly active gyrase complex. MICs and

enzyme inhibition (concentration of drug required to inhibit the supercoiling activity of the enzyme by 50% = IC50) were determined for moxifloxacin, gatifloxacin, ofloxacin, levofloxacin and enoxacin.
Results: Two men aged 24 and 45 years old, both with a previous story of TB, born in Algeria and Congo respectively, had MDR TB caused by Mtb strain carrying a G81A mutation in GyrA. One had cavitary pulmonary TB, was AFB positive and HIV negative. The other one had both pulmonary and extrapulmonary TB, was AFB positive and HIV positive. Both had been treated by fluoroquinolone within the months before the diagnosis of MDR-TB. MICs and IC50s of quinolones were 2 to 16 fold and 3 to 32 fold higher than for the WT for the GyrA G81A mutant and for the GyrA G81C mutant, respectively.
Conclusion: We demonstrated unequivocally that modifying the amino acid at position 81 (whatever G81A or G81C) in GyrA subunit of DNA gyrase lead to quinolone resistance in Mtb. This point contributes to clarify the nature of the drug-enzyme binding pocket in Mtb, which most likely include the aminoacid 81.

Viruses and viral diagnostics

O58

HCMV infects and replicates in the adrenal gland

M. Trevisan, G. Masi, R. Cusinato, K. Fincati, A. Loregian, G. Palù, L. Barzon *(Padua, IT)*

Although one of the clinical pictures of human cytomegalovirus (HCMV) infection is adrenal involvement reported especially in AIDS patients, the association of this herpesvirus with adrenal gland disease has never been cleared. Moreover, recent investigations have shown that a higher frequency of HCMV in malignant tumour tissues, but its role in the pathogenesis of adrenal tumours has not been explored so far. Therefore, in this study we investigated the presence of HCMV in a large series of normal and tumour adrenal samples and the effect of HCMV infection on adrenocortical carcinoma (ACC) cells *in vitro*. A total of 100 adrenal tissues (53 adrenocortical adenomas, 11 ACCs, 17 pheochromocytomas, 2 myelolipomas, 1 primary adrenal non-Hodgkin's lymphoma, and 16 normal adrenal tissues) were examined using quantitative PCR. HCMV DNA was detected in 19% adrenocortical adenomas, 12% pheochromocytomas and 1 normal adrenal tissue, but not in malignant tumours. The positive samples were confirmed by immunohistochemical evaluation of HCMV immediate-early gene expression. To verify the ability of HCMV to infect adrenocortical cell lines *in vitro*, the NCI-H295 and SW13 ACC cell lines and primary ACC cell cultures were infected with clinical isolates of HCMV and with the HCMV strain AD169. All the HCMV strains employed resulted in productive replication in ACC cells, as demonstrated by the expression of both early (pp72) and late (pp65) viral antigens. Analysis of the kinetics of viral replication in ACC cells showed efficient production of infectious viral particles, even if at lower levels than in MRC5 cells. Viral and cellular gene expression profile after AD169 infection of ACC cell lines were assessed by quantitative RT-PCR and DNA microarray analysis, respectively, in a time-course experiment. Evaluation of a total of 22 000 human genes with microarray analysis demonstrated that AD169 infection induced genes promoting cell proliferation and repressed genes that prevent cell migration and invasiveness. These results demonstrates that the human adrenal gland is a target for HCMV infection. HCMV modulates several cellular pathways *in vitro*, including cell growth and stress response. These data represent the basis for further investigation on the effect of HCMV on adrenal function and diseases.

O59

Simultaneous detection of the human cytomegalovirus DNA and RNA by targeting the UL73/74 gene region using multiplex real-time polymerase chain reaction assays

Y.-J. Chan, J.-C. Lin, C.-H. Tsai, W.-S. Weng, D.M-T. Ho *(Taipei, TW)*

Objectives: Reactivation of cytomegalovirus (CMV) among immunocompromised patients may result in severe complications. A timely and accurate diagnostic method for CMV infections is crucial. Polymerase chain reaction (PCR)-based methods have been reported but are complicated by potential contamination, latent CMV infection and strain variations. A quantitative real-time PCR method that detects both CMV DNA and RNA may be one of the solutions.
Methods: By cDNA cloning, we obtained clones encompassing the CMV UL73/74 region and encoding a spliced mRNA, which was used as the standard. This region contained both conserved and variable sequences. We designed primers and analysed 21 clinical viral isolates covering the four major CMV UL 73 genotypes in the junction region of the UL73/74 mRNA by restriction mapping and sequencing. We designed primers and junction TaqMan probes to detect 104 clinical buffy coat samples by real-time reverse transcriptase PCR system (rRT-PCR). The performance of the rRT-PCR was compared to our established in-house real-time PCR (ir-PCR) using the same UL73/74 control standard. Finally, we developed a multiplex real-time PCR and RT-PCR assay (mr-PCR/RT-PCR) to simultaneously detect the existence of both CMV DNA and RNA for 6 clinically samples suspecting CMV infections.
Results: Of the 21 clinical CMV isolates, genotyping revealed all four known genotypes in the ratio of 5:6:4:5 (one possible mix infection) with two junction sequences. The rRT-PCR successfully detected spliced mRNA from different genotypes. The sensitivity for rRT-PCR was 10 copies of the plasmid standard, which was comparable to our previous developed ir-PCR. Good linearity was observed between 100 to 1 000 000 copies. Of the 104 clinical samples, 13 samples were positive by both rRT-PCR and ir-PCR (mean, 97 300 copies/ml). There were 43 samples tested positive by the ir-PCR (mean, 1585 copies/ml) and negative by the rRT-PCR, but in no case was the rRT-PCR result positive and the ir-PCR result negative. There were 48 samples tested negative by both methods. The mr-PCR/RT-PCR simultaneously detected both DNA (mean, 281 000 copies/ml) and RNA (mean, 76 600 copies/ml) for all the 6 clinical samples.
Conclusion: The rRT-PCR is as sensitive as the ir-PCR and detected CMV spliced RNA. A multiplex real-time PCR and RT-PCR is feasible for the UL73/74 region in detecting CMV for clinical samples. It might be helpful to monitor the clinical CMV activity by this new real-time system.

O60

Prospective comparison of real-time quantitative PCR on blood with rapid culture of urine and throat samples for monitoring cytomegalovirus infection in haematopoietic stem cell transplant recipients

I. Thoelen, W. Laffut, K. Van Vaerenbergh, M. Develter, K. Theunissen, J. Maertens, J. Van Eldere *(Leuven, BE)*

Objectives: Human cytomegalovirus (CMV) remains a significant cause of morbidity and mortality in immunocompromised patients. Effective pre-emptive antiviral treatment depends on early detection of CMV infection, before the onset of symptoms. In this study, we prospectively evaluated the use of real-time quantitative PCR (RT-PCR) on whole blood versus virus isolation from urine and upper respiratory tract (URT) samples for monitoring CMV infection in haematopoietic stem cell transplant (HSCT) patients.
Methods: 84 HSCT patients were included in the study. At 998 time points, blood, urine and URT samples were simultaneously taken. An in-house CMV-specific RT-PCR on whole blood and virus culture on the urine and URT specimens, using the rapid shell vial technique, were performed.
Results: In 889 of 998 linked samples both PCR and virus isolations were negative. Of 90 linked samples with a positive RT-PCR, only 3 had a positive virus culture from both isolation sites (urine and URT) and 78 had negative culture results both for urine and throat samples. Copy numbers in the RT-PCR positive and urine and throat culture negative cases ranged from 500 to 1 100 000 copies/ml. Whereas discordant cases with low RT-PCR copy numbers might have been due to the low sensitivity of the culture methods, discordant cases with high copy numbers could have been due to concurrent antiviral therapy. Of 78 linked samples, 24 had high copy numbers (more than 5000) and negative cultures. Of these 24 linked samples, 20 were taken under antiviral treatment (treatment with IV/peroral ganciclovir (6 cases), IV foscavir (4 cases) or IV cidofovir (10 cases)). Positive RT-PCR and positive culture results for one sample were observed in 9 cases (all urine positive isolations). A negative RT-PCR combined with one or two positive isolations was observed in 19 of 998 linked samples. 15 of these samples had a positive urine culture with negative RT-PCR and negative throat cultures. These samples were from 2 patients with intermittently positive urine cultures.
Conclusions: Our data suggest that virus isolation by the shell vial technique should not be used for monitoring CMV infection in HSCT patients because of the high number of false negative results due to low sensitivity of the culture technique or concurrent antiviral therapy. RT-PCR on whole blood seems a more sensitive tool to guide pre-emptive therapy.

O61

Diagnostic relevance of screening clinical specimens from patients attending infectious diseases and haematology units for HHV6 DNA: a two-year experience

G. Parruti, A. Manna, A. Di Girolamo, M. Ciotti, D. Racciatti, G. D'Amico, C. Favalli, E. Pizzigallo, D. D'Antonio *(Pescara, Chieti, Rome, IT)*

Objectives: HHV6 (Human Herpes Virus 6) is a member of the βherpesvirinae subfamily in the Herpesviridae family. It has a yet incompletely defined cell tropism and its clinical spectrum is currently being clarified, both in the immunocompetent and in the immunocompromised host. We introduced the use of tools for molecular diagnosis of HHV6 active replication into routine clinical practice at our Institutions late in 2003. Here we summarize the preliminary results of our 2-year experience in terms of diagnostic efficiency.
Methods: Sample preparation for DNA isolation, commercially available kits were used, according to manufacturers' instructions. DNA extraction from large clinical samples (such as plasma, serum, cerebrospinal fluid, urine, sputum, broncoalveolar lavage fluid) was mainly performed using the QIAamp DNA Mini Kit (QIAgen GmbH, Hilden, Germany). Detection of positive samples for HHV6:Clinical specimens were scored as positive for HHV6 when they tested as positive for the Herpesvirus-Consensus PCR (Argene Biosoft) amplification product, again positive for the HHV6 amplification product and negative for HSV type 1, HSV type 2, Varicella-Zoster virus, Epstein-Barr virus and Cytomegalovirus amplifications. Quantisation of HHV-6 DNA. Quantisation of HHV6 DNA was performed using standard curves generated by TaqMan probes and primers in an ABI 7000 machine (Q-HHV-6 Real Time System -Amplimedical Bioline).
Results: During 2004 and 2005 (January through October), 1207 samples were processed at our Institutions, 987 of which (81.7%) collected at Haematological Units. Thirty-two samples (2.7%) from 19 patients turned out to be positive: 16 of them were from immunocompetent patients (8 patients), the remaining 16 were from immunocompromised patients (11 patients, 1 of whom was HIV positive). Among immunocompetent patients, 2 had persistent fever with rash; one had persistent fever only, while the remaining 5 had recurrent or persistent stomatitis. Among immunocompromised patients, 9 had a diagnosis of haematological malignancy.
Conclusion: Molecular diagnosis of HHV6 active replication seems to be an interesting tool to diagnose a wide range of persistent or recurring clinical conditions. It seems to be particularly promising when used to screen previously unrecognized conditions in the immunocompetent host, as in the case of recurrent stomatitis or persistent fever with rash.

O62

Echovirus 9: a prominent enterovirus causing CNS disease in Kuwait

A. Dalwai, S. Ahmad, A. Pacsa, W. Al-Nakib *(Kuwait, KW)*

Objectives: Enteroviruses in general are recognized as the major cause of aseptic meningitis particularly in children; however, they appear to vary in their neurotropism and neurovirulence. Some types may be overrepresented as etiological agents of CNS disease than others. The aim of this study was to genotype the prevalent enteroviruses causing CNS disease in Kuwait and to study the association between a particular enterovirus with the severity of the disease.
Methods: Detection of enteroviral RNA was based on semi-nested RT-PCR amplification of a portion of the 5'UTR of the enteroviral genome followed by Southern hybridization with an enterovirus specific probe to confirm the results. The enterovirus was genotyped by sequencing of the 5'UTR and the VP4 encoding region, and the sequence was analysed by BLAST analysis, CLUSTALW alignment and PHYLIP phylogenetic analysis package.
Results: Enteroviral RNA was detected in the CSF of 26% (168/647) of the CNS disease cases with a majority of the enterovirus positive cases (82%; 138/168) being children less than 12 years of age. Of the 8 different enteroviruses identified, echovirus 9 was the most common (45%; 66 of 147 genotyped), followed by

echovirus 11 (18%; 27/147), coxsackievirus A7 (17%; 25/147) and coxsackievirus B5 (10%; 14/147). In the severe CNS disease cases (encephalitis and those cases presenting with febrile convulsions/seizures), the occurrence of echovirus 9 was significantly higher (68%; 24 of 35 genotyped) than that observed in less severe CNS disease cases presenting with aseptic meningitis (37%; 42 of 112 genotyped). Echovirus 11 and coxsackievirus B5, on the other hand were more frequent in less severe CNS disease than in severe CNS disease cases.
Conclusions: Echovirus 9 was found to be the predominant enterovirus associated with CNS disease in Kuwait and data obtained through this study suggests that infection with echovirus 9 may lead to a more severe CNS disease outcome.
Supported by Research Administration project grants MI 04/01, YM 03/02 and College of Graduate Studies, Kuwait University.

O63

Detection of respiratory pathogens by real-time PCR in clinical practice

J.W.A. Rossen, E.G. Klein Breteler, A.J. Weersink, R. Schuurman, A.M. van Loon *(Utrecht, NL)*

Objectives: Few hospitals have introduced nucleic amplification techniques for the routine diagnosis of all respiratory tract infections in adults. These diagnostic tests are costly, and the diagnostic yield and feasibility of implementation in routine diagnostic work-up has not been evaluated sufficiently. The diagnostic value of real-time PCR compared to conventional virus detection methods is evaluated in a prospective study during one respiratory season.
Methods: Nose-throat swabs, nasopharyngeal washes, nasopharyngeal aspirates, sputum samples, and bronchoalveolar lavages of patients admitted to our general hospital or to the children's hospital were evaluated by virus culture, direct immunofluorescence assays (DIF) for adenovirus, influenzavirus A/B, parainfluenzavirus 1 to 3 and RSV virus and by real-time PCR for adenoviruses, coronavirus OC43, 229E and NL63, influenzavirus A/B, parainfluenzavirus 1 to 4, rhinoviruses, RS virus A/B, human metapneumovirus and *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*.
Results: Samples of 110 adults and 240 children were included in this study. In adults, respiratory viruses were detected by virus culture in 4 patients (4%) and by real-time PCR in 65 patients (59%). Most frequent pathogens detected were: rhinovirus (n = 22), influenzavirus (n = 20) and RSV (n = 12). In 10 of the 65 positive patients (15%) more than one virus was detected by real-time PCR. In children, respiratory viruses were detected by virus culture in 34 patients (14%), by DIF-assays in 63 patients (26%) and by real-time PCR in 179 patients (75%). The most frequent pathogens detected were: RSV (n = 74), rhinovirus (n = 67), coronaviruses (n = 32) and influenzavirus (n = 24). In 57 of the 179 positive patients (32%) more than one virus was detected by real-time PCR. Real-time PCR for RSV and adenovirus revealed significant differences in Ct-values between DIF positive and DIF negative samples.
Conclusion: The sensitivity of real-time PCR is significantly higher compared to that of virus culture and DIF-assays. Moreover, mixed infections were only detected using real-time PCR and were not found by virus culture and/or DIF assays. Thus, rapid detection of respiratory viruses by means of real-time PCR increased the number of detected pathogens considerably. The results of this study will be used to develop a diagnostic algorithm for selective detection of specific respiratory pathogens in different patient groups.

O64

Nucleic acid sequence based amplification and molecular beacon detection for the real-time identification of human metapneumovirus in paediatric respiratory specimens

R. Manji, F. Zhang, C. Ginocchio *(Lake Success, US)*

Background: Human metapneumovirus (hMPV) may account for about 10% of pediatric upper and/or lower respiratory tract disease in which a respiratory virus, such as RSV, influenza or parainfluenza, could not be detected. Studies have also suggested that hMPV may contribute to increased severity of disease when present with other viral pathogens. Most laboratories cannot routinely diagnose hMPV infections since the virus is slow growing and there are limited reagents for confirmation. The rapid diagnosis of hMPV infections is of central importance for patient management (rational use of antibiotics and antiviral agents), hospital infection control and for understanding the epidemiological disease patterns of hMPV. This study included a technical validation and retrospective clinical evaluation of a real time NASBA assay for the detection of hMPV in pediatric respiratory samples.
Methods: Samples tested included: dilution panels of an hMPV viral isolate, isolates of common respiratory pathogens, and frozen respiratory specimens (nasopharyngeal aspirates, washes or swabs) from 232 children (age range: 5 days to 2 years) who presented with respiratory disease. Nucleic acid (NA) isolation, amplification and detection were performed using NucliSens EasyQ Basic Kit and NucliSens EasyQ hMPV reagents (bioMérieux). Specimen nucleic acids and an hMPV specific internal RNA control (IC) were co-extracted using NucliSens Magnetic Extraction Reagents and the NucliSens miniMAG instrument (bioMérieux) and co-amplified using a single hMPV specific primer pair. Included in the reaction were an hMPV specific molecular beacon (5'-FAM) and an IC specific molecular beacon (5'-ROX). Target amplification and continuous monitoring of emitted fluorescence were performed using a NucliSens EasyQ analyser (bioMérieux).
Results: The limit of detection for hMPV was 2.5 TCID50 per reaction and the 90% detection rate was 12.5 TCID50 per reaction. The assay was 100% specific for hMPV, with no cross reactivity detected to a panel of viral and bacterial respiratory pathogens. The overall hMPV prevalence rate was 2.6% and the rate for samples with no other viral pathogen detected was 3.8%. One sample was also positive for RSV.
Conclusions: The NucliSens EasyQ hMPV assay demonstrated excellent sensitivity and specificity. The assay was easy to use, required minimal hands on time (1 hour) and easily provided a same day result. Additional year round studies will provide important epidemiologic data.

O65

Development of a real-time NASBA for human metapneumovirus

E. Bufflier, H. Savelli, F. Jacobs, C. Moore, P. Van de Wiel *(Grenoble, FR; Boxtel, NL; Cardiff, UK)*

Objective: The aim was to develop and evaluate human Metapneumovirus (hMPV) reagents based on NASBA amplification and real-time detection with molecular beacons.
Methods: hMPV RNA is isolated using a semi automated magnetic extraction method (NucliSens® miniMag). An internal control is added to the sample prior to nucleic acid extraction. Primers are directed against the M-gene region of the hMPV

genome (subtypes A1, A2, B1 and B2), and against the internal control molecule. A FAM-labelled molecular beacon probe is used to detect hMPV amplicons, while a ROX-labelled molecular beacon is designed to detect the internal control amplicons, amplified in the same reaction. Amplification and real-time detection reactions are performed in a NucliSens® EasyQ Analyser. The extraction and amplification-detection reactions to detect hMPV in nasal swab samples can be performed within approximately 3.0 hours.

Results: The analytical sensitivity of the NASBA reaction was determined to be close to 10 copies per input (90% hit rate) using serial dilutions of *in vitro* hMPV RNA in direct amplification, while in combination with extraction, the sensitivity was found to be 263 copies per input (90% hit rate). No cross reactivity was observed with PIV 1, PIV 2, PIV 3, PIV 4, RSV A, RSV B, Sars CoV, *Influenza* A and *Influenza* B. The NASBA real time reagents to detect both hMPV and RSV were further investigated in a clinical study performed in Cardiff in 2004–2005. Amongst 1158 clinical respiratory samples tested (Nasopharyngeal Swabs, Nasal Swabs, Throat Swabs and Nasopharyngeal Aspirates) the NASBA allowed to detect 22 hMPV positive samples (ca. 2%). Finally, the efficiency of the method to detect the four A1, A2, B1 and B2 subtypes of hMPV was demonstrated on viral stocks.

Conclusions: The data showed that the real-time hMPV reagents allows a rapid and sensitive qualitative detection of hMPV. The use of standardized reagents offers considerable benefits in a routine setting for the clinical management of patients with hMPV infections.

O66

Viral induced asthma exacerbations not associated with delayed viral clearance and increased viral load

A.P.E. Sachs, L.J.R. van Elden, A.M. van Loon, G. Kimman, T.J.M. Verheij, M. Nijhuis *(Utrecht, Bilthoven, NL)*

Introduction: Viral respiratory tract infections (RTI) are the most common cause of asthma exacerbations. Especially human rhino viruses (HRV) infections lead to more severe and longer duration of lower respiratory tract (LRT) symptoms in asthmatics than in non-asthmatics. Up-regulation of rhinovirus specific intracellular adhesion molecule (ICAM-1) receptors in asthmatics might be responsible for increased susceptibility to HRV, as well as differences in immunological response, resulting in delayed viral clearance.

Aim: Assessing the magnitude and duration of viral replication and severity of symptoms in relation to various cytokines in nasal washes in asthmatics and controls during naturally occurring viral RTI.

Methods: In a prospective study, asthmatics aged 18–45 years, were recruited according the Dutch asthma guidelines from general practices, together with 44 healthy controls. Both groups daily recorded signs and symptoms of the upper respiratory tract (URT) and of the LRT, rated from 1 (mild) to 3 (severe). Asthmatics recorded peak expiratory flow (PEF) twice daily. Participants contacted investigators if URT symptoms totalled 4, and/or if LRT symptoms >5. Next, participants were visited within 48 hours, with follow-up visits at day 3/4, day 5/6, after 2 and 8 weeks (baseline). During each visit a nasal wash was collected from each nostril for viral cultures RT-PCR, and assays for cytokines.

Results: 44 asthmatics and 44 controls enrolled in the study and 43/88 persons reported 57 episodes of RTI. In 33/57 (58%) episodes a virus was found by PCR. Viral cultures had no additional diagnostic value. No significant differences observed between number of U/LRTI caused by viruses in asthmatics (16/33, 48%) and controls (17/33, 52%). Majority of URTIs (n = 33) were caused by HRV. After first symptoms of U/LRT symptoms, in both groups, simultaneous clearance of viruses in the URT has been observed. No differences in proinflammatory cytokines can be determined in the URT in both groups. LRT symptoms in asthmatics persisted for more than 1 week after clearance of viruses. The actual URTI precedes the PEF reduction as well as LRT symptoms.

Conclusion: Viral induced asthma exacerbations are not associated with delayed clearance and increased viral load. Incremental effects during maintenance therapy of intranasal anti-viral and anti-inflammatory medication need to be studied.

O67

Quantitative detection of herpes simplex virus DNA in the lower respiratory tract

J. Gooskens, K. Templeton, E. Claas, M. van Bussel, V. Smit, A. Kroes *(Leiden, NL)*

Objectives: To compare quantitative herpes simplex virus (HSV) DNA results from adult bronchoalveolar lavage (BAL) specimens to clinical outcome.

Methods: An internally controlled, quantitative real-time PCR (qPCR) assay targeting the UL30 gene encoding HSV DNA polymerase was developed and validated. Adult BAL specimens obtained during a 1-year period (Feb 2003–Feb 2004) were collected. HSV qPCR was retrospectively performed on these specimens and results were compared to patient characteristics and outcome. Differentiation of HSV1 and HSV2 was performed by PCR. In addition, the presence of Varicella-Zoster virus (VZV), cytomegalovirus (CMV), human herpesvirus 6 (HHV-6) and Epstein-Barr virus (EBV) were determined by qPCR. Results were not used to assist clinical management.

Results: A total of 57 BAL specimens were included, of which 41 of 57 (72%) were obtained from immunocompromised patients. HSV DNA was detected in 11 of 57 specimens (19%) with a mean viral load of 5.6 log (10) genome equivalents/ml (range, log 2.9–log 8.1). Of these specimens, 8 contained HSV1, 1 contained HSV2, 2 contained undetermined HSV and none contained both types. HSV DNA level equal or higher than log 5.5 (n = 6, 11%) was significantly associated with 30 day mortality (OR 7.5, 95%CI 1.22–46.0). All patients with HSV levels equal or higher than log 7.5 had severe respiratory failure. HSV pneumonia was histologically proven in one patient with log 8.0 HSV DNA in BAL fluid at autopsy. No patient with HSV DNA levels below log 5.5 (n = 5) or with high levels of CMV (n = 2), EBV (n = 7), VZV (n = 0) or HHV6 (n = 0) died within 30 days of hospital admission.

Conclusion: Detection of high levels of HSV DNA in BAL fluid was associated with severe respiratory failure and with fatal outcome among mainly immunocompromised patients. These results suggest quantification of HSV DNA is a potential diagnostic tool in BAL fluid of high-risk patients.

ISSN: 1470-9465

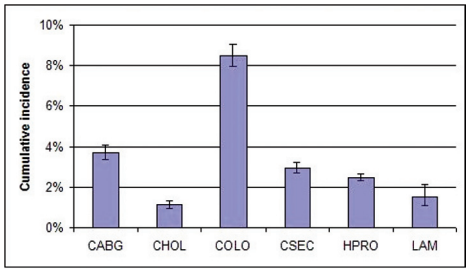
Clinical epidemiology of nosocomial infections

O68

Surgical site infections in Europe: results from HELICS surveillance 2004

C. De Laet, C. Suetens, J. Fabry *(Brussels, BE; Lyon, FR)*

Objectives: The HELICS project is a European-wide surveillance programme for healthcare-associated infections. It is a 'network of networks' with contributions from national surveillance systems, using a minimal common dataset (http://ipse.univ-lyon1.fr). In previous years we tested and reported on the comparability of data. In 2004 routine surveillance started.
Methods: Data from 88 986 surgical procedures, routinely collected by 14 European networks were analysed. Procedures included are CABG, cholecystectomy, colon surgery, caesarean section, hip prosthesis and laminectomy. Infection rates are expressed as the proportion of surgical procedures leading to surgical site infections (SSI) within 30 days. Incidence was also expressed as incidence density: the number of in-hospital SSI per 1000 patient-days. Incidences were calculated by surgical procedure and stratified by NNIS risk index group. Time dependent SSI rates were calculated using survival time analysis to further enhance the interpretation of inter-country differences.
Results: Overall thirty-day cumulative incidence of SSI ranged from 1.1% (95%CI 1.0–1.3) for cholecystectomy to 8.5% (7.9–9.0) for colon surgery (figure). Using incidence density the relative importance of SSI increased for procedures associated with short durations of stay (cholecystectomie and caesarean section). Important inter-country differences were observed although not all statistically significant. Incidence increased with increasing NNIS risk index levels. For all procedures, rates increased during the first days after surgery reaching a peak at day 5–6, and decreasing thereafter. There were important differences in length of stay between countries but comparison of time dependent infection rates during the first week showed that significant differences remained after this adjustment.



Conclusion: The reported incidences are of a similar magnitude as reported for previous years. Inter-country comparisons are important to identify opportunities for improvement, but the observation of different SSI rates cannot be simply assumed to be due to the quality of care. Differences in duration of hospital stay and different procedures for post-discharge surveillance can lead to artificial differences. Moreover, rates are not constant but vary with time since operation. It is important to recognise the limitations of any single metric describing the SSI risk, especially in the presence of different lengths of stay and different post-discharge surveillance.

O69

Deep sternal wound infections following coronary artery bypass graft surgery: risk factors and implications

M. Paul, C. Madar, E. Raz, R. Holinger, A. Fraser, L. Leibovici, B. Rubinovitch *(Petah Tikva, IL)*

Objectives: To assess the risk factors for Deep sternal wound infections (DSWIs) following coronary artery bypass graft (CABG), accounting for peri-operative glucose, oxygenation, and temperature control.
Methods: Prospective cohort study conducted in Rabin Medical Center, Israel including consecutive patients undergoing CABG during 2004. The dataset comprised of 154 peri-operative variables. Data were collected by daily chart review on ward using a case report form and merged with hospitals' electronic records. Continuous measurements of oxygen and temperature throughout surgery were included in the analysis. Follow-up for DSWIs was performed 60 days following surgery. DSWIs were defined by CDC criteria. Dichotomous variables were compared using a chi-square test; continuous variables were compared using nonparametric tests. A multivariate stepwise logistic regression model was constructed using variables found significant on univariate analysis ($p < 0.05$). Odds ratios (95% confidence intervals) are reported.
Results: 809 patients (79% male) undergoing CABG were included. CABG was elective in 31%. An additional operation, mostly valve replacement, was performed in 15%. Off-pump CABG was performed in 22%. Median patient age was 67 years (31–91); 21% were above 75. Diabetes and chronic obstructive pulmonary disease (COPD) necessitating chronic therapy were present in 41% and 6% of patients, respectively. DSWIs occurred in 29 patients (3.6%). Variables independently associated with DSWIs included intra-operative glucose (OR 6.0, 1.3–26.5, for mean glucose > 140 mg/dl); COPD (OR 4.4, 1.4–13.7); and an operation additional to CABG (OR 3.3, 1.2–8.7). Haemoglobin A1C levels, hypothermia and post-operative hypoxemia were not significantly associated with DSWIs. The Euroscore was associated with DSWIs on univariate, but not with multivariate analysis. Post-operative hospital stay was longer among patients with DSWI compared to those without DSWI (median 31 vs. 5 days, respectively, $p < 0.001$). Thirty-day mortality was not significantly different with or without DSWI (0% vs. 5%,

Multivariate analysis model for DSWIs:

Variable	OR (95% CI)	P-value
Variables in the final model		
Additional operation to CABG	3.3 (1.2-8.7)	0.018
COPD	4.4 (1.4-13.7)	0.010
Mean intra-operative glucose >140 mg/dl	6.0 (1.3-26.5)	0.019
Variables not included in the final model		
Adequate antibiotic prophylaxis		0.708
Intra-operative administration of blood products		0.075
Euro Score		0.192
Mean pre-operative glucose >140 mg/dl		0.542
Mean post-operative day 2 glucose >140 mg/dl		0.889
Median operation duration > 240 minutes		0.577
Pulmonary hypertension		0.375
Severe left ventricular dysfunction		0.866

* Low, intermediate and high risk defined as Euroscore 0-2, 2-5, and >5, respectively.

respectively), while 6 months mortality was significantly higher with DSWI (34.5% vs. 7.6%, $p < 0.001$).
Conclusion: DSWIs following CABG have grave implications. Modifiable risk factors include intra-operative glucose control and, potentially, COPD. Further studies should assess whether better preparation of COPD patients lowers DSWI rates.

O70

Diagnosis and microbiology of prosthetic shoulder infection

K. Piper, P. Anguita Alonso, M. Jacobson, A. Trampuz, R. Cofield, J. Sperling, J. Sanchez Sotelo, D. Osmon, R. Patel *(Rochester, US)*

Objectives: Criteria for the diagnosis of prosthetic shoulder infection (PSI) have not been established. We evaluated characteristics of prosthetic shoulder infection and analysed the accuracy of various diagnostic criteria.
Methods: Patients undergoing shoulder arthroplasty revision or resection at Mayo Clinic, Rochester MN from August 2004 through October 2005 were studied. PSI was diagnosed if at least one of the following was present: intraoperative purulence, histopathologic acute inflammation, a sinus tract, a positive synovial fluid culture or at least two periprosthetic tissue cultures positive with the same organism. Medical records were abstracted for age, gender, time to prosthesis failure, synovial fluid leukocyte count and differential (aspirated < 3 months before surgery), and synovial fluid and periprosthetic tissue culture results. In addition, the explanted shoulder implants were vortexed and sonicated in 400 ml Ringers solution (herein defined as sonicate fluid), followed by a 1000-fold concentration and indirect immunofluorescence with monoclonal antibodies against staphylococci and *Propionibacterium acnes*.
Results: Of the 42 patients studied, 28 had aseptic failure and 14 PSI. The median age was 67 years (range, 40–81 years); 43% were female. The median time to clinical failure of the prosthesis was 39 months (range, 0–17 years). Causative organisms were identified in 10/14 infected patients (71%) and included *P. acnes* (n = 7), *Staphylococcus aureus* (n = 1), coagulase negative *Staphylococcus* sp. (n = 1) and *Pseudomonas aeruginosa* (n = 1). Four infected patients (29%) with *P. acnes* had no histopathologic acute inflammation, intraoperative purulence or a sinus tract. Synovial fluid leukocyte count and differential were not predictive of infection. The sensitivity for infection of immunofluorescence on concentrated sonicate fluid was 89% (8/9); specificity was 100%.
Conclusions: While inflammatory response markers such as histopathology, synovial fluid leukocyte count, and purulence are good predictors of prosthetic hip and knee infection, they were not sensitive for diagnosing PSI (i.e., 29% of infections would have been missed using these criteria). This likely relates to low virulence of the predominant pathogen, *P. acnes*. For accurate diagnosis of infection, synovial fluid and periprosthetic tissue cultures were needed. Indirect immunofluorescent microscopy on concentrated sonicate fluid is a promising diagnostic test and deserves further investigation.

O71

The association between ventilator-associated pneumonia and ICU-mortality: approximation of attributable mortality

M. Koeman, E. Hak, A. de Smet, G. van der Heyden, M.J.M. Bonten *(Utrecht, NL)*

Background: Estimates of attributable mortality (AM) due to ventilator-associated pneumonia (VAP), based upon risk factor and matched-cohort analyses have ranged from 0–76%. We used associations between relative risk reductions (RRR) of VAP incidence (due to prevention) and ICU-mortality to determine AM. If AM would be 100%, a 50% RRR of VAP should yield a 50% RRR of ICU-mortality. Our objective, therefore, was to estimate crude relative AM of VAP upon outcomes of randomized studies on VAP prevention.
Methods: A systematic search through MEDLINE, Cochrane Library and reference lists of relevant studies to identify randomized VAP prevention trials published until 2003 providing data on VAP incidence and ICU-mortality. For each study, the methodological study quality (MQS) was assessed (ranging from 0–13 points), based on allocation, concealment, blinding of the intervention, patient selection and characteristics, and on the definition of pneumonia. RRR of VAP and ICU-mortality were calculated. Main outcome measures were crude relative attributable ICU-mortality of VAP, which was estimated by the ratio of RRRs of mortality and VAP, as well as on crude patient data of pooled trial populations.
Results: Sixty trials were identified, with 11 176 patients and 2126 VAP events. Six trials compared two intervention groups to control patients and, therefore, 66 group comparisons were included. Significant RRR of VAP were reported in 30 of 66 trials, whereas 3 of 66 trials reported significant RRR of ICU-mortality due to preventive measures. Overall trial quality was 8.4 ± 2.1. RRR of VAP were highest for antibiotic containing strategies (0.55 (95%CI: 0.47–0.64) for SDD and 0.64 (95%CI: 0.46–0.82) for other types of antibiotic prophylaxis), whereas the lowest were found among stress ulcus prophylaxis interventions with RRRvap of 0.12 (95%CI: 0.02–0.23). No significant VAP reduction was found for ventilator circuit changing and enteral feeding modulation. A significant RRR for mortality was only found for the pooled SDD studies ; 0.14 (95%CI: 0.06–0.23). No significant associations between MQS and RRR of VAP or ICU-mortality and relative attributable mortality were found. The pooled crude relative attributable mortality of VAP (CAM), based upon all individual trials, was 23%, with wide variation between individual studies.
Conclusions: The crude relative attributable mortality of VAP was estimated to be 23%.

O72

Pattern and dynamics of airways colonisation in mechanically ventilated patients

J-E. Berdal, J. Bjørnholdt, A. Blomfeldt, G. Bukholm, N. Smith-Erichsen *(Nordbyhagen, NO)*

Objective: To investigate patterns and dynamics of microbial colonization of the upper and lower airways in ventilated patients.
Methods: Seventy-four mechanically ventilated patients were consecutively recruited, and oropharyngeal, tracheal and bronchoalveolar fluid specimens were collected 48 hours after intubation and thereafter every 72 hours until a total of 5 sample sets had been collected or the patient was extubated. Samples were quantitatively analysed and the dominant isolates genotyped.
Results: Percentage of samples showing growth above the accepted threshold for Ventilator Associated Pneumonia (> 104 cfu/ml) was 36% to 54%, median 47%, from day 2 to 14 after the initiation of mechanical ventilation. Correlation analysis showed a high degree of correlation for the dominant isolate from the three sampling sites on each sampling occasion. The same isolate was found with a high degree of correlation on two subsequent sampling occasions i.e. sampled 72 hours apart.

However no correlation was found when comparing isolates in individual patients sampled more than 72 hours apart.
Conclusion: On all sampling occasions, a high percentage of ventilated patients showed growth above the accepted threshold values for Ventilator Associated Pneumonia, questioning the specificity of this diagnostic criterion for the diagnosis of VAP. The microbial flora of the airways in ventilated is stable over a period of 72 hours, however when samples are collected more than 72 hours apart, results and thus choice of antibiotic treatment cannot be predicted based on results of previous samplings. On a genotypic level there is a high correlation between the dominant isolate found in oropharyngeal, tracheal, and bronchoalveolar fluid. Further studies are warranted to investigate whether less invasive sampling (i.e. oropharyngeal) is sufficient to determine the most likely involved pathogen in ventilator-associated pneumonia.

O73

Factors influenced treatment failure in pneumococcal pneumonia with bacteraemia

V. Huang, I. Pencu, S. Oprea, M. Zervos *(Atlanta, Royal Oak, Detroit, US)*

Objective: To conduct a retrospective study to compare outcomes of patients with pneumococcal pneumonia with bacteraemia over a 5-year period.
Methods: We identified 128 consecutive patients with clinical criteria for pneumonia and positive blood cultures. There were 70 females and 58 males. The majority of patients were elderly with mean age of 70 years (28–98 years). In addition, these patients have history of smoking (51%), underlying medical condition (67%), and most common being chronic lung disease (26%). Six patients were treated as outpatients.
Results: Overall 28% of patients failed initial therapy. Death occurred in 12.5%. The failure rate among bacteraemic patients with cardiovascular disease was 7.7 times higher than the failure rate among patients with no underlying medical conditions, 54% vs. 7% ($p = 0.001$). Patients with no underlying conditions had the lowest failure rate. Rates of *Streptococcus pneumoniae* resistance to penicillin were 22 % and macrolides 20%. Multiple logistic regression analysis demonstrated the cure rate for groups treated with diverse antibiotics were not significantly different: 70% in levofloxacin group (n = 51), 89% in ceftriaxone plus azithromycin IV followed by oral azithromycin group (n = 56), and 84 % in azithromycin alone group (n = 21). Overall, the failure rate in patients with a resistant pathogen was similar to patients with pathogens susceptible to the initial agent, 28% vs. 29%. The majority of patients (83%), who failed therapy, had coexistent illnesses and high severity risk PSI scores (63%). No patients failed outpatient therapy.
Conclusion: The results from this study demonstrated that antimicrobial resistance was not a factor associated with treatment failure. Underlying disease is the most important predictor of outcome and mortality in community-acquired pneumonia due to *S. pneumoniae* complicated by bacteraemia.

O74

Efficacy of the use of through-catheter leukocyte cytospin blood culture for monitoring haemodialysis catheter colonization. A prospective study designed to prevent bloodstream-related infections

J.L. del Pozo, A. Aguinaga, M. Alonso, A. Serrera, S. Hernaez, M.J. Garrido, N. García-Fernández, J. Leiva *(Pamplona, ES)*

Objectives: Permanent tunnelled central venous catheters are commonly used to provide vascular access in patients on maintenance haemodialysis. Infection is one of the leading complications. According to several authors, catheter colonization precedes bloodstream infection. The aim of this study was to detect catheter colonization, and establish a pre-emptive therapy based in catheter antibiotic lock in order to prevent development of catheter-related bloodstream infection (CRBI). Besides, we evaluated the impact of this monitoring system on the life spam of the catheters.
Methods: During a 24-month period (July 2003 to July 2005), 34 haemodialysis patients with tunnelled catheters were prospectively followed up. The study was carried out in a 350-bed university hospital in which 50 patients are maintained on chronic intermittent haemodialysis. All patients were evaluated by extracting a through-catheter leukocyte cytospin blood culture every 15 days. If the culture was positive, paired quantitative blood cultures were obtained through the two lumens of the catheter and a peripheral vein, in order to assess absence of related bacteraemia. A pre-emptive therapy consisting in a 21 days antibiotic lock regimen was then started if coagulase negative *staphylococci* (CNS) were isolated. For isolates other than CNS, decision about conservative treatment was individually taken.
Results: A total of 37 haemodialysis catheters inserted in 34 patients were evaluated. There were 31 episodes of catheter colonization occurred in 13 patients. At the time of colonization, the catheters were in place for a mean of 562 days (range: 16 to 1475 days). Coagulase negative *staphylococci* were the most common microorganism isolated (90% of all isolated microorganisms). Pre-emptive therapy with teicoplanin (10 mg/ml) locks was able to eradicate catheter colonization in 29% of the cases. Relapsing colonization occurred in 61.2% of the cases. Only 1 patient required catheter removal due to an infectious complication (CRBI). Catheter was removed in other two patients due to non-infectious complications. The mean duration of catheter use was 239 days (range: 9 to 483 days) after treatment of a colonization episode.
Conclusion: This study shows the utility of intracatheter leukocyte cytospin blood culture to early detection of haemodialysis catheter colonization, and shows that eradication of biofilm-related microorganisms is possible without removal of the catheter.

O75

Risk factors and treatment outcome of bloodstream infections caused by *Pseudomonas aeruginosa* isolates producing the PER-1 extended-spectrum β-lactamase

A. Endimiani, B. Pini, F. Luzzaro, G. Amicosante, G.M. Rossolini, A. Toniolo *(Varese, L'Aquila, Siena, IT)*

Objectives: Clinical significance of bloodstream infection (BSI) due to *Pseudomonas aeruginosa* (Pa) has received large attention. In contrast, the clinical impact of BSI caused by ESBL-positive

isolates has not been investigated. The PER-1 ESBL is a common enzyme conferring high-level resistance to anti-*pseudomonal cephalosporins*. This study was initiated to evaluate risk factors and treatment outcome of BSI episodes caused by PER-1-positive *Pa* strains (PER-1-P-*Pa*).

Methods: From January 1998 to September 2004, 26 BSI cases due to ceftazidime-resistant *Pa* strains were observed at the Ospedale di Circolo, Varese, Italy. MIC values of anti-pseudomonal drugs were determined by the E-test method (AB Biodisk, Solna, Sweden). The double-disk synergy test was used to detect ESBL production. Molecular methods (PCR amplification and DNA sequencing) were used to characterize ESBL types. Clinical records of BSI-patients were examined retrospectively. Demographic data, underlying diseases (according to McCabe-Jackson classification and Charlson weighted index), risk factors, antimicrobial therapy, and treatment outcome were investigated by comparing cases due to ESBL-positive to those due to ESBL-negative *Pa* isolates. Unpaired Student's t-test, Mann-Whitney U-test, and Chi-square were used for statistical analysis.

Results: Nine *Pa* isolates were found to express the PER-1 ESBL whereas the remaining 17 were PER-1-negative (PER-1-N-*Pa*). Severe sepsis ($P = 0.03$), bladder and intravascular catheters (both, $P = 0.01$), immunosuppressive therapy ($P = 0.04$), and mechanical ventilation ($P = 0.03$) were significantly associated with BSI due to PER-1-P-*Pa*. Empirical treatment ($P = 0.02$) and treatment after ID/AST ($P < 0.01$) were less adequate in PER-1-P-*Pa* cases. Hospital admission and longer mean length of hospital stay after BSI onset ($P = 0.07$ and $P = 0.08$, respectively) was possibly related to expression of the PER-1 enzyme. Overall, 77.8% BSI cases due to PER-1-P-*Pa* vs. 28.6% cases due to PER-1-N-*Pa* isolates failed to respond ($P < 0.03$). Notably, all cases due to PER-1-P-*Pa* that were treated with carbapenems failed to respond. In contrast, 7/8 cases due to PER-1-N-*Pa* given carbapenems were responders.

Conclusions: Therapeutic failure and poor outcome are associated with BSI episodes caused by PER-1-P-*Pa* strains. Thus, recognition and prompt reporting of ESBL-production appears a critical factor for the management of patients with serious *P. aeruginosa* infections.

O76

Is transmission of MRSA frequent between patients sharing the same hospital room?

D.S. Blanc, C. Balmelli, C. Petignat, G. Zanetti, P. Francioli
(Lausanne, CH)

Objectives: To evaluate the transmission of MRSA between patients hospitalized in the same room.

Methods: Analysis of the results of screening for MRSA of roommates of patients found to be MRSA positive. PFGE typing of MRSA isolates from the index case and the roommates.

Setting: Tertiary care hospital of 890 beds, with a proportion of 11 % of *S. aureus* that were resistant to methicillin (MRSA).

Results: During the year 2004, there were 73 occasions in which a patient was found to be MRSA positive (index case) while hospitalized with other patients in the same room. A total of 210 roommates were screened for MRSA, of whom 21 (10%) were found to be positive. Comparison of isolates of the index cases and of MRSA positive roommates revealed that the PFGE patterns were indistinguishable in 11/21 instances, suggesting transmission. However, in 10/21 instances, isolates were not indistinguishable, suggesting that the roommates were contaminated by another source than the index case. Thus, MRSA was probably acquired in only 11/210 (5.2%) patients who were sharing a room with an index case.

Conclusion: In our setting, transmission of MRSA between patients hospitalized in the same room does not seem to be frequent. However, this study also revealed that half of the MRSA positive roommates had isolates different from the index patient. Thus, interpretation of the screening results in terms of transmission should be cautious.

O77

Outbreak of *Burkolderia cenocepacia* bacteraemia associated with disinfection napkin contamination in haemodialysis

M. Solbiati, B. Allegranzi, G. Lo Cascio, M.G. Bonora, C. Loschiavo, N. Tessitore, R. Fontana, E. Concia *(Verona, IT)*

Objective: We investigated an outbreak of bacteraemia involving 38 patients at two Haemodialysis Units located in two different hospitals in Verona and occurring from November 2004 to January 2005.

Methods: Blood cultures were performed from patients having intra- and post-dialytic chills and fever. Investigations to identify source of infection, including echocardiography, were performed. As soon as *Burkholderia cepacia* complex was isolated from the first two blood samples, investigation cultures were carried out on deionised and tap water, dialysate fluid, dialyser blood compartments, dialysate concentrate, disinfectant solution. Afterwards samples were taken from disinfection napkins contained in a commercial sterile dressing kit used to handle central venous catheters (CVCs). Phenotypic and molecular identification of *B. cepacia* were performed by standard biochemical procedures and by restriction fragment length polymorphism-PCR (RFLP-PCR) analysis respectively. Strain clonality was analysed by pulsed-field gel electrophoresis (PFGE).

Results: Seventy-nine episodes of bacteraemia occurred in 38 patients undergoing haemodialysis and having CVC. One case of endocarditis was diagnosed. CVC was removed in 33/38 patients. *B. cepacia* complex was isolated from all blood cultures and disinfection napkins from one batch. RFLP-PCR analysis confirmed this finding from blood and napkin cultures and *B. cenocepacia* (genomovar III B) was identified and demonstrated to be clonally identical or strictly related using PFGE. Following CVC substitution and 5-day combination antibiotic therapy, all patients improved, but 32 relapsed after a mean of 2 weeks and needed 6–8 weeks of treatment to be cured. No death attributable to the infection was observed.

Conclusions: Chronic haemodialysis patients are at high risk of infection, frequently caused by typical nosocomial bacteria and hepatitis viruses, commonly transmitted by contamination of the haemodialysis system and/or the vascular catheter from environmental surfaces or hands of personnel. Only few cases of infection due to *B. cenocepacia* in haemodialysis patients are reported in the literature. Our investigation showed that commercial napkins soaked in disinfectant were the source of infection and that contamination occurred at the time of manufacture. Despite antibiotic susceptibility *in vitro*, the detected infections were difficult to eradicate, probably due to bacteria persistence in biofilm on CVCs.

ISSN: 1470-9465

Biofilms – a highly organised community

O93

AGR-genotyping of biofilm producing *Staphylococcus aureus*

V. Cafiso, T. Bertuccio, M. Santagati, V. Demelio, D. Spina, S. Stefani *(Catania, IT)*

Objectives: This study investigated the possible correlation between biofilm production and the accessory regulatory genes (*agr*) in a sample of *S.aureus* isolated from catheter-associated infections.
Methods: We analysed: (i) the ability to produce biofilm in a static system by a specthrophotometrical quantitative assay; (ii) the *agr*-genotype and (iii) the expression of ica-operon and its correlation with agr functionality and the activity of the two transcriptional regulators *sar*A and *rsb*U by RT-PCR.
Results: Our results, applying a new ScaI-RFLP method for agr-typing, show that strong biofilm producing strains belong predominantly to the *agr*-type I and II and are found in methicillin-resistant strains. We found two *agr*-variants, and sequence analysis revealed that these isolates contained an IS256 copy within the *agr*-locus I: in two strains, indicated as IA– variant, this element is located in the intergenic region between *agr*D and *agr*C and has the same transcription direction as the P2 *agr*-operon; on the contrary, in one strain (IB-variant), this insertion sequence is integrated into *agr*C and caused the interruption of this gene at the nucleotide position 1114 of the *agr* published sequence (GeneBanK AJ617710) but the IS has an opposite transcriptional direction with respect to the *agr* genes. The study of *ica* expression in all *agr*-types, including the three *agr*-variants, was performed and correlated with *agr* functionality and with two transcriptional regulators *sar*A and *rsb*U. Our results suggest that *agr* polymorph II and IB-variant strains produce large amounts of biofilm, possess a defective *agr* system in which *sar*A may activate the *ica*-operon expression by repressing *ica*R transcription, but they are, at the same time, defective in haemolysin activity and lack the transcription of the óB activator *rsb*U.
Conclusion: It is possible to hypothesize that, in *agr* polymorph I and II strains, which are the most diffused in nosocomial settings, biofilms represent a selective advantage in catheter-associated and chronic-infections because microrganisms are able to thrive in the hosts contributing significantly to pathogenesis.

O94

Structural comparison of biofilms formed by *Staphylococcus aureus*, *Staphylococcus epidermidis*, and other coagulase-negative *staphylococci*

M.A. Horstkotte, S. Stepanovic, M. Lang, D. Mack, J.K. Knobloch *(Hamburg, DE; Belgrade, CS; Swansea, UK)*

Objectives: Due to their ability to form multi-layered biofilms *S. aureus* and *S. epidermidis* as well as other coagulase-negative *staphylococci* are the most prevalent pathogens in foreign body related infections. However, biofilm formation of different staphylococcal species seem to be mediated by different mechanisms.
Methods: The biofilm structure as well as single cells embedded within the biofilm were analysed using confocal laser scanning microscopy. Clinically relevant isolates of *S. aureus*, *S. epidermidis*, *S. lugdunensis*, *S. sciuri*, *S. hominis*, *S. haemolyticus*, and *S. simulans* were investigated. Cells were stained with SYTO 9 and N-acetylglucosamine as a marker for the polysaccharide intercellular adhesin (PIA), was detected by staining with TRITC labelled wheat germ agglutinin (WGA). PIA-synthesis of individual strains was confirmed by dot blot analysis using a PIA-specific antiserum.
Results: All strains investigated displayed highly structured, 10 to 30 μm thick biofilms. However, significant amounts of PIA were only detected in biofilms of *S. epidermidis* and *S. aureus*. Large masses of PIA were distributed irregularly within *S. epidermidis* biofilms, whereas in *S. aureus* biofilms, all cells were homogenously surrounded by a thin PIA layer. In PIA-dependent biofilms the cells were rigidly coupled to each other. For biofilms of *S. sciuri* a compact structure with motionless cells was observed, too. However, PIA was not detected in this species. In contrast, to the biofilms with rigid cell-to-cell contacts biofilms of *S. simulans* displayed a sponge-like structure in which individual cells or smaller cell clusters were fixed together by elastic cell-to-cell contacts. Within these biofilms few structures were stained by WGA but PIA could not be detected with PIA-antibodies, indicating that a PIA independent polysaccharide could be involved in biofilm formation in this species. In biofilms formed by *S. haemolyticus*, *S. hominis*, and *S. lugdunensis* large rigid cell clusters could be detected to which single cells or smaller cell clusters were fixed by elastic cell-to-cell contacts. Thereby, the ratio between motionless and elastic fixed cells varied between various species.
Conclusions: At least three different types of cell-to-cell contact within staphylococcal biofilms were detected among the species investigated. Thereby, the PIA independent rigid and elastic cell-to-cell contacts could be observed in parallel in two species.

O95

Differential importance of protein and polysaccharide intercellular adhesin mediated biofilm formation in *Staphylococcus aureus* and *Staphylococcus epidermidis* from prosthetic joint infections

H. Rohde, E. Burandt, L. Frommelt, N. Siemssen, C. Burdelski, C.-J. Wang, S. Scherpe, J.K.-M. Knobloch, J. Kaplan, D. Mack *(Hamburg, DE; Newark, US; Swansea, UK)*

Objectives: Nosocomial staphylococcal foreign-body infections are a major problem in modern medicine, demanding new therapeutic and preventive strategies. The usefulness of known virulence factors as vaccine candidates is critically restricted by their prevalence in natural staphylococcal populations. As biofilm formation is essential for the pathogenesis of *Staphylococcus epidermidis* and *Staphylococcus aureus* foreign-body infections this study examined the distribution of genes involved in biofilm formation, the biofilm phenotype and production of polysaccharide intercellular adhesin (PIA) in clonal independent *S. aureus* and *S. epidermidis* strains isolated from persistent prosthetic joint infections after total hip arthroplasty (THA) or total knee arthroplasty (TKA).
Results: A biofilm-positive phenotype was detected in all *S. aureus* and 69.2 % of *S. epidermidis* strains. There were no major differences in the distribution of factors mediating primary attachment between *S. aureus* and *S. epidermidis*

strains from TKA and THA. *ica*ADBC, encoding the synthesis apparatus for PIA, was detected in all *S. aureus* strains, but 38.5% of *S. epidermidis* strains were *ica*ADBC-negative. 50 % of those produced trypsin sensitive biofilms, indicating that in these strains protein factors like the highly prevalent accumulation associated protein (Aap) is sufficient for intercellular adhesion. Protease-sensitive biofilms were exclusively found in *S. epidermidis* strains from THA, representing 37 % of all biofilms in this population. In contrast, both trypsin and PIA-cleaving dispersin B disintegrated *S. aureus* biofilms, indicating that in this species PIA and proteins act cooperatively in biofilm formation regardless of the infection site.

Conclusion: Our findings suggest that PIA and protein factors are of differential importance for the pathogenesis of *S. epidermidis* in PJI after THA and TKA, implicating *ica*ADBC cannot serve as a general virulence marker in this species. In *S. aureus* biofilm formation proteins are of overall importance and future work should focus on the identification of functionally active molecules.

O96

The role of the quorum-sensing ''target of RNAIII activating protein'' and ''RNAIII-inhibiting peptide'' in staphylococcal biofilm formation and control *in vitro*

C.A. Fux, D. Nguyen, S. Wilson, N. Balaban, P. Stoodley *(Berne, CH; Pittsburgh, Bozeman, North Grafton, US)*

Objectives: *Staphylococci* are the leading cause of device-related infections. By forming biofilms, involved bacteria increase tolerance to antibiotics and host immunity. Recently, the ''RNAIII-inhibiting peptide'' RIP was found to reduce staphylococcal surface attachment and biofilm-related infections. Its mode of action remains unclear. RIP inhibits the phosphorylation of the ''target of RNAIII activating protein'' TRAP and thus the expression of *agr*. Agr knockout strains, on the other hand, have been associated with increased surface adhesion and biofilm formation. This discrepancy suggests that RIP and TRAP might be more directly involved in biofilm control. We evaluated their role in biofilm growth under static and flow conditions.

Methods: We compared *S. aureus* wild type (WT), the WT with exogenous RIP, and a TRAP- mutant. Also, we tested the effect of RIP on 120 hours biofilm in a catheter infection model. Surface area coverage (SAC), biofilm thickness, roughness and biomass were quantified by digital time lapse and confocal microscopy as well as bacterial counts.

Results: Neither TRAP inactivation nor the addition of RIP affected planktonic growth, in both the static and the flow-cell biofilm model, TRAP inactivation and to a lesser extent the addition of RIP significantly increased surface attachment and biofilm biomass for up to 24 hours. At later timepoints up to 96 hours, however, no significant differences in SAC, biofilm thickness or biomass could be found. Interestingly, the WT strain always grew rougher (i.e. less homogeneous) biofilm. Measuring exclusive static growth, bacterial counts of WT biofilms were significantly higher at 24 hours and 96 hours ($p < 0.01$). The addition of RIP to vancomycin did not improve bacterial clearance of mature catheter biofilms.

Conclusion: The reported effectiveness of RIP against infections in animal models is not due to the abolishment of biofilm formation per se, but is more likely related to a reduction of initial surface attachment, the down-regulation of protective and aggressive virulence factors as well as an altered biofilm architecture. It is not clear at this point how switching from a rough to a flat architecture may reduce virulence and increase antibiotic susceptibility.

O97

Using an efficient anti-biofilm procedure to improve the safety of pacifiers

E. Comina, K. Marion, F. Renaud, E. Bergeron, J. Freney *(Lyon, FR)*

Objectives: Many parents refuse the use of pacifiers because they consider them as non-hygienic supplies. However, recent publications have highlighted the role of pacifiers in the prevention of Sudden Infant Death. The aim of this study was first to assess the bacterial contamination of the surface of pacifier and then, to develop a cleaning method to avoid biofilm growth and improve the safety of pacifiers.

Methods: 25 used latex or silicone pacifier nipples were provided by various day care centers. Biofilm was searched on their surface using first direct staining with crystal violet microscopy and image analysis, and then bacterial enumeration after scrapping. A biofilm reactor system was set-up. It was filled with a contaminated media made of artificial saliva supplemented with LB broth and inoculated with 3 different strains. This reactor was used to test 4 different cleaning procedures (stasis, brushing, spray and steam sterilization) the three first treatments including the Pronetron®, a new anti-biofilm product.

Results: 80% of the pacifiers studied presented a biofilm with living cells on their surface, 80% of them had a surface coverage upto 50%. The two main bacterial genus isolated were *Staphylococcus* and *Candida*. Latex was more contaminated than silicone, 89% of latex nipples contained more than 1000 cfu/cm^2. The biofilm reactor system filled with artificial saliva and 10% of LB broth enabled a massive biofilm growth within 7 days on latex nipples, which covered a surface of almost 80% and contained 8.10E7 cfu/cm^2. The most efficient treatment for bacterial kills was water steam sterilization but led to the lowest biofilm detachment. Brushing with Pronetron® had the best efficacy both on biofilm detachment and residual living cells. This treatment was reproduced on 4 different samples under more realistic conditions (LB broth reduced to 1%). The thickness of the biofilm formed on the control was significantly reduced (55% coverage and 2.10E5 cfu/cm^2). Analysis revealed no biofilm on the treated surfaces. Only a very few isolated living cells remained. Averages and standard deviations were calculated for each parameter ($n = 4$). The low standard deviations showed a good reproducibility of the cleaning method.

Conclusion: A daily cleaning with a very quick and easy system made of a simple dental brush combined to an efficient anti-biofilm agent will give a boost to the use of pacifiers, whose advantages seam to be underestimated.

ISSN: 1470-9465

Emerging viral infections

O98

Clinical features and molecular epidemiology of coronavirus HKU1 associated community-acquired pneumonia

P.C.Y. Woo, S.K.P. Lau, H-W. Tsoi, Y. Huang, R.W.S. Poon, C-M. Chu, R.A. Lee, W-K. Luk, G.K.M. Wong, B.H.L. Wong, V.C.C. Cheng, B.S.F. Tang, A.K.L. Wu, R.W.H. Yung, H. Chen, Y. Guan, K-H. Chan, K-Y. Yuen *(Hong Kong, HK)*

Objectives: Recently, we described the discovery of a novel group 2 coronavirus, coronavirus HKU1 (CoV-HKU1), from a patient with pneumonia. In this study, we examined the clinical features and molecular epidemiology of CoV-HKU1-associated community-acquired pneumonia.
Methods: We prospectively collected nasopharyngeal aspirates (NPAs) from patients with community-acquired pneumonia during a 12-month period. A 453-bp fragment of the RNA-dependent RNA polymerase (*pol*) gene of CoV-HKU1 was amplified from the extracted RNA by RT-PCR using CoV-HKU1 specific primers. The epidemiological, clinical, laboratory and radiological features of patients with pneumonia associated with CoV-HKU1 were analysed. Specific antibodies were detected using a recombinant CoV-HKU1 nucleocapsid protein based ELISA. The complete *pol*, spike and nucleocapsid genes of the CoV-HKU1 were amplified and sequenced. RNA extracted from 208 nasopharyngeal swabs and faecal samples from 56 wild and domestic animals in Hong Kong and southern China were subject to RT-PCR of *pol* gene of CoV-HKU1 using CoV-HKU1 specific primers.
Results: RNA extracted from NPAs in 10 (2.5%) out of 418 patients with community-acquired pneumonia was positive for CoV-HKU1. No epidemiological linkage was identified among the 10 cases. All cases occurred in spring and winter (January–May). Four patients had underlying diseases of the respiratory tracts. Without a diagnostic test, the illness was not distinguishable from other community-acquired pneumonia clinically. Only two had symptoms of the upper respiratory tract and one had extra-pulmonary symptoms. In the six patients with serum samples available, all showed a four-fold change in IgG titre and/or the presence of IgM against CoV-HKU1. No other respiratory pathogen was detected in any of the patients. The two patients who died had lower haemoglobin concentration ($P = 0.04$), monocyte count ($P = 0.04$), serum albumin ($P = 0.04$) and oxygen saturation on admission ($P = 0.03$) and bilateral involvement ($P = 0.003$) and more number of zones involved ($P = 0.01$) on chest radiograph. Sequence analysis of the complete *pol*, spike and nucleocapsid genes revealed the presence of two genotypes of CoV-HKU1. None of the animal samples was positive for CoV-HKU1 RNA.
Conclusions: CoV-HKU1 accounts for 2.5% of community-acquired pneumonia, with two genotypes in the study population. Without diagnostic tests, the illness was clinically indistinguishable from other community-acquired pneumonia.

O99

Stability of SARS coronavirus to high temperature and high relative humidity

W.H. Seto, K.H. Chan, S.Y. Lam, M. Peiris *(Hong Kong, HK)*

The stability of the novel SARS coronavirus (SARS CoV) virus at different temperatures and relative humidity was evaluated. Ten microlitre of 107 TCID50 per ml of virus was incubated at different temperatures (38°C, 33°C, 28°C) at different relative humidity (> 95%, 80–89%) for 3 hr, 7 hr, 11 hr, 13 hr and 24 hr, and the residual viral infectivity was titrated. High relative humidity (> 95%) at comparatively low temperature (28°C and 33°C) didn't affect the virus stability significantly. Higher temperature at 80–90% relative humidity affected the virus infectivity 0.25–2 log 10 loss of titre for up to 24 hr. However if the relative humidity is maintained at > 95%, virus stability was adversely affected with further 1.5 log loss of titre for each time point up to 24 hr (0.38–3.38 log 10). We conclude that high temperature at high relative humidity has synergistic effect on inactivation of SARS CoV viability. This may help explain the low transmissibilility of SARS in tropical areas like Malaysia and Indonesia where patients are placed in naturally ventilated wards, while Singapore, with air-conditioned wards had a relatively large outbreak. Similarly large outbreaks occur in the winter of non-tropical areas like Hong Kong when the humidity and temperature was low.

O100

First identification of Toscana virus and other phleboviruses in sandflies from southern France

R.N. Charrel, A. Izri, S. Temmam, P. Delaunay, I. Toga, P. Marty, H. Dumon, X. de Lamballerie, P. Parola *(Marseille, Paris, Nice, FR)*

Toscana virus (TOSV) is an arthropod-borne virus vectored by sand flies. TOSV infection has long been recognized in central Italy. However, it is an emerging cause of meningitis and encephalitis in Spain and France. However, the ecology and epidemiology of TOSV in France is poorly understood.
Objectives: During summer 2005, sand flies captures were organized in the surroundings of two large cities (Marseilles and Nice), in the vicinity of dog kennels and horse stables. Pools were constituted in respect of the trapping location, sex and species identification with a maximum of 30 individuals per pool. PCR assays targeting three genomic regions were used, and final identification was done via sequencing.
Methods: Pools were constituted in respect of the trapping origin, sex and species. They were tested by RT-PCR with primers targeting either the polymerase gene of Phleboviruses or specifically TOSV, or the nucleoprotein gene.
Results and discussion: A total of 725 sand flies were trapped and identified by morphological keys. Positive results were obtained with TOSV specific primers for 3 pools collected in Marseille. Sequence analysis showed that these pools contained a virus most closely related to but distinct from TOSV since an 82.3% and 96% identity at the nucleotide and amino acid level respectively was observed with the TOSV strain isolated in Italy. This is the first time that TOSV is detected in sandflies from southern France. One pool included *Sergentomyia minuta* sand flies, and this constitutes the first detection of TOSV in this species. *S. minuta* readily bite reptiles, but its affinity to bite humans is poorly known. This sandfly could however play a role in the maintenance of TOSV in the nature. Three pools were found to be positive with Phlebovirus generic primers whereas they were negative with TOSV specific primers. Sequence analysis indicated a high genetic diversity with recognized phleboviruses for which sequence data are available. This indicates that this virus could represent a new species within the genus Phlebovirus. However another phlebovirus, Arbia virus, has been repeatedly isolated in Italy, though no sequence

data is available. Arbia virus is currently being sequenced to determine the nature of the phlebovirus detected in these 3 pools. This study showed that TOSV (and another phlebovirus) are distributed throughout coastal south-eastern France.

O101

Imported cases of chikungunya virus infection from eastern Africa

R.N. Charrel, P. Brouqui, H. Tissot-Dupont, X. de Lamballerie *(Marseille, FR)*

On April 5th 2005, ProMED posted a report on an outbreak of chikungunya virus (CHIKV) infection confirmed in Ngazidia, the largest island of Comoros located off the east coast of Africa. On May 18th 2005, cases of CHIKV infection were laboratory-confirmed in Mauritius and Reunion islands. From January 2005 and during 5–6 months period, approximately 5000 cases were recorded in the different islands of Comoros. In the Reunion island (700 000 inhabitants) most cases occurred in April and May 2005 in urban settings (cities of Saint-Denis, Le Port and Saint-Pierre). Clinical manifestations were characterized by fever (99%), arthralgia (98%), myalgias (97%), headache (81%) and skin rash (49%). A total of almost 3200 cases were registered, with more than 880 confirmed via serological testing. In Mauritius island, a total number cases estimated at 3600 occurred between April and late June 2005.

Objectives: Marseille city hosts the second larger community of Comorian in the world with a total number of 70 000 individuals. Since they operate frequent back and forth travels between Comoros islands and Marseille, the aim of this study was to investigate the impact of CHIKV African outbreak onto the population residing in Marseille.

Methods: CHIKV (strain Ross) was used to infect Vero cells and to produce an immunofluorescence assay to detect anti-CHIKV IgG and IgM. Sera were diluted 1:20 in PBS before testing. A total of 97 sera collected from patients returning from Comoros, Reunion, and Mauritius islands admitted in one of the infectious disease units of the Public Hospital System of Marseille were tested. Early serum specimens were tested via PCR and virus isolation was attempted in C6/36 cells.

Results: Of these 97 sera, 15 were found to have antibody against CHIKV with 10 showing either IgM or seroconversion. The results of PCR testing and virus culture will be discussed. Interestingly, the clinical features in those returning patients were not similar to those reported in Reunion island. Hospital admission was motivated by fever, but a minority complained about arthralgia. This will be discussed.

O102

Crimean-Congo haemorrhagic fever in the East Black Sea region of Turkey: a report of 78 cases

N. Sucu, K. Aydin, I. Köksal, R. Caylan, G. Yilmaz *(Trabzon, TR)*

Objectives: Crimean-Congo haemorrhagic fever virus (CCHFV) belongs to the genus Nairovirus in the Bunyaviridae family and causes a potentially fatal disease. After a short incubation period, Crimean-Congo haemorrhagic fever (CCHF) is characterized by acute febrile syndrome (a sudden onset of high fever, severe headache, dizziness and myalgia). In severe cases, haemorrhagic manifestations develop. Two outbreak of CCHF occurred in the East Black Sea region of Turkey during spring and summer of 2004 and 2005 years. In this study, we report 78 cases who suspected as CCHF during the outbreak.

Methods: Patients with acute febrile syndrome were admitted to our hospital between May and August 2004–2005. Seventy-eight patients were analysed for the epidemiological and clinical features and mortality. The diagnosis was confirmed with ELISA and/or RT-PCR.

Results: Fifty-six (72%) of patients were female and twenty-two (28%) were male. The mean age was 47.7 (range 12–77). Malaise (98.7%), fever (84.6%) and muscle ache (57.6%) were the most common presenting symptoms. Sixty percent of patients had history of tick bite, and 83% of handling livestock. At admittance, white blood cell and platelet count were lower than normal value, generally. But alanine aminotransferase (ALT), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) levels were higher than normal level (mean value: $2.5 \times 103/\mu l$, $69 \times 103/\mu l$, 191 U/l, 475 U/l, 1125 U/l, respectively). Thirty-nine (50%) patients were treated with oral ribavirin. Thirty (34%) patients were replaced with platelet and/or erythrocyte suspensions. Fifty-four (70%) patients had immunoglobulin M antibodies and/or PCR results positive for CCHFV in the serum samples. Three (3.8%) patients died.

Conclusion: CCHF is being epidemic in recent years in Turkey. East Black Sea Region is also very important region for this disease. Our CCHF cases had lower mortality rate than other reports. It could be explained by early defining of the disease during the outbreak, supportive therapy.

The year in infectious diseases medicine

S103

The year in infectious diseases medicine

S. Esposito, S. Bosis, N. Principi *(Milan, IT)*

This year had significant achievements in pediatric infectious diseases, including substantial progress in the area of respiratory infections and vaccines. The severe acute respiratory syndrome (SARS) was defeated, with the World Health Organization that showed great leadership during a period of crisis. Avian influenza virus strains have caused recent localized outbreaks, and threaten to escalate to pandemic status. Remarkable progress has been made in studies of epidemiology and pathogenesis of human metapneumovirus since its discovery in 2001. Novel coronaviruses have been identified in Europe and the U.S. that appear to play an important and previously unrecognized role in pediatric diseases. The growing use of specialised diagnostic techniques has allowed a considerable amount of new information to be obtained concerning the role of *Mycoplasma pneumoniae* and *Chlamydia pneumoniae*: they seem to play a more significant role in causing respiratory tract infections than previously thought; they have been associated with tonsillitis and they are also frequent in children aged less than five years. Regarding vaccines, it has been demonstrated that the increased use of influenza vaccine among children could reduce illness in household or community contacts. Clinical trials have also highlighted that pneumococcal conjugate vaccines prevent invasive disease, pneumonia, and acute otitis media in vaccinated children and reduce nasopharyngeal carriage of vaccine-type *pneumococci* with dramatic effects on adult disease. Moreover, phase III trials on human papillomavirus vaccines as well

ISSN: 1470-9465

as rotavirus vaccines are now in progress and there is wide spread optimism for the prospects of regulatory approval for general distribution of these vaccines in 1–2 years. Furthermore, substantial progress has been made in management of children and adolescents with HIV infection as well as in the approach to infections in pediatric transplant recipients. The year also introduced several new challenges, such as the emergence of community strains of methicillin resistant *Staphylococcus aureus*, problems related to nosocomial infections and the evolution of travel diseases.

The hyper-virulent strain of *Clostridium difficile*: sharing experiences in Europe and North America (Symposium arranged with ESGCD)

S107

Spread of the new variant 027 of *Clostridium difficile* in the Netherlands and Europe

E.J. Kuijper, M. Delmée, F. Barbut, M. Rupnik, J. Brazier *(Leiden, NL; Maribor, SI; Paris, FR; Brussels, BE; Cardiff, UK)*

A new strain of *Clostridium difficile* has been recently reported from Canada and the United States as a cause of outbreaks often associated with increased disease severity. The increased virulence is considered to be associated with the production of a binary toxin and an increased production of toxins A and B. Characterization of this strain revealed that it belongs to toxinotype III, pulsed-field gel electrophoresis (PFGE) type NAP1, restriction endonuclease analyis group BI and PCR ribotype 027. In the period February until June 2004, this strain was also recognized in the UK at an outbreak involving 150 patients with 12 deaths at the Stoke Mandeville Hospital. Since then, 302 isolates of type 027 were referred to the Reference laboratory in Cardiff from 44 hospitals. After the recognition of the first outbreak of *Clostridium difficile*-associated diarrhoea (CDAD) in July 2005 due to type 027 among 45 patients at St. Jansdal hospital in Harderwijk with 3 deaths, the strain thus far caused outbreaks in 8 hospitals and 1 nursing home in the Netherlands. In 2 additional hospitals, the strain has been found in isolated cases of CDAD without an outbreak. In total, 86 isolates belonging to 027 have been found in the Netherlands. In September 2005, the PCR ribotype 027 strain was isolated from 4 patients with CDAD in Ieper, Belgium. Subsequently, the same pattern was identified among strains from three outbreaks that had occurred in Brussels in 2003–2004 involving 17, 6 and 5 patients, respectively. In Ostende (Belgium) another outbreak was found with 4 patients. The PCR ribotype 027, toxinotype III strain has a characteristic antimicrobial susceptibility pattern, since it is resistant to the newer fluoroquinolones and to erythromycin, but susceptible to clindamycin. The strain also has the binary toxin genes and contains an 18bp deletion in a toxin regulator gene (*tcd*C). Genes encoding TcdA and TcdB demonstrate numerous nucleotide sequence changes compared to strains belonging to toxinotype 0. The European Study Group of *Clostridium difficile* (ESGCD) has undertaken a survey and collected 494 strains from 14 European countries, which are currently under investigation. Preliminary results indicate that of 168 toxinogenic isolates derived from 4 countries, toxinotype III was found in 13 (7.7%). We conclude that *C. difficile* ribotype 027, toxinotype III is already in Europe since 2003 and is now rapidly spreading.

S108

Clostridium difficile-associated disease: a growing threat to U.S. public health

C. McDonald *(Atlanta, US)*

Several lines of evidence suggest a changing epidemiology of *Clostridium difficile*-associated disease (CDAD). First, there have been increased reports of individual hospital outbreaks, in many instances associated with increased deaths and colectomies. Second, there has been a sharp increase since 2000 in the number and rate of U.S. acute care hospital discharges coded for CDAD. Finally, national surveys conducted over the past year and a half suggest that 30–40% of U.S. infectious disease physicians have perceived increases in the incidence, severity, and likelihood of CDAD recurrence. Possible reasons for this increase in incidence and disease severity could involve trends in antimicrobial or other drug (i.e., stomach acid suppressing medication) usage, changes in infection control practices (i.e., hand hygiene or environmental surface disinfection), or the emergence of one or more strains of *C. difficile* with increased antimicrobial resistance and/or virulence. Indeed just such a novel strain has emerged and appears responsible for outbreaks in the United States, Canada, and, most recently, England. This strain possesses somewhat unique toxin gene variations, including a deletion in a putative negative regulator (*tcd*C) for toxins A and B and genes for an additional toxin known as binary toxin. Although this strain has been isolated infrequently from U.S. patients over the past 20 years, it has recently (since 2000) appeared as a cause of major outbreaks; one reason for this may be the emergence of increased resistance to fluoroquinolones in this strain, possibly providing it a selective advantage over other strains. There is also evidence to suggest that this strain produces much greater quantities of both toxins A and B and that this strain produces these toxins earlier during log phase growth. Experiences to date suggest that control of hospital outbreaks caused by this strain can be difficult to achieve and will likely require multifaceted interventions including careful attention to infection control practices as well as some form of antimicrobial usage restrictions. Finally, the most recent development in changing epidemiology of CDAD is what appears to be increasingly severe disease in previously low risk populations including individuals with little or no exposure to either healthcare settings or antimicrobials. Improved surveillance will be necessary to more fully understand the magnitude of community-acquired CDAD and possible reasons for recent increases in its incidence.

Blood-borne viruses in health care facilities-the silent enemy?

S109

An overview of transmission of BBV in healthcare facilities

S. Mehtar *on behalf of HSRC, MRC & CADRE*

There are an estimated 5 million HIV infected people in South Africa, and until recently, sexual transmission was considered to be the main, if not only, route of transmission for blood borne viruses such as HIV. The WHO reported that unsafe injections (and other health delivery practice) might contribute up to 15% of these infections particularly in children. Following a published report from Tygerberg Hospital, Stellenbosch Uni, where 14 children were found to be HIV infected whose biological mothers were HIV uninfected, a large cross-sectional with a nested case control study was undertaken to identify risk factors in children under the age of 9 years old who were HIV infected with an uninfected biological mother. Evaluation of infection control practices in all dental and mother and child healthcare facilities were undertaken in one province of South Africa. Fifteen percent of the children who had been tested between the ages of 2–9 years were found to be HIV infected, however there were 7 children (1.4%) who were HIV infected yet their biological mothers were HIV uninfected. The infection control (IC) study protocol assessed knowledge of the health staff, provision for IC and safe practice at ward or unit level and actual clinical practice among dental and maternity staff. Cleaning and sterilization of critical items were tested for the presence of occult blood as a surrogate marker for IC practice. Expressed breast milk (EBM) was sent for viral load estimation. Findings suggest that transmission via unsafe injections, improper cleaning and sterilization of instruments (25% of items were found to be contaminated with blood) and poorly labelled expressed breast milk (RNA viral load of 1500 copies/ml or higher found in 30% of EBM) from an HIV infected mother could contribute to transmission of HIV and other blood borne viruses in health care facilities. Africa is not unique in this regard. Reports of hepatitis B and C from the reuse of multi-dose vials have been reported from Sweden, Denmark and the United States. There is clearly a risk of transmission of blood borne viruses in both high and low income countries, and it is most likely due to the lack of proper care during healthcare procedures than economics.

Viral congenital paediatric diseases

S111

Congenital CMV infection: current controversies

S. Stagno *(Birmingham, US)*

CMV is the leading cause of congenital viral infections worldwide with an incidence ranging from 0.2 to 2 percent of all live births. Only 5 to 10 percent of all cases of congenital CMV infection are symptomatic at birth and the majority of these children go on to develop severe neurologic sequelae. In contrast, only 8 to 15 percent of patients with clinically inapparent infection in the neonatal period develop sequelae. Sensorineural hearing loss is the most significant complication. It is bilateral in 50 percent, progressive in 50 percent, and late appearing in 20 percent of patients. Predictors of hearing loss include the presence of petechiae and thrombocytopenia, hepatosplenomegaly with laboratory evidence of hepatitis, and intracranial calcifications. The progressive nature and delayed onset of hearing loss demonstrate the inadequacy of mass screening for hearing based on testing newborn infants. Prenatal diagnosis can be achieved by testing amniotic fluid with PCR or culture obtained after 20 weeks of gestation. Quantitative PCR has a good predictive value for symptomatic infection. Children with congenital CMV infection following first trimester maternal infection are more likely to have CNS sequelae, especially hearing loss, than those whose mothers were infected later in pregnancy. Mothers known to have been infected with CMV months to years before conception have a 1/100 to 1/300 risk of having a congenitally infected infant. It appears that maternal reinfection with different strains and short duration of preconceptional immunity influence outcome. Preconceptional maternal immunity is less than perfect in preventing foetal infection and foetal damage. This issue raises concerns about the future development of a vaccine. However, there is provocative new data from non-randomized studies suggesting that treatment with CMV-specific hyperimmune globulin and ganciclovir for primary CMV infection during pregnancy may be effective in the prevention and treatment of congenital CMV infection.

Brucellosis

S113

Molecular methods in the diagnosis of human brucellosis

J. Solera, M.J. Castaño, E. Navarro *(Albacete, ES)*

Brucella spp. is endemic worldwide, and infection causes significant morbidity in developing countries. Due to the heterogeneous and non-specific clinical symptoms of brucellosis, diagnosis always requires laboratory confirmation. The diagnosis of brucellosis is currently based on isolation of *Brucella* or detection of an immune response by classical serological methods and the presence of characteristic clinical findings. However, in situations in which these criteria are unsatisfactory, new and improved diagnostic methods are needed. Real-time PCR has revolutionized the way clinical microbiology laboratories diagnose many human microbial infections. In the last year, three real-time PCR assays for detection and quantification of *Brucella* spp. and *B. melitensis* have been developed using the LightCycler ® (Roche). Overall, use of real-time PCR for *Brucella* suffers from a lack of sensitivity, a lack of standardization, and variable results depending on the laboratory in which it is performed. The use of real-time PCR for detection and quan-

ISSN: 1470-9465

tification of *Brucella* DNA, and its utility in the diagnosis and follow-up of patients with brucellosis will be reviewed. Recently, a quantitative real-time PCR (Q-PCR) technique has been developed in our laboratory to monitor evolution of *B. melitensis* DNA load from initial diagnosis through to post-therapy follow-up in brucellosis patients. We collected 180 peripheral-blood samples from 18 brucellosis patients. Sensitivity of Q-PCR for both initial infections and relapses was 100%, similar to the results obtained by Queipo-Ortuño *et al*. Despite positive response to treatment and sharp decline in bacteria load after initiating therapy, 50% of patients (7 relapse and 2 non-relapse subjects) were low level-positive Q-PCR upon finalizing treatment. At conclusion of follow-up, almost 40% of patients (4 relapse and 3 non-relapse cases), most of them asymptomatic, continued with low bacteria DNA loads. In conclusion, our data suggest that brucellosis may be a chronic, relapsing disease comparable with those caused by intracellular pathogens such as *Mycobacterium tuberculosis*, where the bacteria persist during and after therapy, despite appropriate antibiotic treatment and good host response. In order to better understand the course of brucellosis, the significance of the persistence of *B. melitensis* DNA in brucellosis patients and the nature of relapse, prospective studies including larger, more homogeneously treated samples should focus on monitoring the long-term post-therapy course of the disease.

S114

What's new in the treatment of human brucellosis

G. Pappas *(Ioannina, GR)*

Brucellosis is the most common zoonotic disease worldwide, recently re-emerging in new foci of the developing world, and entering the developed world through the evolution of international travel and food trafficking. Its treatment principles have remained unchanged since the last issue of the World Health Organization recommendations, although various clinical and *in vitro* trials have attempted to improve the overall outcome, which is currently marred by relapse rates at about 10% overall, and difficulties in treating serious disease complications. The advances in treating brucellosis can be categorized as follows: (i) use of alternative regimens incorporating other aminoglycosides; (ii) use of alternative regimens incorporating classic and newer quinolones; (iii) modification of the duration of treatment; (iv) re-appraisal of traditional alternative agents as co-trimoxazole; (v) attempts at monotherapy; (vi) evaluation of newer agents as tigecycline and (vii) Modification of major pathogenetic events of the disease. Although these aspects have been the subjects of debate for the past decade, definite conclusions cannot be drawn since the applicability of laboratory studies to clinical practice is limited in brucellosis, and the existing clinical trials are often illdesigned and conflicting in their results.

Dissemination of molecular resistance traits

O115

Comparative genomic analysis of community-acquired ST80-SCC*mec* IV *Staphylococcus aureus* isolates by high-density microarray

R.V. Goering, A.R. Larsen, R. Skov, F.C. Tenover, K. Anderson, P.M. Dunman *(Omaha, US; Copenhagen, DK; Atlanta, US)*

Objectives: Community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) isolates producing Panton-Valentine leucocidin (PVL) are now a worldwide concern. In Europe, ST80-SCC*mec* IV (ST80-IV) isolates are the predominant concern while, in the United States, these strains have appeared as pulsed-field types USA400 (ST1-SCC*mec* IV) and more recently described USA300 (ST8-SCC*mec* IV). To better understand the European situation, we compared the genomic content of ST80-IV isolates with other MRSA strains by high-density microarray analysis.
Methods: The genomic composition of two ST80-IV isolates was assessed using Affymetrix *S. aureus* GeneChips (Affymetrix, Santa Clara, CA). Purified chromosomal DNA from each isolate was queried by hybridization for the presence or absence of the 7775 loci represented on the chip. These included resistance determinants, exoenzymes, exo- or enterotoxins, and a variety of virulence regulators and cell surface factors. The results were compared to microarray data recently obtained for *S. aureus* USA100, 300, 400 and 500 isolates (Tenover, *et al*., J. Clin. Microbiol., in press, 2005).
Results: Overall, the ST80-IV isolates were 97% related, differing primarily in conserved hypothetical open reading frames and intergenic sequences. In general, ST80-IV isolates were more similar to USA400 than to USA300 isolates. For example, both ST80-IV and USA400 isolates appear to share the same capsular polysaccharide synthesis type and *agr* type. However, important differences between these two strains were observed as in the case of fibronectin-binding proteins A and B (*fnb*A and *fnb*B), which were absent from USA400 but present in ST80-IV isolates. Analysis of all loci suggested the presence of gene combinations potentially important in facilitating the persistence and spread of these strains in a community environment.
Conclusion: High-density microarray analysis related ST80-IV isolates more closely to other community-acquired, rather than hospital-acquired, MRSA–consistent with clinical experience. The similarities and differences noted between these and other epidemic MRSA strains represent an important resource for identifying gene combinations specific to ST80-IV isolates. These data should help in strain identification, epidemiological analysis, and in providing a greater understanding of the mechanisms by which clonal lineages such as ST80-IV persist and spread in the community setting.

O116

Presence of PVL among erythromycin- and methicillin-resistant community-acquired *Staphylococcus aureus* isolated from children in Texas from 1999 to 2002

N. Aydin, T. Bogdanovich, S. Chavez-Bueno, G. McCracken, B. Bozdogan, P.C. Appelbaum *(Aydin, TR; Hershey, Dallas, US)*

Objectives: Panton-Valentin leucocidin (PVL) is a toxin found in *Staphylococcus aureus* isolates associated with serious invasive infections. The aim of this study was to determine the prevalence of PVL among erythromycin-and methicillin-resistant and clindamycin-susceptible community-acquired *S. aureus* isolated from children in Texas during 1999–2002.
Methods: Erythromycin and methicillin resistant but clindamycin susceptible strains (a total of 197) were tested. Presence of PVL and the SCC type of strains were studied. Relation between presence of PVL and macrolide resistance mechanism was also studied.

Results: Of 197 MRSA 11 (5.5%) were SCC II, and 185 (94%) were SCC IV. The SCC type of one strain could not be determined with the method used. The 11 SCC II strains were all PVL (-) and carried *erm*(A) gene alone (9 strains) or with *msr*(A) (2 strains). Among SCC IV strains, 173 were PVL (+) and 12 were PVL (-). Of 12 PVL (-) strains 2 carried *erm* genes: 1 *erm* (C) alone and 1 *erm* (C) with *msr* (A) gene. Four strains carried *msr* (A) alone. Of 173 PVL (+) strains, 128 had *msr* (A) alone, and 2 had *erm* (C); 20 strains had *erm* (C) and 1 strain was *erm* (A)-positive. The prevalence of PVL in community-acquired strains has been increasing progressively over 3 years: from 66.6% in 2000 to 90.3% in 2001 and 98.7% in 2002.
Conclusion: PVL is commonly present between macrolide-resistant and clindamycin-susceptible SCC IV MRSA but absent among SCC II strains with *erm* (A), isolated from children in Texas.

O117

Identity of macrolide-resistant clones of *Streptococcus pyogenes* in Portugal supports Europe-wide dissemination of a few lineages

C. Silva-Costa, M. Ramirez, J. Melo-Cristino *(Lisbon, PT)*

Objectives: Although overall macrolide resistance has remained stable in Portugal (27%), a rapid inversion of the dominant phenotypes was noted with a sharp decrease in the MLSB phenotype paralleled by an increase in the M phenotype. To gain further insights into these changes we characterized 325 macrolide resistant isolates, previously characterized by T and emm typing.
Methods: A combination pulsed-field gel electrophoresis (PFGE) and multi-locus sequence typing (MLST) was used. The use of Cfr9I, an isoschizomer of SmaI to digest strains of the M phenotype refractory to SmaI digestion allowed the direct comparison of both MLSB and M isolates. The Bionumerics software was used to create UPGMA dendrograms of SmaI/Cfr9I fragment patterns. The Dice similarity coefficient was used with optimization and position tolerance settings of 1.0 and 1.5 respectively. PFGE clusters included isolates with $\geq 80\%$ relatedness on the dendrograms. MLST analysis was performed on representatives of each major lineage. Bacitracin susceptibility was determined for all the isolates by disk diffusion.
Results: All the 325 isolates included in this study were typeable by PFGE. PFGE and MLST were highly concordant and identified eight major clones accounting for 92% of the isolates, each exclusively associated with a single macrolide resistance phenotype. Two major clones were found among MLSB isolates characterized by sequence types (ST) 46 (T12/emm22) and 52 (T28/emm28) whereas among M isolates clones ST39 (T4/emm4) and ST28 (T1/emm1) dominated. The clone defined by ST52 corresponded to a bacitracin resistant clone circulating in Europe and a novel variant expressing other surface antigens (T12/emm22) was detected.
Conclusion: Although there was a large diversity among macrolide resistance genotypes, notably among isolates presenting the M phenotype, 8 clones accounted for the majority of the isolates and the 4 most frequent lineages represented 76% of the isolates analysed in the study period. The presence of the four major clones in other European countries was reported previously suggesting a Europe-wide dissemination of a few macrolide resistance lineages.

O118

Rapid dissemination of MDR CTX-M-15-producing *K. pneumoniae* epidemic clone in Hungary

I. Damjanova, Á. Tóth, Z. Ozsvár, E. Bauer, J. Pászti, M. Füzi *(Budapest, Székesfehérvár, HU)*

Objectives: Multi-centre spread of MDR CTX-M-15-producing *K. pneumoniae* epidemic clone was detected in Hungary in 2003. Continuous screening for such isolates was performed from January 2004 until October 2005 and results of this survey were presented.
Methods: Three of 206 (2004) and 124 of 394 (2005) KP clinical isolates submitted to the National ESBL Reference laboratory from 23 Hungarian hospitals were selected according their high resistance to cefotaxime, aminoglycosides, tetracycline and ciprofloxacine. Susceptibility to antimicrobials by disc-diffusion method and ESBL expression were detected according to NCCLS criteria. Molecular typing was performed by TEM-, SHV-, CTX-M, and ISEcp1 PCRs, sequence analysis of PCR amplicons and XbaI-PFGE. Resistance to cefotaxime was transferred by conjugation to rifampin-R *E. coli* J53. Plasmid extraction was performed by alkaline lysis.
Results: A total of 127 ESBL-KP isolates were collected from various hospital infection samples from 23 Hungarian hospitals. Of these 42 isolates were collected from parallel nosocomial outbreaks reported from ICUs of two different hospitals in 2005. In the first outbreak (Fejér county) 29 isolates were recovered from 25 inpatients (44% mortality; median age 64 years, range 43–81; median length of stay was 30 days, range 6–72) from surgical wounds (8), lower respiratory tract (8), urine (6), blood (4), conjunctiva (1), bile (1) and sputum (1). In the second outbreak (Budapest) 13 isolates were recovered from 13 inpatients (median age 64.2 years, range 44–74; mean length of stay was 27.3 days, range 11–87) from blood (8), urine (2), trachea (1), sputum (1), vaginal secretion (1). Environmental samples have not discerned any apparent source for these outbreaks. PFGE analysis revealed strong clonal link between the 127 isolates and those of previously described epidemic clone. *bla*CTX-M-15 was detected on similar large plasmids in all selected isolates and their transconjugants. ISEcp1 was not found.
Conclusions: Till the end of 2004 the SHV was the most common β-lactamase detected in KP nosocomial isolates in Hungary. Since the beginning of 2005 the ESBL-KP clinical isolates were predominantly CTX-M-15 producers. There was evidence that the background of this ESBL change is the countrywide spread of an MDR CTX-M-15-producing KP epidemic clone. Their eruptive dissemination in 23 geographically distinct hospitals emphasizes the necessity of immediate intervention and epidemiological monitoring.

O119

Evidence for low rates of ciprofloxacin and rifampicin resistance in persistent *Helicobacter pylori* infection

S.A. Chisholm, R.J. Owen *(London, UK)*

Objective: This study aimed to determine the rates of ciprofloxacin (CIP) and rifampicin (RIF) resistance in English dyspeptic patients with refractive *H. pylori* infection. These agents are proposed components of ''rescue'' therapies in instances when recommended first-line, and possibly second-line, therapies fail. However, few studies have monitored levels

ISSN: 1470-9465

of resistance to these, particularly in patients that have failed eradication therapy.
Methods: Primary antibiotic resistance was determined for all isolates/specimens referred to our specialist unit from 2002–2004. Isolates that were dually resistant to metronidazole (MTZ) and clarithromycin (CLA) and recovered from patients that had failed at least one eradication attempt (n = 25) were examined by E-test for susceptibility to CIP and RIF. Additionally, CIP and RIF susceptibilities were determined for eight-fullysensitive isolates (n = 8), recovered pre-treatment.
Results: Of 138 isolates referred from 2002–2004, 64% (n = 89) and 49% (n = 67) were MTZ and CLA resistant, respectively, and 41% (n = 57) were dually resistant. The incidence of dual resistance was higher (76%) in the 54 patients for whom previous treatment failure was documented. In the treatment-failure group, CIP MICs ranged from 0.016–0.125 mg/l for the 23 isolates that were CIP sensitive (< 1.0 mg/l). The two remaining isolates were CIP resistant, with MICs of 2.0 mg/l and > 32 mg/l. All eight of the pre-treatment isolates were CIP sensitive. RIF MICs ranged from < 0.016–2.0 mg/l but all were lower than the proposed resistance breakpoint (> 4 mg/l). Raised MICs to RIF (> 1.0 mg/l) were observed in both the treatment failure (n = 5) and the pre-treatment groups (n = 2). Interestingly, the isolate that displayed high-level CIP resistance (> 32 mg/l) and a RIF MIC of 1.0 mg/l showed reduced susceptibility to tetracycline (MIC = 3.0 mg/l) in addition to MTZ and CLA resistance.
Conclusions: Resistance to CIP and RIF was rare in dyspeptic patients from the UK, even in patients infected with dually resistant strains of *H. pylori* that had already failed at least one eradication therapy. The identification of multi-resistant strains demonstrates the importance of testing susceptibilities of refractive *H. pylori* to a wider range of therapeutic agents.

Diabetes and infection

O120

Stress hyperglycaemia as a prognostic factor in patients with severe sepsis

L. Leonidou, M. Michalaki, A. Leonardou, E. Polyzogopoulou, K. Fouka, M. Gerolimos, P. Leonardos, E. Giannoulaki, V. Kyriazopoulou, C.A. Gogos *(Patras, GR)*

Objectives: Stress hyperglycaemia is a condition related to transient elevation of the blood glucose levels due to various kinds of stress, while severe sepsis, as a result of infection may sometimes lead to organ dysfunction and death. The aim of the present study was to investigate the impact of stress hyperglycaemia on the survival of non-diabetic patients with severe sepsis.
Patients and methods: The study included 242 non diabetic patients (124 women and 118 men) with severe sepsis hospitalized in three peripheral Hospitals in South-Western Greece during an one-year period. The patients were divided into two groups according to their glycaemic profile at admission: patients with baseline hyperglycaemia (group H) and patients with normal glucose level (group N). Hyperglycaemia was defined as an admission or in-hospital fasting glucose level of 126 mg/dl or more or a random blood glucose level of 200 mg/dl or more, on 2 or more determinations. Immunosuppressed patients were excluded. The severity of sepsis was classified by the sepsis-related organ failure assessment score (SOFA).
Results: Forty nine out of 242 patients (20.2%) had stress hyperglycaemia. Patients in, group H were older than group N (73.4 ± 13.8 years vs. 65.7 ± 21.4 years). The mean glucose plasma level at admission in, group H was 198 ± 75.3 mg/dl. There was no significant difference in sex, BMI, CRP, blood cultures, Hba1c and hospitalization days between the two groups. Interestingly, 6.1 % of the hyperglycaemic patients had a first degree relative with diabetes, while the percentage in normoglycaemics was 11.1%. Group H had a significantly higher SOFA score than group N (mean 4.9 ± 3.2 vs. 2.9 ± 2.5, $p < 0.05$). The source of infection in all patients was the following: respiratory tract 41.7%, urinary tract 34.5%, intra-abdominal 14.9%, central nevrous system 3.4%, soft tissue 2.6%, endocarditis 1.3%. The causes of infection were similar in both groups. A significantly higher percentage of septic patients with stress hyperglycaemia died compared to patients with normal baseline glucose level (43.8% vs. 13.0%, $p < 0.001$).
Conclusion: Stress hyperglycaemia is a common finding in non-diabetic patients with severe sepsis and is not related to a genetic predisposition to diabetes mellitus. It may be related to cytokine related insulin resistance and seems to be a significant predictor of outcome in non-diabetic septic patients.

O121

Diabetes mellitus and 30-day and 90-day mortality after hospitalization with pneumonia: a population-based follow-up study of 41 793 adult patients

R.W. Thomsen, J. Brommann, A. Riis, H.-H. Lervang, H.C. Schønheyder, H.T. Sørensen *(Aalborg, Aarhus, DK)*

Objectives: Diabetes may be associated with increased mortality after pneumonia due to decreased immunity, impaired lung function, and hyperglycaemic derangement. We examined diabetes as a predictor of 30-day and 90-day mortality in a large cohort of adult patients hospitalized with pneumonia.
Methods: Population-based cohort study in the three Danish counties of North Jutland, Viborg and Aarhus between 1994 and 2003. Patients with a first-time hospital discharge diagnosis of pneumonia were identified in hospital discharge registries. Information on diabetes and comorbidities was obtained from discharge registries and prescription databases. All patients were followed for 90 days after hospitalization through the Danish Civil Registration System. We compared mortality rates in patients with and without diabetes, adjusted for gender, age, and comorbidity.
Results: A total of 41 793 adult patients were hospitalized with pneumonia (median age 74 years); of these, 3928 patients (9.6%) had diabetes. Overall mortality in diabetic patients compared with other patients with pneumonia was 18.7% vs. 14.8% after 30 days, and 26.5% vs. 21.4% after 90 days. After adjustment, mortality rates in patients with diabetes were only marginally increased (mortality rate ratio = 1.12 (95%CI: 1.04–1.21) after 30 days, and 1.09 (95% CI: 1.02–1.16) after 90 days) compared with non-diabetic pneumonia patients.
Conclusion: Our results corroborate previous reports suggesting that diabetes has only a very limited influence on outcome in patients hospitalized with pneumonia.

O122

A clinical prediction rule for a complicated course of urinary tract infections in patients with type 2 diabetes in primary care

L.M.A.J. Muller, K.J. Gorter, G.E.H.M. Rutten, A.I.M. Hoepelman, E. Hak *(Utrecht, NL)*

Objective: Although it has been demonstrated that type 2 diabetes patients (DM2) have an increased risk of urinary tract infections (UTIs), not much is known about predictors of a complicated course. A safe and feasible management strategy for patients at high or low risk of UTIs could improve efficient antimicrobial use and may direct preventive measures at those who need it most. We developed a prediction rule for complicated UTIs in DM2 patients in primary care.
Methods: We conducted a 12-month prospective cohort study, including DM2 patients aged 45 years or older from the Second Dutch National Survey of General Practice carried out in the period 2000–2001. The primary outcome measure was a complicated course, which was a composite of recurrent cystitis, acute pyelonephritis and prostatitis. Multivariate logistic regression analysis was performed to produce a clinical prediction rule and area under the receiver-operating curve (AUC) characteristics was calculated as a measure of discriminative capacity. For score cut-off points, the following test-characteristics were calculated: positive and negative predictive value, sensitivity, specificity and proportion of subjects selected.
Results: Mean age of the 6343 DM2 patients was 67 years (SD 11) and 46% was male. Overall, the incidence rate of the outcome was 3 per 100 patient years (n = 179), 1 per 100 in females and 2 per 100 in males. Independent predictors were increasing age (odds ratio [OR], 1.02; 95% confidence interval [95% CI], 1.00–1.03), male sex (1.72; 1.25–2.35), number of physician contacts (1.06; 1.05–1.07 per class), incontinence of urine (1.87; 1.03–3.38), cerebrovascular disease or dementia (2.14; 1.29–3.56) and renal disease (5.62; 2.11–14.95). The AUC was 0.77 (95%CI 0.73–0.80). Subgroup analyses for gender showed no differences in discriminative ability. Using a cut-off score of 4 or more points (range 0–12) 60% of the DM2 patients would be selected for tailored care whereas only 8% of the patients with a complicated course of UTI would be missed.
Conclusion: We were able to derive an accurate model to predict complicated UTIs in male and female DM2 patients in primary care. If confirmed in external populations, the model may be used to improve management of UTIs in DM2 patients.

O123

Applicability of a clinical prediction rule for lower respiratory tract infections in elderly patients with diabetes mellitus: a subgroup analysis

L.M.A.J. Muller, J. Bont, K.J. Gorter, G.E.H.M. Rutten, T.J.M. Verheij, E. Hak *(Utrecht, NL)*

Objective: Among elderly persons, those with diabetes (DM) have an elevated risk of complications from lower respiratory tract infections (LRTI). Careful risk assessment using an accurate and objective prediction rule could help general practitioners to target management of these infections more efficiently. However such a rule for an unselected population might be inaccurate for this high-risk group. As part of a large retrospective cohort study among elderly persons aged over 65 years, we conducted a subgroup analysis to determine the applicability of a developed prediction rule for the probability of a complicated course of LRTI in those with diabetes.
Methods: In the original cohort study using the computerized database of the University Medical Center Utrecht general practitioners research network, 3166 general practitioner attended episodes of acute bronchitis, exacerbation of COPD or asthma or pneumonia were included. The outcome was a composite of 30-day hospitalization or death following an episode of LRTI. Potential predictors according to the prediction rule in the unselected population were type of LRTI diagnosis, age, presence of heart failure, predisone use, hospitalization in preceding year and antibiotics in previous month. For the high-risk group we were specifically interested in the added value of diabetes specific variables as the presence of cardiovascular co-morbidity and the use of insulin or oral diabetes medications. Calibration was tested with the Hosmer-Lemeshow goodness of fit test and discriminative capacity was estimated with the area under the receiver operating curve (AUC).
Results: Among 445 episodes of LRTI among elderly patients with DM, 68 endpoints occurred (attack rate 15%). Reliability of the model was good (goodness-of-fit test $p = 0.54$). The discriminative properties of the original rule was acceptable (AUC: 0.79, 95% confidence interval: 0.72–0.86). In the low risk group (≤ 2 points), 6% of all patients ended with hospitalization or death and 40% in the high risk group (≥ 6 points).
Conclusion: The prediction rule for the probability of hospitalization or death derived from an unselected elderly population with LRTI appeared to have acceptable discriminative properties in diabetes patients also and may be used to target management of these common diseases. Applying this rule may safely restrict the need for treatment and help GPs to target additional efforts to those patients who need it most.

O124

Diabetic foot infections: the evolving characteristics, microbiology and determinants of mortality

S. Gündes, Z. Yulugkural, M. Biyikli, B. Buyukarslan *(Kocaeli, TR)*

Objectives: Foot infections in patients with diabetes mellitus are among the most common bacterial infections encountered in our clinical practice. The aim of the present study was to evaluate aetiology, medical outcomes, including risks for complications and mortality, in 68 adult patients hospitalized for severe DFI.
Methods: This report describes a retrospective analysis of medical records of 68 hospitalized patients who had been admitted or consultated to Infectious Diseases Department for severe DFI from January 2004 to November 2005 in a University hospital.
Results: There were 40 males and 28 females. Average patient age was 51.8 years. Most of the patients were in level 1, 2, or 3 on Wagner Scale, only 23 (33.8%) of patients had combined osteomyelitis and deep soft tissue infection. Forty-seven bacteria were documented microbiologically in 39 cases (57.3%). *Staphylococcus aureus* and *Enterobacteriaceae* being the most common isolates. Overall, 25 patients (36.7%) were discharged early (< 14 days) and 43 patients (63.2%) were hospitalized for more than 14 days. Of these, 51 required early surgical debridement (75%), 23 (33.8%) underwent at least one digit amputation. Amputation was more common with level 4

ISSN: 1470-9465

patients (62%) compared with those who had SSTI (30%) only. Overall mortality was 14% factors associated with death were male sex, presence of multiple comorbid conditions, congestive hearth failure, morbid obesity, hypoalbuminaemia, renal insufficiency, and shock.

Conclusion: DFIs are serious bacterial diseases that are often underestimated and poorly recognised. Our findings indicate that deep foot infections in patients with diabetes was a heterogeneous entity, in which type and severity is related to choice of treatment strategy and to outcome.

Pump it out and resist

O125

Which Mex efflux pumps are overexpressed in multidrug-resistant *Pseudomonas aeruginosa?*

D. Hocquet, R. Fabre, M. Roussel-Delvallez, J.D. Cavallo, B. Dehecq, P. Plésiat *(Besancon, Bégin Saint-Mandé, Lille, FR)*

Objectives: β-lactams (bLs), aminoglycosides (AGs) and fluoroquinolones (FQs) are substrates for one or several RND efflux pumps (MexAB-OprM, MexCD-OprJ, MexEF-OprN and MexXY/OprM) in *Pseudomonas aeruginosa*. The aim of this study was to determine the prevalence of these efflux mechanisms in resistant clinical strains of *P. aeruginosa*.
Methods: A hundred and seventy strains of *P. aeruginosa* with reduced susceptibility to ticarcillin (MIC > or = 32 mg/l) collected in 15 French hospitals in 2004 were analysed for the overexpression of efflux genes *mex*B, *mex*C, *mex*E and *mex*Y, by real-time RT-PCR. MICs of antibiotics were determined by using the conventional agar dilution method.
Results: 59% (n = 100), 65% (n = 110), 0.5% (n = 1) and 0.5% (n = 1) of the selected isolates were found to overproduce MexAB-OprM, MexXY, MexCD-OprJ, or/and MexEF-OprN, respectively. 59% (61/103) of the strains resistant to both ticarcillin and ciprofloxacin (MIC ≥ 2 mg/l overexpressed MexAB-OprM. 78% (69/88) and 83% (86/103) of the bacteria resistant to amikacin (MIC ≥ 8 mg/l) or ciprofloxacin exhibited up-regulation of MexXY, respectively. Interestingly, 48% (49/103) of the strains that were non susceptible to ciprofloxacin overproduced both MexAB-OprM and MexXY. The prevalence of the MexXY mechanism was correlated with the levels of resistance to ciprofloxacin (39, 50, 64, 89, 93 and 100% of the strains with MIC of ciprofloxacin of 2, 4, 8, 16, 32 and ≥ 64 mg/l were MexXY-overproducers, respectively) and to amikacin (17, 37, 58, 68, 76, 100, 80 and 93 % of the strains with MIC of amikacin of 1, 2, 4, 8, 16 and ≥ 32 mg/l).
Conclusion: These results show that in contrast to MexCD-OprJ and MexEF-OprN, the systems MexAB-OprM and MexXY/OprM are frequently up-regulated among clinical *P. aeruginosa* isolates showing decreased susceptibilities to bLs, AGs and/or FQs. Furthermore, drug efflux mediated by MexXY/OprM is highly prevalent in isolates with strong levels of resistance to AGs or FQs.

O126

Expression of *mex*Y and gene PA5471 in drug-induced amikacin resistant small colony variants of *P. aeruginosa*

S. Islam, A. Vang, B. Wretlind *(Stockholm, SE)*

Objective: In *P. aeruginosa* the MexXY multidrug efflux system contributes to the intrinsic and inducible resistance to a long array of antibiotics including aminoglycosides and fluoroquinolones. *P. aeruginosa* has the ability to change phenotype under special circumstances, such as growth in the lungs of cystic fibrosis patients. One such phenotype is small-colony variants (SCV). This trend of changing to SCV goes towards increased MIC values against antipseudomonal agents, especially aminoglycosides. The inducibility of MexXY is dependent on the drug ribosome interactions which results in higher signal/s levels that upregulate this operon. It has been reported that the operon PA5470–5471 has some role in drug inducible overexpression of MexXY operon. In this study we tried to explain the relationship between expression of PA5471 and aminoglycoside resistance in SCV mutants of *P.aeruginosa.*
Methods: Ten aminoglycoside induced SCV (MIC 48–> 256 mg/l, wild type 2 mg/l) mutants have been isolated and characterized from wild type strain PAO503. The expression of mRNA for MexY pump protein and PA5471 was determined by realtime PCR.
Results: The MexY mRNA is overexpressed in all of the strains tested, ranging from 10 to 100 folds. Two out of 10 strains (MIC amikacin 128 and > 256 mg/l), which overproduced PA5471 mRNA 41 × PAO503 and 83 × PAO503 respectively, have also expressed ≥ 200 × PAO503 MexY mRNA. One strain (MIC 48 mg/l) with 0.4 fold down regulation of PA5471 produced 10 × PAO503 MexY mRNA. The rest of the strains produced PA5471 mRNA similar to the wild type strain.
Conclusion: Elevated expression (> 40 × PAO503) of PA5471 mRNA suggests a role in upregulation of MexXY operon (≥ 200 × PAO503), which is followed by higher MIC value for aminoglycosides. These overall data suggest that PA5471 can play an important role in highly elevated expression of MexY and thereby might be necessary for the drug induced aminoglycoside resistance in *P. aeruginosa* SCV. Similar mechanisms are probably also relevant for *P. aeruginosa* strains from CF patients.

O127

Contribution of efflux pumps overexpression to carbapenem heteroresistance in *Pseudomonas aeruginosa*

A. Ikonomidis, S. Pournaras, N. Spanakis, A. Tsakris, N.J. Legakis, A.N. Maniatis *(Larissa, Athens, GR)*

Objective: To investigate the carbapenem heteroresistance phenotype of *Pseudomonas aeruginosa* and examine its molecular basis.
Materials and methods: Twenty consecutive clinical isolates of *P. aeruginosa* that exhibited colonies grown within the apparent zone of inhibition in disk diffusion were included in the study. The isolates were screened by E-test to determine imipenem and meropenem MICs and genotyped by pulsed-field gel electrophoresis (PFGE). The heteroresistant subpopulations were substantiated by population analysis of each clone. RT-PCR and exponential range analysis were performed to examine the level of transcription of genes coding for efflux pumps, particularly *mex*Y and *mex*B. The contribution of the bacterium cell wall to the heteroresistance was examined by RT-PCR of

*lpx*C gene. The multicopy gene coding for 16S rRNA and the single copy gene *rps*L were used as internal control.
Results: Macrorestriction analysis of the isolates showed at least nine unrelated genotypes. The MICs of the isolates for both imipenem and meropenem ranged from 0.5 to 1 mg/l but all isolates exhibited colonies grown also within the apparent inner zone of inhibition of both imipenem and meropenem E-test strips. Population analysis revealed subpopulations grown in concentrations of 8 mg/l and 16 mg/l of meropenem and imipenem, respectively. RT-PCR and exponential range analysis of one isolate of each clone showed a substantial increase in transcription levels of both *mex*Y and *mex*B in carbapenem subpopulations. No transcription variation of *lpx*C was detected.
Conclusions: Efflux pumps seem to contribute to heteroresistance of these isolates to carbapenems. This phenotype is more readily observed in isolates with lower levels of resistance. It is not yet known whether the use of carbapenems would lead to selection of resistant subpopulations that subsequently cause infections by resistant isolates or to treatment failure.

O128

Identification of novel mutations in MTR locus of clinical isolates of *Neisseria gonorrhoeae*

M. Ali, V. Kumar, P. Sachdeva, U. Chaudhry, D. Saluja *(Delhi, IN)*

Objectives: Active efflux of antimicrobial hydrophobic agents is one of the most important bacterial defence systems against inhibitory host factors. Two well-characterized MDR efflux pumps *mtr*CDE and FarAB-*mtr*E exist in *Neisseria gonorrhoeae*. The *mtr*CDE encoded efflux pump of *Neisseria gonorrhoeae* mediates an energy dependent efflux process of structurally diverse hydrophobic agents in *gonococci*. Strains of *Neisseria gonorrhoeae* that show resistance to hydrophobic agents often contain mutations in the coding region or promoter region of the operon. The product of adjacent *mtr*R gene negatively regulates the expression of *mtr*CDE operon. *mtr*E encodes a putative lipoprotein of 48.5 kda, which is associated with the *mtr* phenotype.
Methods: In an earlier study, we have reported mutations in the QRDR region of *gyr*A and ParC gene in clinical isolates of *Neisseria gonorrhoeae* showing high resistance to ciprofloxacin (MIC ranging from 4 to 32 μg/ml) (1). To account for the variation in MIC values of the samples showing similar mutations in *gyr*A and *par*C gene, we checked for mutations in *mtr*R and *mtr*E genes. The nucleotide sequence of PCR amplified *mtr*R gene was analysed and compared with the nucleotide sequence of wild types *Neisseria gonorrhoeae*.
Results: These isolates show a missense mutation Tyr105-His located downstream of Helix- Turn- Helix motif of *mtr*R and a base pair addition mutation in the promoter region of *mtr*R. The nucleotide sequence of *mtr*E showed distinct mutations in different clinical isolates invariably at the same positions viz. Val204-Glu, Ala303-Gly, Leu455-Pro and Ser429-Ile.
Conclusion: We envisage that the altered DNA-*mtr*R protein interaction, resulting in increased expression of proteins involved in efflux of hydrophobic antibiotics, may be one of the important mechanism by which pathogen becomes resistant to ciprofloxacin.(1). Chaudhry U, Ray K, Bala M, Saluja D. Mutation patterns in *gyr*A and *par*C genes of ciprofloxacin resistant isolates of *Neisseria gonorrhoeae* from India. Sex Transm Infect. 2002; 78:440–4.

O129

Acquired β-lactam resistance in three *Escherichia coli* strains due to overexpression of AcrAB, TolC and PBP3

M.M. Tavío, V. Aquili, N.T. Antunes, J. Sánchez-Cespedes, Z. González-Lama, J. Vila *(Las Palmas de Gran Canaria, Barcelona, ES)*

Objectives: In a previous study, three multidrug resistant *Escherichia coli* mutants were selected *in vitro* (CazE7, CazE11 with ceftazidime and LmfE7 with lomefloxacin) from two *E. coli* susceptible clinical isolates. Mutants increased cyclohexane tolerance and 2–256 fold-β-lactam MICs without increased β-lactamase activity or decreased permeability. β-lactam resistance has been described as the interplay between AcrAB system and β-lactamases. The aim of this work was the study of potential interplay between AcrAB-TolC overexpression and penicillin binding protein (PBP) changes as mechanisms to explain β-lactam resistance in the three mutants.
Methods: Inner and outer membrane proteins (IMP and OMP) were analysed by SDS-PAGE. PBPs were detected with Bocillin FL and Imperial Protein Stain (a formulation of coomassie R-250). Expression of the *acr*A, *acr*B, *mar*A and *sox*S genes was studied by reverse transcription of total RNA and PCR of cDNA (RT-PCR), using *gap*A gene as internal control of expression. PCR products were separated in SDS-PAGE and silver stained. Ag100 strain (induced or non-induced with 5 mM salicylate) was the control strain.
Results: The level of expression of *acr*A and *acr*B genes was the same in mutants and parent strains and higher than that found in the non-induced Ag100 strain. The OMP TolC increased only in the mutants, coinciding with their increased cyclohexane tolerance. Neither parent strains nor any mutant showed *mar*A and *sox*S genes overexpression. NaCl at 1% induced OmpF expression in CazE7, the single OmpF deficient mutant. All three mutants increased expression of PBP3. A 56-kDa IMP was detected in CazE7.
Conclusion: (i) Increased expression of TolC and PBP3 in the three mutants added to loss of OmpF in CazE7 explains 64–256-fold increase in ceftazidime MICs and 4–8-fold increase in aztreonam MICs in the mutants; (ii) Detection of a 56-kDa IMP in CazE7 concurrent with loss of β-lactamase activity suggests MppA over expression, a sensor protein of murein tripeptide level and (iii) As osmoregulation of *omp*F gene expression was intact in CazE7 strain and overexpression of *mar*A and *sox*S genes was not detected in the same strain, an increased expression of rob gene might explain OmpF loss in CazE7.

ISSN: 1470-9465

Mycobacterial infections

O130

Epidemiology and trend of leprosy disease in the Iranian Qazvin province during 45 years (1958–2003)

R. Qassemi Barqi *(Qazvin, IR)*

Introduction: Leprosy is a chronic infectious disease that has been important along the history. Because of its ability to establish disability, social excluding and stealth, leprosy exists in 110 country still, although there are most of leprosy cases in the 6 countries include: India, Brazil, Madagascar, Mozambique, Nepal and Tanzania. Elimination of Leprosy is the goal of WHO. Our country was arrived at the elimination of leprosy phase from 1994.
Objective: The purpose of this study is to determine of Leprosy trend during 45 years in the Qazvin province.
Materials & Methods: In this descriptive study all leprosy cases that had been recorded during 45 years (1958–2003) in the Qazvin province, were studied about age group, sex, clinical symptoms, disability stage, incidence rate and typing based on the documents of Leprosy surveillance system.
Results: During 45 years, 321 patients with leprosy had been treated from them 177 (55%) cases were men and 144 (45%) cases were women. Only 16 (5%) cases were in < 20 years old age group. Most of patients (118 cases: 36.8%) had > 60 years old. Multi Bacillary cases were 264 (82.2%) and Puci Bacillary cases were 57 (17.8%). Lesion of macular and without sensation was the most common sign. The most patients had been recorded during 1971–1990. Leprosy disease has been at the elimination phase (prevalence ≤ 1/10000 population) from 1995 in the Qazvin province.
Discussion & Conclusion: Qazvin province is one of leprosy endemic provinces that it has achieved to goal of the elimination of Leprosy. Also less cases of Leprosy in < 20-year sold age group indicates success of program for leprosy by using MDT in the Qazvin province.

O131

Mycobacterium kansasii: results of a 10-year study in Zaragoza, Spain

S. Terraza, J.A. Amiguet, P. Arazo, C. Ramos, A. Vitoria, M.A. Lezcano, J. Cuesta *(Zaragoza, ES)*

Objectives: Clinical and epidemiological study of disease caused by *Mycobacterium kansasii* in Zaragoza, Spain.
Methods: A retrospective study was carried out between January 1990 and December 1999 in Miguel Servet Hospital, Clínico Lozano Blesa Hospital, and Royo Villanova Hospital. Diagnosis of the disease was performed according to American Thoracic Society criteria.
Results: The patient charts of 94 patients in which an isolate of *M. kansasii* had been recorded were reviewed. Sputum was by far the most common source of isolates, accounting for 79.7%. We considered 34 patients to be suffering from disease caused by *M. kansasii*. The number increased for the second half of the study period (13 vs. 21 cases). Pulmonary disease was the most frequent clinical presentation (98.9%). Seventy nine percent of all patients with disease were male. The mean age was 44 years. The most common risk factors associated with disease due to *M. kansasii* were smoking (82.4%), infected with the human immunodeficiency virus (HIV) (23.5%), chronic obstructive pulmonary disease (17.6%), and tuberculosis (17.6%). The mean CD4 cell count of the HIV-infected patients at diagnosis was 45×106/l. The most frequent clinical features were the respiratory symptoms (85.3%), followed by fever (64.7%), and constitutional symptoms (38.2%). Chest radiographs revealed cavitating pulmonary disease in 82.3% of HIV-negative cases and in 12.5% of HIV-positive cases ($p < 0.01$). The incidence of unilateral disease was 88.2% in HIV-negative patients and 25% in HIV-positive patients ($p < 0.01$). The most common treatment regimen was rifampicin, isoniazid, and ethambutol (85.3%), and the average duration was 13.3 months. Twenty-one patients (70%) were initially treated for *M. tuberculosis*. With treatment, all cases of non-HIV-associated disease had favourable outcomes, but those of HIV-associated pulmonary and disseminated disease were poor (37.5%).
Conclusion: The increasing incidence of disease causing *M. kansasii* is surmised to be due to AIDS, and other factors may be external factors causing either changes in the distribution or virulence of mycobacteria in the environment. Outcomes of therapy in HIV-negative patients were excellent, but in HIV-positive patients were poor.

O132

Comparison of conventional antimicrobial susceptibility and oligonucleotide chip system for detection of drug resistance in *Mycobacterium tuberculosis* isolates

H. Park, E.J. Song, E.S. Song, E.Y. Lee, C.M. Kim, S.H. Jeong, J.H. Shin, J. Jeong, S. Kim, Y.K. Park, G.-H. Bai, C.L. Chang *(Busan, Ulsan, Jinju, Seoul, KR)*

Objectives: Rapid detection of drug resistance is critical to manage tuberculosis patients. The clinical efficacy of the oligonucleotide chip technology for the detection of drug-resistant tubercle *bacilli* was evaluated and compared with the standard drug susceptibility testing.
Methods: Oligonucleotide chip detecting specific mutations in the *rpo*B, and *kat*G and *inh*A genes of *M. tuberculosis* was designed. The probes detecting drug-resistance were as follows; 7 wild-type and 13 mutant-type probes for rifampin, and 2 wild-type and 3 mutant-type probes for isoniazid. Target DNA of *M. tuberculosis* was amplified by polymerase chain reaction, followed by hybridization and scanning. Direct sequencing was performed to verify the results of the oligonucleotide chip. The results of mutations were compared with the results of conventional antimycobacterial susceptibility test by using Lowenstein-Jensen proportion method.
Results: Sixty-eight of seventy-six rifampin-resistant strains (90%) had mutations in the *rpo*B gene. Forty-seven of eighty-one isoniazid-resistant strains (58%) had mutations in the *kat*G gene or *inh*A gene. The mutations were easily detected by the oligonucleotide chip system within a day.
Conclusion: The diagnostic oligonucleotide chip with mutation-specific probes is a reliable and useful tool for the rapid detection of drug-resistance against rifampin and isoniazid in *M. tuberculosis* isolates.

ISSN: 1470-9465

O133

Clinical and microbiological data of patients infected with multidrug-resistant *Mycobacterium tuberculosis* strains carrying gyrase mutations

N. Veziris, J. Robert, C. Truffot-Pernot, V. Jarlier, E. Cambau, A. Aubry *(Paris, Créteil, FR)*

Objectives: In a previous study we demonstrated that mutations in *M. tuberculosis* DNA gyrase are either associated with fluoroquinolone resistance (GyrA at position G81, A83 and D87, GyrB at position N464), normal susceptibility (GyrA T73A) or hypersusceptibility (GyrA T73A and A83G). As fluoroquinolone use is increasing in tuberculosis treatment, we analysed the clinical characteristics and the *M. tuberculosis* (Mtb) resistance pattern of the patients infected by a strain carrying mutations in GyrA or GyrB subunits.

Methods: Retrospective review of characteristics of patients infected with a multidrug resistant (MDR) strain of Mtb carrying a mutation in *gyr*A and/or *gyr*B: (a) 18 patients with quinolone resistant Mtb were included in the study, 16 with a strain mutated in GyrA (A83V (n = 7); D87S (n = 1), A (n = 2), G (n = 2) or N (n = 2); G81A (n = 2)) and 2 with a strain mutated in GyrB (N464T or D) and (b) 5 patients infected with a strain carrying a novel GyrA mutation leading to quinolone hypersusceptibility in 4 cases (T73A + A83G), and in normal susceptibility in 1 case (T73A).

Results: The 23 MDR patients were majority males (13 vs. 10), had a mean age of 35 years were born outside Europe (n = 20). A majority (n = 13) had been previously treated for tuberculosis, 5 were HIV positive, at least 19 had pulmonary tuberculosis and 14 were AFB positive. The mean number of resistance of the 23 Mtb strains was 9.2 resistances including isoniazid and rifampin: 83% were resistant to pyrazinamide, 65% to ethambutol, 65% to streptomycin, 61% to ethionamide, 48% to cycloserine, 22% to kanamycin and 13% to para aminosalicylic acid. Prior treatment history was available for 13 of the 18 patients infected with a fluoroquinolone-resistant strain and all of them received a fluoroquinolone-containing regimen.

Conclusion: MDR-TB patients harbouring strains with gyrase mutations had extreme drug resistant strains and were more likely to be born outside Europe when compared to MDR-TB patients previously reported in France between 1992 and 2002 (87% vs. 64%, $p < 0.05$).

O134

Implementation of automated mycobacterial interspersed repetitive unit–variable number of tandem repeat (MIRU-VNTR) typing in a routine laboratory setting

C. Allix, P. Supply, M. Fauville-Dufaux *(Brussels, BE; Lille, FR)*

Objectives: Rapid detection, adequate therapy and contact tracing to stop further transmission are key factors to control tuberculosis (TB). The use of automated MIRU-VNTR typing technique can greatly facilitate and accelerate the resolution of problems typically encountered in clinical mycobacteriology laboratories or in public health facilities.

Methods: Amplification of 12 MIRU-VNTR genomic loci was performed on heat-inactivated cultures using 4 different multiplex PCR with fluorescent primers. DNA fragments were separated using the 3100-Avant ABI Genetic Analyser. Analysis was performed with Genescan and Genotyper softwares (Applied Biosystems).

Results:

Laboratory cross contamination: Suspicion of laboratory cross contamination is the most frequent cause of genotyping analysis requests by hospital laboratories. By using the MIRU-VNTR method, the delay between the delivery of cultures to our laboratory and a reliable genotyping result is reduced to just one or two days.

Worsening of tuberculosis under treatment: A patient, diagnosed with susceptible tuberculosis, received adequate therapy. Meanwhile, she went to Africa for a journey of 8 weeks. When she came back, her tuberculosis had worsened. The clinician suspected a re-infection by a multi-drug resistant TB in Africa. MIRU-VNTR analysis rapidly showed that the patient was still infected by the same strain.

Detection of unsuspected transmission: All isolates from tuberculosis patients living in Brussels-Capital Region are sent to Pasteur Institute of Brussels for a large-scale epidemiological study. An unsuspected tuberculosis outbreak detected since 2003 and implying at least 41 patients was evidenced by the MIRU-VNTR technique and was subsequently confirmed by IS6110 RFLP.

Conclusion: These examples illustrate how the use of automated MIRU-VNTR technique can help in the resolution of typical issues encountered in clinical mycobacteriology laboratories. False-positive cultures for *M. tuberculosis* are more frequent than suspected and can cause serious adverse effects on clinical management of patients. From culture delivery to the final MIRU-VNTR results, the delay extends from 5 to 30 hours depending on the number of isolates. In average, clear results are obtained for 97% of all loci analysed. MIRU-VNTR swiftness, rapidity and reliability are major arguments in favour of implementation of this technique in reference laboratory, to help clinicians, hospital laboratories and tuberculosis control authorities.

ISSN: 1470-9465

Current aspects on MRSA

S136

The current situation of community-acquired MRSA in Europe

J. Etienne, T. Ferry, A. Tristan, G. Durand, M. Bes, G. Lina, F. Vandenesch *(Lyon, FR)*

The overall percentage of *S. aureus* isolates expressing methicillin resistance has increased significantly in the community during the past 2 decades. Community-MRSA (C-MRSA) infections correspond to those associated to MRSA strains that have emerged recently *de novo* in the community. They contain frequently the Panton Valentine leukocidin genes. These C-MRSA infections have emerged worldwide and are associated with a limited number of clones that are highly epidemic in the community. In Europe, the prevalence is low but increases gradually. These strains tend to infect young patients, especially children and young. Outbreaks of C-MRSA infections, especially of skin and soft tissue infections have been described among members of ''closed populations''. C-MRSA are mainly transmitted from man to man by direct skin-to-skin contact, or indirectly through contact by touching objects contaminated by the infected skin of a person with MRSA. Most of the C-MRSA infections are represented by superficial skin and soft tissue infections. These infections are usually non-severe, although requiring frequently incision and surgical drainage. Deep-seated infections associated with C-MRSA (e.g. necrotising pneumonia) are rare, but are extremely severe and life-threatening infections. Cases of osteomyelitis and septic arthritis caused by C-MRSA have been reported, mainly in children. Recently, some cases of pyomyositis due to C-MRSA have been described. Necrotizing fasciitis due to C-MRSA is also an emergent severe infection. Overall, the evidence of multiple disseminated abscesses in a patient with MRSA bacteraemia is highly speculative to be due to a PVL-positive strain. C-MRSA isolates are typically sensitive to a wide variety of non-?β-lactam antibiotics including trimethoprim-sulfamethoxazole, minocycline, fluoroquinolones, vancomyin, linezolid, quinupristin-dalfopristin, and daptomycin. As C-MRSA are easily transmitted, patients with soft tissue infections should be counselled on the importance of hand hygiene, not sharing personal items such as towels, and appropriate wound care.

S137

MRSA transmission dynamics in hospitals: will control policies finally succeed?

B. Cooper *(London, UK)*

Successful control of a communicable disease requires one thing: that the average number of secondary cases generated by a typical primary case is less than one. When transmission is reduced below this threshold, sustained spread of the pathogen becomes impossible. Evidence form national surveillance data, clinical intervention studies, and mathematical models is reviewed and used to assess the possibilities for reducing transmission below this level leading to the long-term control of MRSA transmission in hospitals. Community MRSA reservoirs, community transmission, misleading surveillance data, limited infection control resources, and over-reliance on control measures of limited effectiveness all represent potential obstacles to the successful control of MRSA in hospitals. The importance of these obstacles will be considered, and means of overcoming them discussed.

Mechanisms of infectious diseases: new science and novel insights

S141

Innate immunity and Streptococcus pneumoniae

R. Read *(Sheffield, UK)*

Streptococcus pneumoniae is a major cause of morbidity and mortality worldwide, and commonly colonizes the upper respiratory tract. In some colonized individuals the organism translocates to other tissues and causes life-threatening diseases including pneumonia, bacteraemia and meningitis. The non-immune host relies upon innate immunity for defence against the *pneumococcus,* and because *S. pneumoniae* is relatively resistant to complement-mediated killing (via the terminal membrane attack complex) phagocytes (initially alveolar macrophages, and subsequently recruited neutrophils) are of critical importance. The major serum and mucosal fluid opsonins apart from immunoglobulin are the complement proteins which on contact with *pneumococci* are activated with resulting stabilisation of C3b on the bacterial surface (which is recognized by phagocyte receptors CR1 and CD11b/CD18). Other potential opsonins include the collectins (e.g. MBL and surfactant proteins with are recognized by collectin receptors) and CRP (probably recognized by FcR). *Pneumococci* possess a number of elements that can modify complement activation and consequent C3b mediated opsonophagocytosis, including the polysaccharide capsule, pneumolysin, PspA and PspC (or the alternative gene product, Hic). The *pneumococcus* can manipulate initial innate immunity by modifying the rate of decay of C3b to iC3b to C3d. If phagocytosis proceeds, a number of cellular mechanisms including generation of reactive oxygen species (ROS) and nitric oxide facilitate general bacterial killing. *Pneumococci* are killed by ROS but some studies have suggested *pneumococci* inhibit spontaneous ROS production. In addition *pneumococci* express manganese superoxide dismutase which limits ROS-mediated killing. NO contributes to both bacterial killing and induction of apoptosis in macrophages. There is no evidence that *pneumococci* can detoxify or metabolize NO. Other microbicidal molecules implicated in pneumococcal killing include cathepsin B and β-defensins. Alveolar macrophages in the process of being overwhelmed by *pneumococci* must recruit assistance from neutrophils to clear the infection. This is initiated by chemokine release by alveolar macrophages and also by alveolar epithelial cells. Chemokine and cytokine induction occurs after activation of Toll-like receptors and Nod proteins in response to a number of pneumococcal products including peptidoglycan, lipoteichoic acid and pneumolysin. Alveolar macrophage apoptosis is a feature of both sub-clinical murine pneumococcal infection models and established pneumococcal pneumonia. Inhibition of *in vivo* macrophage apoptosis by caspase inhibition is associated with decreased bacterial clearance and enhanced rates of invasive pneumococcal disease. Therefore induction of macrophage apoptosis by *pneumococci* may contribute to bacterial killing although the mechanisms underlying this association

remain to be defined. Recent discoveries in each of these elements of innate immunity to *pneumococci* will be discussed.

S142

Brucellosis: novel virulence determinants and cellular organelles

J.-P. Gorvel *(Marseille, FR)*

The facultative intracellular pathogen *Brucella* is the causative agent of brucellosis, a worldwide zoonosis that affects mammals, including humans. Essential to *Brucella* virulence is its ability to survive and replicate inside host macrophages. Understanding the molecular interactions between *Brucella* and its replication niche may lead to new tools useful for vaccine design. We have shown in a model of *Brucella abortus* infection of murine bone marrow derived macrophages that a fraction of the bacteria that survive an initial host cell killing proceed to replicate in a compartment segregated from the endocytic pathway. The maturation of the *Brucella*-containing vacuole involves sustained interactions and fusion with the endoplasmic reticulum (ER), which creates a replicative compartment with ER-like properties. The acquisition of ER membranes by replicating *Brucella* is independent of ER-Golgi COPI vesicular transport, but dependent of the COPII molecular system. A mutant of the VirB type IV secretion system, which is necessary for intracellular survival, was unable to sustain interactions and fuse with the ER, and was killed via eventual fusion with lysosomes. Thus, we demonstrate that live intracellular *Brucella* evade macrophage killing through VirB-dependent sustained interactions with the ER. This leads to hypothesis that *Brucella* effector molecules secreted by the type IV secretion system may be target for future vaccine. Osmoregulated periplasmic glucans (OPGs) are general constituents of the envelopes of gram-negative bacteria with glucose as the sole constitutive. These molecules play a critical biological role as shown by the fact that mutants unable to synthesize them are avirulent in their plant or animal hosts. OPG in *Brucella* genus is constituted by a cyclic *β*-1,2-glucan (CG) glucose backbone. Results suggest an important role of C*β*G in *Brucella* virulence as addition of this periplasmic sugar to liposomes extracted cholesterol, caused haemolysis in erythrocytes and perturbed lipid rafts in peritoneal macrophages. In addition, mutants defective in periplasmic glucan production partially recovered intracellular replication when C*β*G was added to bacterial culture media prior to macrophages infection. These data suggest that C*β*G plays an important role in *Brucella* virulence at the onset of infection and that drugs able to modulate the vacuolar environment may lead to circumvent *Brucella* infection.

Foreign body infections

S145

Orthopedic infections: tricky to diagnose, hard to treat

A. Trampuz *(Basel, CH)*

Orthopedic devices are increasingly used to alleviate pain (joint prostheses) and to fixate bone fractures (intramedullary nails, external-fixation pins, plates and screws). Infections associated with orthopedic implants occur rare, but produce significant morbidity and consume substantial healthcare expenditures. Implant-associated infections are typically caused by microorganisms attached to a surface and embedded in an extracellular matrix. This structure is called microbial biofilm organized as complex communities with structural and functional heterogeneity resembling multicellular organisms. Existence in biofilms renders these microorganisms difficult to detect and hard to eradicate. Prosthetic joint infection is usually defined by the presence one or more of the following criteria: (i) microbial growth from synovial fluid or intraoperative tissue (at least 2 positive cultures are required for low-virulent organisms); (ii) visible purulence and (iii) acute inflammation on histopathological examination or presence of a sinus tract. Infections are classified according to the onset of clinical symptoms after implantation into early (<3 months), delayed (3–24 months) or late (>24 months) infections. Early and delayed infections are usually acquired during implant surgery (perioperatively), whereas late infections are predominantly acquired by haematogenous seeding. A combination of clinical laboratory, histopathology, microbiology and imaging studies are necessary for accurate diagnosis of infection. New techniques to disrupt microbial biofilms (e.g. sonication of removed implants) and molecular methods (e.g. quantitative PCR) can further facilitate diagnostic accuracy. Successful treatment usually requires adequate surgical procedure combined with long-term antimicrobial therapy (3–6 months), ideally with an agent acting on adhering stationary-phase microorganisms. Rifampin has an excellent activity on biofilm *staphylococci*, but must always be combined with another drug (e.g. quinolones, *β*-lactams, glicopeptides) to prevent emergence of resistance. Surgical modalities include debridement with retention of the prosthesis, one-stage or two-stage exchange, resection arthroplasty, arthrodesis and amputation. Debridement with retention is a reasonable option, if the duration of clinical signs and symptoms <3 weeks, the implant is stable, the soft tissue is in good condition, and an agent with activity against biofilm microorganisms is available.

Antimicrobial clinical trials

O147

Skin disinfection with octenidine dihydrochloride for central venous catheter placement and care–a randomized controlled trial

M. Dettenkofer, C. Wilson, A. Gratwohl, D. Bolliger, C. Schmoor, D. Heim, H. Bertz, D. Luft, S. Schulz, F.D. Daschner, A.F. Widmer *(Freiburg, DE; Basel, CH)*

Objective: To investigate the efficacy and tolerability of two commercially available, alcohol-based antiseptic solutions for preparation and care of central venous catheter (CVC) insertion sites. One solution contained the bispyridine octenidine dihydrochloride.

Methods: A double blind, randomized, controlled trial was undertaken in the haematology units of university hospitals, and in one surgical unit. Adult patients with a nontunneled CVC were enrolled prospectively after informed consent and randomly assigned to different skin disinfection regimens at the insertion site: (a) 0.1% octenidine with 30% 1-propanol and 45% 2-propanol, (b) 74% ethanol with 10% 2-propanol. Treatments

were compared with respect to (i) skin colonisation within the first 10 days after CVC insertion; (ii) positivity of the catheter tip (> 15 CFU) and (iii) Occurrence of CVC related sepsis. Quantitative skin cultures were obtained from the insertion site at regular intervals, and cultures of the CVC tip on removal were undertaken with the roll plate technique. CVC-related bloodstream-infection (BSI) was defined according to criteria set up by the Centres for Disease Control and Prevention (CDC). The statistical analysis was performed according to the intention-to-treat principle. Statistical tests (alpha = 5%) of the a-priori ordered hypotheses of equality of the treatment groups with respect to the efficacy criteria were performed by an independent biostatistical department using linear regression for criterion (1), and logistic regression for criteria (2) and (3). Models included centre for adjustment.
Results: Four hundred patients with inserted CVC were enrolled during 5/2002 and 4/2005. Both groups showed no difference in patient characteristics (Table). Skin colonization at the CVC insertion site during the first 10 days showed a highly significant reduction (Group A vs. B: 0.21; CI95: 0.11–0.4, $P < 0.0001$; analysis of 365 patients due to missing values). Positivity of the catheter tip was significantly lower for Group A (7.9%) vs. B (17.8%): odds ratio = 0.40 (CI95: 0.20–0.81, $P = 0.011$; analysis of 322 patients). There was no statistical difference in the occurrence of CVC-related BSI. Side effects (i.e., burning) showed no relevant difference between the groups.

|Table: Patient characteristics

	Group A (with octenidine)	Group B (without octenidine)
Nr. of patients	201	199
Sex (female / male)	66 / 135	70 / 129
Haematopoietic stem cell transpl.	69	68
Surgery	91	90
Antimicrobially coated catheter	153	154
Catheter insertion site (V. jugularis / V. subclavia	167 / 34	159 / 38 a)

a) missing data in 2 cases

Conclusion: Disinfection with an alcoholic skin antiseptic that contains octenidine leads to a significant decrease in skin and catheter tip colonization and is a promising option for prevention of CVC-related infection.

O148

Pooled analysis of the mortality and cardiac safety of moxifloxacin

S. Choudhri, F. Krüsmann, C. Reiter *(West Haven, US; Wuppertal, DE)*

Objectives: Moxifloxacin (MXF) is an established treatment for respiratory tract infections and is used as oral, intravenous, or sequential (intravenous followed by oral) therapy. A pooled analysis of all controlled MXF Phase II, III and IV studies, which were transferred into the global integrated analysis database by 31 August 2005, was conducted to determine the cardiac safety profile and mortality risk for MXF vs. a range of comparator treatments.
Methods: The pooled analysis included 7913 patients treated with oral MXF and 7603 treated with comparator therapy in 34 oral studies and 2535 patients treated with MXF and 2574 patients treated with comparator therapy in 11 sequential (intravenous/oral) studies. All patients included were randomized, had received at least one dose of study drug and were deemed valid for the safety analysis. Comparator drugs included: cefuroxime, clarithromycin, levofloxacin, amoxicillin/clavulanic acid, ofloxacin, cephalexin, doxycycline, azithromycin, amoxicillin, co-trimoxazole and cefixime. Dosages and indications were according to manufacturer's guidelines.
Results: Mortality and cardiac safety data for treatment with oral and sequential MXF vs. comparator drugs are shown in the Table.

	Treatment group			
	Oral MXF (n = 7913)	Comparator (n = 7603)	Sequential MXF (n = 2535)	Comparator (n = 2574)
Total deaths	21 (0.27%)	27 (0.36%)	98 (3.87%)	103 (4.0%)
Deaths within 2 days of treatment start	1 (0.01%)	3 (0.04%)	8 (0.32%)	11 (0.43%)
Deaths within 3 days of stopping study medication	7 (0.09%)	11 (0.14%)	36 (1.42%)	46 (1.79%)
Death and adverse event considered as a clinically-relevant outcome of QTc prolongation	5 (0.06%)	4 (0.05%)	10 (0.39%)	15 (0.58%)
Adverse events considered as clinically relevant outcome of QTc prolongation	20 (0.25%)	16 (0.21%)	27 (1.07%)	29 (1.13%)

There were no clinically significant differences in estimated risk between the MXF and comparator groups for any parameter.

Conclusions: In this large patient population pooled from a range of clinical trials, MXF treatment did not result in an increased risk of cardiac morbidity or mortality or of overall mortality vs. comparator therapies.

O149

Short-course fluoroquinolones vs single dose fosfomycin for acute uncomplicated cystitis: systematic review and meta-analysis

V. Rafalskiy, T. Perepanova, V. Bistryukov *(Smolensk, Moscow, RU)*

Objectives: Acute uncomplicated cystitis (AUC) is one of the most common bacterial infections in adults. Oral fluoroquinolones and fosfomycin are the antimicrobials often recommended for AUC as second or first-line therapy. The aim of the study was to compare the efficacy and safety of quinolones vs. fosfomycin for AUC.
Methods: System review (SR) and meta-analysis (MA) of randomized controlled trials (RCT) was performed. The search via the Cochrane Register of Controlled Trials (Issue 2, 2005), MEDLINE (1966–September 2005), EMBASE (1988–September 2005), reference lists of articles and abstracts from conference proceedings without language restriction was carried out. RCTs comparing short-course (SC) fluoroquinolones (3–7 days) and single dose (SD) fosfomycin in patients with AUC were selected.
Results: Only 3 RCT (Boerema 1990 [1], De Jong 1991 [2], Mon-US-01 [3]) from 16 evaluated were included in MA. Fosfomycin SD was compared with norfloxacin (two studies [1, 2]) or ciprofloxacin (one study [3]) for 3–7 days. Pulled analysis of these three studies was performed (tab). Clinical as well as microbiological efficacy of fluoroquinolones SC was significant higher then fosfomycin SD. Odds ratio (OR) of eradication rate

(at the end of therapy), sustain microbiological response (28–40 days after the end of the therapy) and reinfection rate were 2.33 [1.40, 3.90], p < 0.001, 1.95 [1.36, 2.80], p < 0.0003, 0.62 [0.39, 0.99], p < 0.04. Sustain clinical response (28–40 days after the end of the therapy) was also significantly higher after SC fluoroquinolones– OR –2.59 [1.66, 4.06], p < 0.0001. Frequency of adverse drug reactions during fosfomycin therapy was significant higher then during fluoroquinolones therapy– OR – 0.29 [0.11, 0.76], p <0.01, particularly, the rate of diarrhoea development – OR -0.14 [0.02, 0.77], p < 0.02.

Outcome	Fluoroquinolones SC vs focfomycin SD: pulled OR [95% CI]	P
Sustain clinical response	2.59 [1.66, 4.06]	0.0001
Eradication rate	2.33 [1.40, 3.90]	0.001
Sustain microbiological response	1.95 [1.36, 2.80]	0.0003
Re-infection rate	0.62 [0.39, 0.99]	0.04
Adverse drug reactions	0.29 [0.11, 0.76]	0.01
Diarrhea	0.14 [0.02, 0.77]	0.02

Conclusion: There are significantly higher clinical and bacteriological efficacy rate for short-course fluoroquinolones therapy vs. fosfomycin SD therapy for patients with AUC. Adverse drug reaction particularly diarrhoea appeared significant more frequently during fosfomycin administration.

References:

(1). Boerema J.B., Willems F.T. Infection. 1990; 18 Suppl 2: S80–8.
(2). de Jong Z., Pontonnier F., Plante P. Urol Int. 1991; 46 (4): 344–8.
(3). Patel S.S., Balfour J.A., Bryson H.M. Drugs. 1997 Apr; 53 (4): 637–56.

O150

A clinical and laboratory evaluation of PAR-101 in patients with *Clostridium difficile*-associated diarrhoea

T.J. Louie, M. Miller, C. Donskey, K. Mullane, E.J.C. Goldstein, D.M. Citron, M. Corrado, S.L. Gorbach, P. Sears, S. Shangle, B. Walsh, Y.-K. Shue *(Calgary, Montreal, CA; Cleveland, Chicago, Santa Monica, New Hope, San Diego, US)*

Background: PAR-101 is a new macrocyclic compound in phase 2 clinical development for the treatment of *Clostridium difficile*-associated diarrhoea (CDAD). PAR-101 has strong antimicrobial activity against *Clostridium difficile* as compared to current therapies; MIC90 values vs. *C. difficile* for PAR-101, metronidazole, and vancomycin are 0.125, 0.5, and 2 mcg/ml, respectively.

Methods: This proof of principle study assessed the safety, tolerability, and pharmacokinetics of PAR-101 in 45 evaluable subjects with mild-moderate disease. The oral doses of PAR-101 evaluated were 50, 100, and 200 mg bid administered daily for 10 days. The tolerability/safety was evaluated based on adverse events, vital signs, ECG, clinical laboratory values, and physical examination. The clinical evaluation included relief of symptoms of CDAD, time to resolution of diarrhoea, and clinical recurrence. Plasma and faecal samples were collected to investigate the relationship among dose, concentration, and excretion of PAR-101 in CDAD patients, as well as faecal microbiology.

Results: PAR-101 was well tolerated by all subjects at all doses. Clinical response was very promising; 2 of 15 patients in the two lower dose groups and 0 of 15 in the top dosing group were transferred to conventional therapy for apparent treatment failure (41/45 cured overall). Of the subjects that completed therapy, 2/41 patients had recurrence of symptoms within the 6 weeks of follow-up, one each in the low and high dose groups. After multiple dose oral administrations, plasma concentrations of PAR-101 were typically <5 ng/ml (0.005 mcg/ml) across the dose range, and no plasma level of PAR-101 exceeded 100 ng/ml (0.1 mcg/ml), while faecal concentrations were high, averaging 1433 mcg/g at the top dose, or over 10 000 times the MIC90 for *C. difficile*.

Conclusions: The clinical response, tolerability, and systemic exposure data obtained support the further clinical development of PAR-101 as an oral therapy for *C. difficile* infection.

O151

Therapeutic drug monitoring in adult patients receiving imipenem or cefepime therapy: one-year single-centre experience

S. Vora, A A. Pascual, S. Bolay, P. Francioli, T. Calandra, O. Marchetti *(Lausanne, CH)*

Background: Experimental evidence suggests that time of β-lactam antibiotics blood levels above MIC is a key factor for treatment efficacy. Low levels may be associated with treatment failure, while high levels may increase toxicity, especially in patients with impaired renal function.

Objective: To report the experience with therapeutic drug monitoring (TDM) in adult patients receiving imipenem (IMP) or cefepime (CEF) therapy.

Methods: Retrospective analysis of consecutive IMP or CEF TDM during a 1-year period. Initial drug dosing was based on standard recommendations. IMP or CEF trough blood levels (Cmin) were measured by HPLC. Interpretation of Cmin as appropriate (A), inappropriate low (L) or inappropriate high (H) was based on MIC (when available), clinical and microbiological response, renal function, and drug blood levels reported in clinical studies. For L or H drug levels, interventions were proposed.

Results: 56 drug levels were measured (IMP, n = 30; CEF, n = 26) in 29 haemato-oncology, 11 intensive care, and 16 other adult patients. Treatment indications were microbiologically (27) or clinically (7) documented infections or unexplained fever (21). Daily dosing ranged from 500 to 4000 mg (IMP) and 500 to 6000 mg (CEF). TDM was performed 4 days (median, range 1–25) after treatment initiation: Cmin ranged from 0 to 13 (IMP) and 0.7 to 61 mg/l (CEF). Assessment of appropriateness of blood levels is shown in table 1. Interventions in patients with L IMP levels (range 0–1.5 mg/l) were: prolongation perfusion time (n = 1), increase daily dosing (n = 8), repetition TDM (n = 1) or change antibiotic (n = 1). All patients with H CEF levels (range 5–61 mg/l) had impaired renal function (creatinine clearance <70 ml/min). To prevent toxicity daily dose was decreased (n = 5) or antibiotic was changed (n = 1); no data (n = 2).

Table 1

	IMP (n=30)	CEF (n=26)
Appropriate	18 (60%)	16 (61%)
Inappropriate low (L)	11 (37%)	2 (8%)
Inappropriate high (H)	1 (3%)	8 (31%)

Conclusions: Inter-individual variability of IMP or CEF blood levels is large. Low or high drug levels were observed in a high proportion of patients suggesting that TDM may help to ensure that dosing of antibiotic therapy is appropriate. Prospective studies are needed to confirm the clinical utility of TDM.

O152

Integrated analysis of efficacy of faropenem medoxomil in the treatment of uncomplicated skin and skin structure infections

E.E.L. Wang, R. Echols, R. Tosiello *(Louisville, US)*

Faropenem medoxomil (FM) is an oral penem with potent activity against *S. pyogenes* and methicillin-susceptible *S. aureus*. This integrated analysis was conducted to describe the efficacy of FM based on 2 non-inferiority design randomized clinical trials (RCT).

Methods: Non-inferiority was defined as the lower limit of the 95% CI being >10%. 7 days of 300 mg BID of FM was compared with 7 days of either amoxicillin-clavulanate (AC) or cephalexin (C). One study was conducted in Europe, Israel, and S. Africa and the other in the USA.

Results: A total of 1180 subjects were enrolled consisting of 305 cellulitis, 249 impetigo, 277 furunculosis, and 349 simple abscess cases. In study 1, FM was non-inferior to AC. In study 2, FM did not meet the protocol-defined criteria for non-inferiority. For the pooled population from the 2 studies, non-inferiority of FM to comparators was established for PP, ITT and microbiologically evaluable populations. Clinical response for cellulitis was 86.8% FM vs. 88.8 Comp; impetigo 87.6 FM vs. 95.6 Comp; furunculosis 90.7 vs. 88.2 Comp; and simple abscess 87.8 FM vs. 93.9 Comp. Microbiologic eradication for *S. aureus* was 91.7 FM vs. 91.6 Comp; for *S. pyogenes* was 95.1 FM vs. 97.8 Comp.

Study	Clinical response PP (%)	Diff (95% CI)	Clinical Response ITT (%)	Diff (95% CI)	Clinical Response – Microb Evaluable (%)
1 FM	224/246 (91.1)	-0.1 (-5.3, 5.0)	258/298 (86.6)	0.5 (-5.1, 6.0)	139/154 (90.3)
1 AC	207/227 (91.2)		254/295 (86.1)		125/139 (89.9)
2 FM	210/246 (85.4)	-6.5 (-12.1, -0.9)	220/296 (74.3)	-4.1 (-10.9, 2.8)	132/151 (87.4)
2 C	226/246 (91.9)		228/291 (78.4)		139/150 (92.7)
All FM	434/492 (88.2)	-3.3 (-7.1, 0.5)	478/594 (80.5)	-1.8 (-6.2, 2.7)	271/305 (88.9)
Comp	433/473 (91.5)		482/586 (82.3)		264/289 (91.3)

Conclusion: Although FM did not meet the non-inferiority criteria in one RCT, it met it in the other, and in the pooled analysis. FM is effective in all four clinical infection types making up uncomplicated skin and skin structure infections (uSSSI) and it is highly efficacious for both *S. aureus* and *S. pyogenes*, the major pathogens of uSSSI.

O153

One-week once-daily triple therapy with esomeprazole, moxifloxacin and rifabutin is effective in the treatment of *Helicobacter pylori* resistant to both metronidazole and clarithromycin

S. Miehlke, W. Schneider-Brachert, A. Morgner, A. Madisch, C. Kirsch, E. Bästlein, C. Haferland, C. Jebens, H. Knoth, M. Stolte, N. Lehn *(Dresden, Regensburg, Cologne, Görlitz, Jülich, Bayreuth, DE)*

Objectives: Failure of standard triple therapy aiming at eradication of *H. pylori* often leads to post-treatment resistance to metronidazole (MET) and/or clarithromycin (CLA). Subsequent treatment of these patients remains a clinical challenge. We evaluated the efficacy and tolerability of a convenient triple therapy using esomeprazole, moxifloxacin and rifabutin for eradication of *H. pylori* resistant to both MET and CLA in a prospective open-label study.

Methods: Consecutive patients with *H. pylori* infection resistant to both MET & CLA (E-test) were treated with esomeprazole 40 mg, moxifloxacin 400 mg and rifabutin 300 mg, each given orally once daily in the morning for 7 days. Follow-up endoscopy including histology and *H. pylori* culture was performed 6 to 8 weeks after treatment.

Results: Between January 2004 and October 2005, 86 patients were enrolled (56 female, 30 male, median age 52 years). 62 patients (72.1%) had a history of two or more previous treatment failures. Three patients (3.5%) discontinued treatment prematurely due to adverse events. 73 patients (85%) are currently available for per protocol efficacy analysis. Successful eradication was confirmed in 58 patients (79.5%). In the patients with clinical failure (n = 15), post-treatment resistance to the test drugs rifampicin and ciprofloxacin were detected in 5 and in 3 patients, respectively.

Conclusion: One-week once-daily triple therapy with esomeprazole, moxifloxacin and rifabutin is effective and safe for eradication of *H. pylori* resistant to both metronidazole and clarithromycin in patients with a history of previous treatment failures.

O154

Prospective randomized trial comparing rifabutin-based triple therapy and high-dose dual therapy for eradication of *Helicobacter pylori* resistant to both metronidazole and clarithromycin

S. Miehlke, K. Hansky, W. Schneider-Brachert, A. Madisch, A. Morgner, C. Kirsch, C. Haferland, E. Bästlein, M. Buchner, E. Jacobs, M. Stolte, N. Lehn *(Dresden, Regensburg, Gorlitz, Cologne, Ribnitz-Damgarten, Bayreuth, DE)*

Objectives: After failure of standard anti-*H. pylori* therapy post-treatment resistance to metronidazole (MET) and/or clarithromycin (CLA) frequently occurs. We investigated and compared the effectiveness of a one-week triple therapy containing esomeprazole, rifabutin and amoxicillin with a two-week high-dose dual therapy containing omeprazole and amoxicillin for eradication of *H. pylori* resistant to both MET and CLA.

Methods: Patients with *H. pylori* infection resistant to both MET & CLA (E-test) were randomized to receive esomeprazole 20 mg bid, rifabutin 150 mg bid, and amoxicillin 1 g bid for 7 days (ERA), or omeprazole 40 mg tid and amoxicillin 1 g tid for 14 days (HD-OA). Follow-up endoscopy was performed 6 to 8 weeks after treatment. Cross-over therapy was performed in cases of persistent *H. pylori* infection.

Results: 145 patients were randomized (91 females, median age 50 years, peptic ulcer disease 40%). Per protocol and intention to treat eradication rates in the ERA and HD-OA group were 78% vs. 75% and 72.6% vs. 69.5% ($p > 0.05$). Two patients of the ERA group (2.7%) and 4 patients of the HD-OA group (5.5%) discontinued prematurely due to side effects. Seven of 10 patients who failed HD-OA therapy were cured by cross-over ERA therapy. Six of 8 patients who failed ERA therapy were cured by cross over HD-OA therapy. Posttreatment resistance to amoxicillin or rifabutin was not detected.

Conclusions: One-week triple therapy with esomeprazole, rifabutin and amoxicillin is effective for eradication of *H. pylori*

resistant to both metronidazole and clarithromycin. High-dose dual therapy with omeprazole and amoxcillin is a valuable alternative.

O155

A randomized study of cefuroxime vs. ampicillin-sulbactam prophylaxis for obstetrical and gynaecological operations: an effect on Gram-negative anaerobic bacteria associated postoperative surgical site infections

E. Ziogos, K. Kanellakopoulou, A. Dervisoglou, N. Galanakis, A. Papadimitriou, H. Giamarellou *(Nikaia, Haidari, GR)*

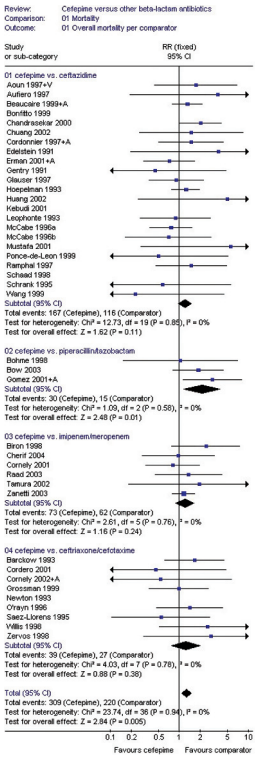
Objectives: Cephalosporins are not active against gram-negative anaerobic bacteria. Nevertheless they have been widely used and considered to be clinically effective as prophylaxis in obstetrics and gynecology surgery. We performed a prospective randomized study to compare use of cefuroxime vs. ampicillin-sulbactam (due to its activity against gram negative anaerobic bacteria), as single dose prophylaxis in obstetrical and gynecological operations.
Methods: During the time period from June 2004 through October 2004, 120 patients who underwent elective or urgent obstetrical or gynecological operation (either open or laparoscopic), were randomized to receive a single dose of either cefuroxime 1.5 gr iv (Group A), or ampicillin-sulbactam 3 gr iv (Group B), intraoperatively. All patients underwent prospective assessment for development of postoperative infection.
Results: The median age of the patients was 37.53 years old, and median hospitalization time 4.64 days. Concerning chemoprophylaxis, 62/120 (51.67%) were belonging to Group A and 58/120 (48.33%) to Group B. Surgical site infection was observed in 5/120 (4.17%) patients (4 in Group A and 1 in group B). From them 3 (60%) patients suffered from intra-abdominal infection (2 in Group A and 1 in group B). Also, 3/5 (60%) had postoperative infection caused by gram-negative anaerobic bacteria. All of them were Group A patients.
Conclusion: The patients were too few to come in any safe conclusion. Nevertheless, it is worth to mention that all postoperative surgical site infections due to gram-negative anaerobic bacteria were observed in patients who received cefuroxim as prophylaxis.

O156

The efficacy and safety of cefepime: systematic review and meta-analysis

M. Paul, D. Yahav, A. Fraser, N. Sarid, L. Leibovici *(Petah Tikva, IL)*

Objectives: To assess the safety and efficacy of cefepime, a fourth generation cephalosporin widely recommended for several indications.
Methods: Systematic review and meta-analysis of randomized controlled trials. All trials comparing cefepime to another β-lactam antibiotic, alone or with the addition of the same non-β-lactam antibiotic to both study arms were included. We searched CENTRAL, PUBMED, EMBASE, LILACS, new Federal Drug Administration drug applications, conference proceedings and references of included studies. Two reviewers independently performed search and data extraction. Missing data were sought from authors. No date, language, age or publication limits were imposed. The primary outcome assessed was 30-day all-cause mortality. Relative risks (RR) with 95% confidence intervals (CI) are reported.
Results: Fifty-five trials were included. All-cause mortality was higher with cefepime compared to other β-lactams, RR 1.26 (95%CI 1.08–1.49), p = 0.005, with no heterogeneity. Mortality was higher with cefepime vs. several comparators (Fig.). Cefepime was assessed for different indications, including febrile neutropenia, pneumonia, sepsis, bacteraemia, meningitis, skin/ soft tissue and uro-gynecological infections. All cause mortality was significantly higher with cefepime in febrile neutropenia, RR 1.42 (95%CI 1.09–1.84). Studies of higher methodological quality were associated with a greater disadvantage to cefepime, RR 1.36 (95%CI 1.09–1.70) for studies reporting adequate allocation concealment and RR 1.52 (95%CI 1.20–1.92) with adequate allocation generation. No significant differences between cefepime and other β-lactams were observed for clinical failure, RR 0.99 (95%CI 0.94–1.04), microbiological failure RR 0.93 (95%CI 0.85–1.02), bacterial superinfections, RR 1.01 (95%CI 0.74–1.38) and adverse events requiring treatment discontinuation, RR 1.12 (95%CI 0.86–1.46). Enlisting of specific adverse events reported did not reveal a specific adverse event potentially responsible for the increased mortality.



Conclusions: Our study shows a significant 24% increase in all-cause mortality among patients treated with cefepime in randomized trials. Despite a broader *in vitro* spectrum of coverage than most comparators assessed in these trials and claims for reduced resistance induction, no advantage to cefepime was observed with regard to clinical or microbiological failure and bacterial superinfections. The use of cefepime should be reconsidered.

ISSN: 1470-9465

Epidemic influenza

O158

Fatal cases of influenza-associated encephalopathy in the Netherlands

J. Gooskens, T. Kuiken, E. Claas, H. Harinck, H. Baelde, W. Spaan, A. Kroes *(Leiden, Rotterdam, NL)*

Objectives: To investigate clinical, pathological and molecular aspects of fatal cases of influenza-associated encephalopathy (IAE) in the Netherlands.
Methods: Two fatal cases of IAE, occurring in 2004 and 2005, were analysed for clinical details by review of medical records and by pathological and molecular analysis on post-mortem fresh and paraffin-embedded tissue in one case. A 5-year hospital database search (2000–2005) and a MEDLINE literature search (1955–2005) were performed for documented fatal cases in the hospital and the Netherlands respectively.
Results: Two unrelated native Dutch patients, a 17-year-old male adolescent and a 9-year-old girl, were admitted to the hospital in shock and coma, with signs of profuse bleeding from the gastrointestinal or respiratory tract. The patients had a short prodromal illness consisting of flu-like symptoms, vomiting and increasing drowsiness. Influenza A virus H3N2 was cultured from the respiratory tract of both patients. Only the 9-year-old girl had respiratory symptoms on physical examination and an infiltrate on the chest radiograph. Neuroimaging was normal in both patients. Both patients died within 24 hours after admission following rapidly progressive multi-organ failure (MOF), disseminated intravascular coagulation and shock. Post-mortem analysis of the lungs of the 17-year-old male revealed extensive haemorrhages, bronchitis, early diffuse alveolar damage and bronchial epithelial cells positive for influenza A virus by immunohistochemistry. No pathological changes or immunohistochemical positive cells were observed in other organs. Influenza RNA was detected in multiple organs including the brain, but not in blood specimens. No additional fatal cases were uncovered in the hospital from 2000–2005. To our knowledge, no fatal cases of IAE are documented in the Netherlands after the 1957 Asian influenza pandemic, when 68 cases were reported.
Conclusions: Fatal IAE is extremely rare, except among Japanese children with 50–100 deaths each year during 1995–2001. These cases are commonly preceded by rapidly progressive coma, MOF and severe thrombocytopenia. A similar presentation described in two previously healthy unvaccinated Dutch children warrants monitoring of severe influenza to determine the true incidence of fatal IAE. Influenza RNA was detected in multiple organs and the brain of one patient, suggesting a causal relationship between viral dissemination and severe neurological and systemic disease.

O159

Lack of discriminating signs in clinical diagnosis of influenza

M. van der Hoeven, M. Scholing, P. Wever, M. Hermans, R. Fijnheer, P. Schneeberger *('s-Hertogenbosch, NL)*

Objectives: Rapid diagnosis of influenza in hospitalized patients is important to prevent the transmission of the infection in the hospital. This prospective study was designed to determine the relationship between the clinical diagnosis of influenza made by the physician at admission and the presence of influenza virus in patients with respiratory tract infections.
Methods: The study was conducted in a large Dutch teaching hospital during the influenza season in 2005 in a period of three weeks. All patients of 18 years and older, admitted with respiratory tract infections were included in the study. Clinical and laboratory parameters, chest radiograph, blood and sputum cultures and nasopharyngeal swab for polymerase chain reaction (PCR) were obtained for each patient. In addition, the physicians opinion at admission whether this patient had influenza was recorded.
Results: A total of 78 patients were hospitalized with respiratory tract infections. In 41 (53%) of them influenza virus was detected by PCR. Among the patients with PCR proven presence of influenza virus, the clinical diagnosis was made in 18 cases (44%). No false positive cases, i.e. clinical diagnosis of influenza but no virus detected, were observed. Neither C-reactive protein, nor leucocytes an infiltrate on chest radiograph could discriminate between viral and bacterial infections of the respiratory tract.
Discussion: The present findings failed to demonstrate a significant relationship between the clinical diagnosis of influenza and PCR detection of the virus. More specifically, the virus was present at least twice more often than influenza was clinically diagnosed. As a consequence, the decision to take protective measures to control spread of the virus should not rely on the clinical diagnosis.

O160

Unusual summer influenza outbreak in a nursing home. Diagnosis and prophylactic measures

J. Gaillat, G. Dennetiere, M. Valette, E. Raffin-Bru *(Annecy, Lyon, FR)*

Objectives: Despite vaccination flu epidemic in nursing homes does happen occasionally, but when it occurs during summer time it is a rare event. To describe an influenza outbreak and to assess the prophylactic measures in such a situation.
Description of the epidemic: The outbreak started on the 27 June and lasted 7 days up to 3 July 2005 in a nursing home of 81 residents (R) (mean age 88 years) and 51 health care workers (HCW) Fever, cough and wheezing were reported as main symptoms among 32/81 R and 6/48 HCW on duty during the mentioned period. On day 4, one patient was hospitalized. The knowledge of several similar cases and the clinical features (short incubation, fever, wheezing and cough with normal pulmonary Xray) gave grounds to perform a rapid diagnosis test for influenza on a rhinopharyngeal sampling (RPS) despite the season. The results turn out to be positive. At the same day, 10 symptomatic residents have had RPS and of which 3 rapid diagnostic tests were performed at the time and all were positive. In collaboration with the departmental medical authorities and the staff of the nursing home, isolation of patients plus wearing of surgical masks (HCW, visitors and ambulatory residents) were decided and oseltamivir was prescribed. and started on the 01/07/05. For 19 patients and for 5/6 HCW symptomatic for <48 hours received oseltamivir (O) at 75 mg BID for 5 days. A prophylactic treatment, 75 mg OD 7 days, was given to 47 residents as well as to the 42 remaining HCW. The epidemic was stopped 24 hours later. The viral strain was similar to the winter epidemic strain of the 2004–2005 season: H3N2A/New York/55/2004. Case fatality rate was 15.6 (5/32) (2 had received O, the former only one dose, the later 15 days after the end of treatment due to a myocardial

infection), 3 others had symptoms for more than 48 hours and did not receive O. The flu vaccine coverage in the resident was 93.5%. Because of the midsummer heat alert, all residents had stayed in a single air-conditioned room from 26 to 28/06. It can explain the high and rapid attack rate.
Conclusion: The rapid diagnosis test has been a clue to rapidly intervene in the nursing home and to confirm the flu epidemic. As soon as the outbreak was recognized, a crisis cell has been put in place to apply the measures to control the epidemic as described in the national instructions particularly O prescription. The rapid control response was found to be quite effective.

O161

Effects of statin therapy on the risk of serious outcomes during influenza epidemics

E. Hak, T. Verheij, G. van Essen, M.J.M. Bonten, A. Hoes *on behalf of ESPRIT*

Background: Studies have shown immune-modulatory and anti-inflammatory properties of statins, therefore statins could benefit patients during influenza outbreaks.
Methods: The effects of statins were assessed in patients aged 50 years and older from the University Medical Center Utrecht primary care network who were followed up during four epidemic and non-epidemic influenza seasons from 1996 until 2004. Primary endpoint was a composite of community-acquired pneumonia, prednisolone-treated acute respiratory disease, myocardial infarction, stroke and death from all causes. To control and quantify confounding, we obtained estimates of reductions in outcome and control events (dermatomycosis, irritable bowel syndrome) associated with statin therapy after adjustments for demographic and health characteristics.
Results: In the study, 22 638 persons provided 130 558 persons-periods and statin therapy was used in 5.3%. In 3.2% of person-periods the primary endpoint occurred and most events were respiratory (72%). During influenza epidemics, statin therapy was associated with a 33% reduction in the primary endpoint (relative risk [RR] 0.67; 95% confidence interval [95%CI]: 0.54–0.83 [P < 0.001]), a 28% reduction in respiratory disease (RR 0.72; 95%CI: 0.57–0.90 [P = 0.004]) and 51% reduction in all-cause mortality (RR 0.49; 95%CI: 0.29–0.82 [P = 0.007]). The findings were consistent across subgroups according to age, cardiovascular disease or exposure to influenza vaccination. In non-epidemic influenza seasons, no significant reduction in the primary endpoint was observed while statin therapy was not associated with reductions of the control event rates (n = 1.240) in either season.
Conclusions: Statin therapy was associated with substantial reductions in the risk of serious, mainly respiratory, outcomes during influenza epidemics and these findings should direct future studies into the potential implications during pandemics.

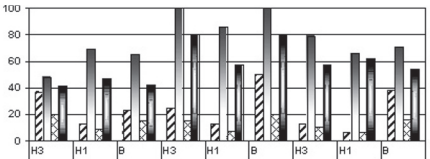
O162

Influenza vaccination in health workers included in high-risk group population

A. Piñón, S. Oropeza, B. Acosta, C. Aragones, B. Galindo *(Havana, CU)*

Influenza is a highly contagious acute respiratory disease of global importance. A vaccination with inactivated Influenza virus is currently the most effective measure for reducing the impact of the disease and prevents its complications and mortality in persons at risk.

Objectives: Determine antibody responses against vacunal strains; identify antibody protector levels against each one of the strains; detection of seroconvertion protection level in the study population and identify differences of the response in the different high-risk groups.
Methods: To evaluate the humoral immune response by Influenza vaccination in high-risk groups, we conducted a prospective longitudinal descriptive study. Haemagglutination inhibition test was used to determine antibody levels against the three strains included in the vaccine.
Results and conclusions: The studied population had antibodies before vaccination against all the strains at the vaccine, which is because of the circulation of similar strains in the season and is in concordance with international reports. Pre-vaccination GMTs agree with those results. We detected a high percent of protection against the three strains as a response to vaccination; GMTs increased to values higher than 40. The three groups included in the study (asthmatic, diabetic and hypersthenes) had a good response to vaccination and diabetics are better responders than the other two groups, with 100% of protection against two strains of the vaccine (AH3N2 and B).



	Asthma			Diabetes			Hypertension		
	H3	H1	B	H3	H1	B	H3	H1	B
Seroprotection Pre	37	13	23	25	13	50	13	6	38
Seroprotection Post	48	69	65	100	86	100	79	66	71
GMT Pre	20	9	15	15	7	20	10	6	16
GMT Post	41	47	42	80	57	80	57	62	54

Figure: Seroprotection rates and Geometric mean titers for anti-HI antibodies in asthmatic, diabetic and hypertsense subjects.

References:
(1) Hilleman MR. Realities and enigmas of human viral influenza: pathogenesis, epidemiology and control. Vaccine 2002; 20:3068–87.
(2) Bridges CB, Harper SA, Fukuda K, Uyeki TM, Cox NJ, Singleton JA. Prevention and control of influenza. Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep 2003; 52:1–34.
(3) Nancy JC, Kanta, S. Influenza. Lancet 1999; 354:1277–82.

O163

Efficacy of influenza vaccination in patients with liver cirrhosis

H.J. Cheong, C.W. Park, S.Y. Ki, Y.M. Cho, J.Y. Song, J.E. Yeon, K.S. Byun, W.J. Kim *(Seoul, KR)*

Objectives: Cirrhosis is a major chronic disease in many Asian countries. An estimate of the prevalence of cirrhosis cases in Korea is around 460,000 cases and constitutes 15% among 6 major chronic diseases in Korea. Though WHO does not include cirrhotic patients among influenza vaccine-target groups, they have been considered as one of the priority groups in Korea. However, available data on the impact of influenza in liver cirrhosis is extremely limited up to now. Therefore, we decided to evaluate the efficacy of influenza vaccine in chronic liver disease, and intended to study the clinical outcomes of influenza in patients with liver cirrhosis.

Methods: Two hundred sixty-five patients with liver cirrhosis were, in October of 2004, persuaded to be vaccinated against influenza. Among them, 198 patients were vaccinated with a trivalent influenza vaccine and the others were not vaccinated. Both of the vaccinated and unvaccinated groups were observed until May of 2005; self reporting system was operated. If influenza-like illness (ILI) was suspected in those patients, diagnostic tests were performed with rapid diagnosis kit (Quick View™) and viral culture. We compared the incidence of ILI and positive rates of diagnostic tests between vaccinated and non-vaccinated groups.
Results: Most of demographic data were indistinguishable between vaccinated and unvaccinated groups. However, the proportion of patients with severe hepatic dysfunction was somewhat larger in vaccinated group compared to those of the unvaccinated: Child-pugh B and C (77.1% in vaccinees vs. 57.7% in non-vaccinees). Overall incidences of ILI (P = 0.064) and culture confirmed influenza (P = 0.009) were significantly higher in unvaccinated group than those were in vaccinated group. Most of the patients with influenza complained of febrile sense (91.6%) and myalgia (83.3%), but typical manifestations such as cough (41.6%) and sore throat (25.0%) were not frequently noted. In some patients, influenza was accompanied with aggravation of underlying hepatic dysfunction: hepatic encephalopathy (16.6%), uncontrolled ascites (25.0%) and oliguria (33.3%). One influenza patient was complicated by secondary bacterial pneumonia.
Conclusions: Influenza vaccination against cirrhotic patients was effective to prevent disease occurrence. Influenza in cirrhotic patients showed atypical clinical presentation and caused aggravation of underlying hepatic dysfunction though it was not direct cause of mortality.

O164

Clinical effectiveness of influenza vaccination in adults with diabetes mellitus

I. Looijmans-van den akker, T. Verheij, E. Buskens, G. Rutten, E. Hak *(Utrecht, NL)*

Objectives: Evidence is conflicting regarding the potential benefits of influenza vaccination in patients with diabetes. As part of the PRISMA study (Hak E, et al. Arch Intern Med, 2005) we assessed the clinical effectiveness of influenza vaccination in adults with diabetes and specifically examined potential modification of effect by age and prior influenza vaccination.
Methods: We conducted a case-control study nested in a large cohort of patients recommended for influenza vaccination during the 1999–2000 influenza A epidemic (n = 75,000). We selected cases and controls with a diagnosis of diabetes from the original study population (n = 9,238). Vaccination status was recorded by the general practitioner. The primary endpoint was the composite of all-cause mortality and hospitalization for diabetes events, acute respiratory or cardiovascular disease during the epidemic, and the separate components were secondary endpoints. We evaluated the effect of vaccination and the influence of prior vaccination by means of logistic regression analysis controlling for age, gender, health insurance coverage, prior health care use, medication use and co-morbid conditions.
Results: We observed 131 hospitalizations and 61 deaths. In all, the combined endpoint was reduced by 56% (95% confidence interval: 36–70%), hospitalizations by 52% (95% CI 22 to 70%) and deaths by 58% (95% CI 13 to 80%. Among persons with diabetes aged 18 to 64 years we observed somewhat higher reductions in the primary endpoint than elderly persons with diabetes over 65 years of age (72% versus 39%). In those vaccinated for the first time, the primary endpoint was reduced by 47% (95% CI 0.2% to 72%) and in those who received prior vaccination the reduction was 58% (95% CI 4 to 81%).
Conclusion: Adults with diabetes alike other recommended risk groups as healthy elderly persons and patients with cardiovascular or pulmonary disease benefit considerably from influenza vaccination and no difference in vaccine effectiveness was observed between first time and repeat vaccination.

O165

Immunogenicity of baculovirus-expressed trivalent influenza HA vaccine: randomised, double-blind, dose-escalation single institution study in adult patients with B-cell lymphoma

A. Safdar, A. Rodriguez, F. Luis, J. Romaguera, F. Hagemeister, P. McLaughlin, L. Kwak, R.B. Couch *(Houston, US)*

Background: Immunogenic response of conventional influenza vaccine is markedly impaired in patients with hematologic malignancies. We sought to evaluate possible beneficial effect on increasing vaccine dose on specific neutralizing immunoglobulin response among patients with non-Hodgkin's lymphoma.
Methods: This prospective study was performed during the later half of 2004 and was sponsored by the National Institutes of Health (NIH). The randomization was undertaken by an independent NIH designated agency. Pre- and 4 week post-vaccination antibody titers were measured under a code at the BCM. Test viruses for neutralizing assays were A/Moscow/10/99 (H3N2) (antigenically similar to A/Panama/H3N2 virus) was used in influenza A/H3N2 tests. A/New Caledonia/20/99 (H1N1), and B/Hong Kong/330/2004.
Results: Please refer to the table attached.All patients visiting outpatient lymphoma clinic were screened and those who had not received antineoplastic therapy within 3 months and/or rituximab in the past 6 months were considered for the study. Splenctomized patient, and those receiving systemic corticosteroids were not included. There were no serous adverse reactions noted in any of the 27 enrollees on 6 months

		A/H3		
Vaccine	Dose (μg)	Pre-Antibodies	No.	No. Rise (%)
rHO	15	4.5-8.0[1]	5	2 (40)
rHO	45	4.5-8.0	5	2 (40)
rHO	135	4.5-8.0	5	3 (60)
Standard	15	4.5-8.0	4	0 (0)

[1]Log base 2

		A/H1		
Vaccine	Dose (μg)	Pre-Antibodies	No.	No. Rise (%)
rHO	15	<1-4[1]	4	0 (0)
rHO	45	<1-4	4	2 (50)
rHO	135	<1-4	3	2 (67)
Standard	15	<1-4	5	2 (40)

[1]Log base 2

		B		
Vaccine	Dose (μg)	Pre-Antibodies	No.	No. Rise (%)
rHO	15	<1-2[1]	6	1 (17)
rHO	45	<1-2	5	0 (0)
rHO	135	<1-2	4	2 (50)
Standard	15	<1-2	5	2 (40)

[1]Log base 2

post-vaccination follow up. Among influenza A/H1N1 and A/H3N2, higher doses were associated with increased strain-specific protective neutralizing antibody titers. Whereas, this beneficial effect was not seen in patients receiving higher doses of influenza B vaccine.

Conclusions: Recombinant Baculovirus-Expressed influenza vaccine was safe in our compromised patients with non-Hodgkin's B-cell lymphoma. These preliminary results indicate potential immunogenic benefit associated with influenza A recombinant vaccine dose-escalation.

Rapid methods for the identification of bacterial clones and pathogens

O166

Mixed whole genome microarray technology discerns a hospital-adapted subtype of *Enterococcus faecium*, comparable to multilocus sequence typing clonal complex (CC) 17

H.L. Leavis, W.J.B. van Wamel, R.J.L. Willems, F.H. Schuren, A.C. Fluit, M.J.M. Bonten *(Utrecht, Zeist, NL)*

Objectives: Previously we identified a genotypically distinct subset of epidemical *Enterococcus faecium* (Efm), designated CC17 using multilocus sequence typing (MLST). We investigated whether a whole genome microarray approach would type strains similarly and if epidemic specific markers could be identified.
Methods: A shotgun library was constructed of 9 Efm strains of which 5700 ± 1.2 kb DNA fragments were spotted on a slide. Cy5 labeled DNA of 100 MLST typed Efm isolates from different sources (outbreaks, infections, surveys and animals) and 5 continents, vancomycin resistant and susceptible, were hybridized to the array together with Cy3 labelled DNA of the library strains. 2919 spots met quality criteria and were included in normalization and EPP data transformation (Kim et al. 2002). Transversal hierarchical clustering (HC) was performed based on strains and spots (inserts). Inserts differentiating between CC17 and non-CC17 were identified from the visualized data matrix, PCR amplified and sequenced. Sequences were blasted in Genebank (NCBI).
Results: HC resulted in 9 different clusters and a small cluster of 2 strains and resembled MLST genogrouping. Four clusters consisted of mainly CC17 strains, including all but two epidemic strains. Approximately 60 % of inserts was conserved while 413 inserts (14.1%) were associated with the epidemic clusters. From these, 190 were selected for sequencing. This revealed that differentiating inserts were gene fragments encoding potential virulence factors (24), resistance determinants (3), transposons or IS elements (32), proteins involved in replication (13) or regulation (3), as well as hypothetical proteins (10). 44 (23%) of hits showed no significant homology.
Conclusion: Comparative genomics of Efm based on a mixed whole genome microarray discerned the epidemic MLST subpopulation. From the analysis 14.1% of inserts were identified differential for CC17 including both regulatory and potential virulence genes. This technology may provide new targets for diagnostics and for the development of selective drugs for hospital-adapted Efm.

O167

Genetic relationship between *Streptococcus pneumoniae* isolates responsible for a case of meningitis and for asymptomatic carriage in children attending a day-care centre in Lisbon

T. Figueiredo, M. Ramirez, I. Santos-Sanches, S. Nunes, A. Brito-Avô, R.M. Barros, I. Peres, H. de Lencastre *(Lisbon, Oeiras, PT; New York, US)*

Objectives: Colonisation of the nasopharyngx by *Streptococcus pneumoniae* although asymptomatic, is a prerequisite for the development of a clinical infection. However, the underlying mechanisms that turn the benign state of colonization into clinical disease are poorly understood. Thus, in order to understand the molecular mechanisms that define the virulence of *S. pneumoniae*, a clear picture of the genetic relationship between invasive and carried pneumococci is needed.
Methods: We characterised 2 pneumococcal strains recovered from the blood and cerebrospinal fluid (CSF) of a child with meningitis and 23 nasopharyngeal isolates from asymptomatic carriers attending the same room in a day care centre (DCC), using antimicrobial susceptibility profiling, serotyping, PFGE and MLST. We also investigated the presence of lysogenic phage since these were shown to be associated with virulence determinants in other streptococci.
Results: Strains recovered from the sick child and seven carriage isolates were fully susceptible, expressed serotype 6B and belonged to a new clone by PFGE and MLST (ST896). However, there was a band difference in the PFGE pattern of the isolates from the CSF and carriage. The *in vivo* loss of a lysogenic phage in the CSF isolate accounted for this difference and suggested an involvement of the phage in the transition between colonization and disease. However, the localization of the attB in an intergenic region and the putative functions of the adjacent genes do not support this hypothesis. The remaining 16 isolates recovered from asymptomatic carriers belonged to other clones including internationally clones previously found associated with invasive disease (e.g. Spain23F-1).
Conclusions: The same pneumococcal clone was simultaneously responsible for carriage and for invasive disease in different hosts. The *in vivo* loss of a lysogenic prophage, between the isolate recovered from the blood and the one from the CSF, suggested an involvement of the bacteriophage in this transition. The localization of the attB site in an intergenic region and the suggested functions of the adjacent genes do not support this hypothesis.

ISSN: 1470-9465

O168

Changes in the distribution of hypervirulent complexes of Neisseria meningitidis in the Czech Republic

P. Kriz, J. Kalmusova, M. Musilek, J. Felsberg, R. Haugvicova, D. Caugant, K. Jolley, M. Maiden *(Prague, CZ; Oslo, NO; Oxford, UK)*

Objectives: The distribution of major hypervirulent complexes causing invasive meningococcal disease (IMD) in the Czech Republic in the period 1993–2005 is presented. The case fatality rate caused by individual hypervirulent complexes is analysed and a corresponding vaccination strategy recommended.
Methods: Sequence types (ST) were identified by multilocus sequence typing (MLST) presented on the MLST website (http://pubmlst.org/neisseria/). The first MLST was done in Oxford, later in Oslo and finally in Prague. The total number of *N. meningitidis* isolated from IMD in the Czech Republic in the period 1993–2005 and investigated by MLST was more than 450.
Results: Four major hypervirulent complexes causing IMD in the Czech Republic in the period 1993–2005 were detected: ST-11 complex (representing 32% of the whole collection of strains), ST-18 complex (14%), ST-32 complex (10%) and ST-23 complex (3%). ST-11 complex was represented mainly by strains of serogroup C, ST-18 and ST-32 complexes by strains of serogroup B and ST-23 complex by strains of serogroup Y. ST-11 complex reached its maximum in the period 1993–2002, ST-18 complex in the period 2000–2003, ST-32 complex in the period 1993–1996 and ST-23 complex in the period 2001–2005. The case fatality rate recently caused by *N. meningitidis* Y belonging to ST-23 complex is extremely high (33%). Since 2005, meningococcal tetravalent polysaccharide vaccine A, C, Y, W135 has been imported to the Czech Republic and is recommended for vaccination of children before they reach 14 years of age. Registration of conjugate tetravalent vaccine A, C, Y, W135 is desirable in the Czech Republic.
Conclusion: The prevalence of hypervirulent complexes has changed in the Czech Republic in the period 1993–2005. A decrease of *N. meningitidis* C belonging to ST-11 complex and an increase of *N. meningitifis* Y belonging to ST-23 complex occurred recently. In addition to the conjugate MenC vaccine, tetravalent polysaccharide vaccine A, C, Y, W135 is recommended for children.
Acknowledgement: This work was supported by Ministry of Health, by research grants A8688-3/2005 and NJ/7458-3 of the Internal Grant Agency of Ministry of Health of the Czech Republic and research grant EU-MenNet Contract No. QLK2-CT-2001-01436 and made use of the Multi Locus Sequence Typing website (http://pubmlst.org/neisseria/) sited at the University of Oxford and funded by the Wellcome Trust and European Union.

O169

Rapid diagnosis of bacterial vaginosis by terminal restriction fragment length polymorphism profiling

F. Thies, W. König, B. König *(Magdeburg, DE)*

Objectives: Bacterial vaginosis (BV) is an ill-defined disorder of the vaginal microflora associated with impaired health conditions of women and, possibly, of fetuses and newborns. As cultivation is of limited value, the diagnosis of BV is currently based on microscopical techniques (e.g., Nugent's Gram stain criteria). Molecular identification methods are supposed to provide more detailed insight into the bacterial composition of the disturbed vaginal flora. However, most techniques described so far, as sequencing of 16S rDNA clone libraries, are time-consuming and not suitable for routine purposes. Therefore, we adapted a culture-independent method for bacterial community analysis, called terminal restriction fragment length polymorphism (T-RFLP), to the needs of a routine microbiology laboratory.
Methods: Vaginal swabs were collected from 50 patients with BV and, as control, from 25 healthy subjects. Disease status was assessed using Nugent's criteria. We analysed the bacterial community directly from the swab specimens by means of the T-RFLP method. T-RFLP analysis is based on the restriction endonuclease digestion of fluorescently end-labelled PCR amplificates (derived from the 16S rRNA gene). For the identification of bacteria from T-RFLP raw data, we developed an in-house computer software.
Results: Using T-RFLP, analysis of the vaginal microflora could be accomplished within 12 hours. In each BV sample, we found, on average, 6 to 8 typical fingerprints corresponding to definite bacterial species. Atopobium vaginae (95 % of all BV samples) and Gardnerella vaginalis (60 %) proved to be the predominant species, followed by *Lactobacillus iners* (50 %), *Prevotella* sp. (30 %) and *Peptoniphilus* sp. (15%). At least 3 different, and so far uncharacterized, bacteria from the Clostridiales order could be regularly detected, with one of them (a presumed *Megasphaera* sp.) at a frequency of 70 %. In healthy controls, only lactobacilli were detected.
Conclusion: Molecular tools are currently favoured for the analysis of the vaginal microflora. As such a tool, T-RFLP proved to be very promising for the rapid and detailed analysis of the bacterial communities inhabiting the vagina. This technique may be especially helpful in the context of clinical studies, when collecting reliable and extensive microbiological data may be crucial for the interpretation of study results.

O170

Development and validation of real-time PCR assays for a multi-centre study to assess the prevalence and epidemiology of shiga-toxin producing *Escherichia coli* in the Netherlands

T. Schuurman, A. Roovers, W.K. van der Zwaluw, A.A. van Zwet, L.J.M. Sabbe, A.M.D. Kooistra-Smid, Y.T.H.P. van Duynhoven *(Groningen, Goes, Bilthoven, Arnhem, NL)*

Objectives: Traditionally, laboratory surveillance for shiga-toxin producing *Escherichia coli* (STEC) has focused on the detection of *E. coli* O157 by stool culture on sorbitol-MacConkey agar (SMAC). Infections by *E. coli* O157 range from mild, self-limiting diarrhea to hemorrhagic colitis and hemolytic-uremic syndrome (HUS). Recently, non-O157 strains are increasingly associated with diarrhea and HUS in several European countries. Therefore, a nationwide screening program will take place in the Netherlands from November 2005 to November 2006. This screening will be real-time PCR-based (RT-PCR), with subsequent STEC isolation from the positive stools. Prior to the start of this program, RT- PCR assays were developed and validated.
Methods: Assays targeting the stx1 and stx2 genes were developed for both LightCycler (LC) and TaqMan (TM). The LC assay has been described by Bellin et al (J. Clin. Microbiol. 2001 370–374). The TM stx1 assay was adapted from Jinneman et al. (Appl. Environ. Microbiol. 2003 6327–6333), whereas the stx2 assay was a new design. Stools were processed by a miniMAG stool protocol. The phocine herpes virus-1 was used as an internal control (IC). Both assays were validated with a panel of

well characterized *E. coli* strains (n = 31) and non-*E. coli* strains (n = 38). Intra-, inter-assay variation and analytical sensitivity were assessed by dilution series (n = 8), spiked in 2 faecal matrices, analysed in 5-fold on the same day and once daily on 4 subsequent days.

Results: Both assays proved specific for stx1 and stx2 genes and no cross-reaction was observed. The TM assay was capable of detecting approximately 10000 CFU/g of stool with a 100% hit rate for both semi-solid and liquid stools. Lower hit rates were observed at approximately 1000 CFU/g (22% and 67%). The LC assay proved to be 1 log less sensitive (100% hit rate) compared to the TM assay for semi-solid stools. Furthermore, the LC assay did not detect approximately 1000 CFU/g. Coefficients of variation (CV) were < 5% for both TM and LC assays.

Conclusion: Both TM and LC assays proved to be very reproducible, although their sensitivities were not equal. The lower sensitivity of the LC assay compared to TM is however still in line with other published fecal RT-PCR assays. Probably, it will not be detrimental to the screening, since it is still 1 to 2 log more sensitive compared to the reported sensitivity for SMAC screening. Both RT-PCR assays are currently in use in the Dutch STEC screening program.

O171

Based upon repeat patterns: variable number of tandem repeat pattern analysis to infer clonal groupings of *Staphylococcus aureus* strains from spa typing data

T. Weniger, C. Berssenbruegge, A. Mellmann, J. Rothgänger, M. Sammeth, J. Stoye, D. Harmsen *(Munster, Wurzburg, Bielefeld, DE)*

Objectives: For molecular typing of *Staphylococcus aureus* and in particular MRSA, DNA sequencing of the polymorphic X region of the protein A (spa) gene is a well established and highly discriminatory method. This spa region consists of a variable number of 21–27 bp long repeats varying in composition that result in the different spa types. It was hypothesized that this region reflects not only short- but also long-term epidemiology due to two different mechanisms of evolution, i.e. repeat-duplication/-deletion and point mutations. However, until today, no algorithm exists to infer the clonal relatedness from different spa types. Therefore, the Based Upon Repeat Patterns (BURP) algorithm was developed and evaluated in this study.

Methods: For evaluation of BURP, 36 *S. aureus* strains that were previously characterized by whole genome micro-array, spa, multi locus enzyme electrophoresis (MLEE), pulsed-field gel electrophoresis (PFGE), and coagulase (coa) gene typing were used (Koreen et al., J. Clin. Microbiol. 42:792–9, 2004). BURP clusters (spa-CC) from these strains were determined using the Ridom StaphType version 1.2 (Ridom GmbH, Würzburg, Germany) software. This algorithm takes repeat-duplication/-deletion and point mutation events into account when calculating the relatedness of different spa types. spa types shorter than 6 repeats were excluded from analysis because no reliable evolutionary signal could be extracted. Subsequently, spa-CCs were compared to the clustering of the various other typing methods by using concordance analysis.

Results: The maximum achievable concordance with various parameters (exclude spa types shorter than ''x'', cluster spa types into spa-CC if cost distances are less than ''y'') between BURP spa-CCs and the other methods were: for whole-genome micro-array lineages 97.1% (0.81 Pearson correlation coefficient), 92.2% (0.5), and 89.0% (0.31), for MLEE lineages 85.9%, for MLEE types 91.3%, for PFGE types 98.3%, for coa lineages 96.6%, and for coa types 95.6%. If 3 very short spa types were excluded from analysis, a maximum of 99.8% concordance between spa-CCs and manually by Koreen et al. grouped spa lineages was obtained.

Conclusion: BURP was able to extract automatically an evolutionary signal from spa repeat patterns rather congruent to signals found by other typing approaches. Therefore, by using just single variable number of tandem repeat sequences of *S. aureus* - for the first time - a meaningful clustering could be realized.

O172

Multiple locus variable number tandem repeat assays do not differentiate between community-associated and healthcare-associated lineages of methicillin-resistant *Staphylococcus aureus*

F. Tenover, R. Vaughn, L. McDougal, G. Fosheim, J. McGowan Jr. *(Atlanta, US)*

Objective: The ability to type methicillin-resistant *Staphylococcus aureus* (MRSA) strains rapidly in clinical laboratories and differentiate between multi-resistant healthcare-associated (HA) MRSA strains and the more susceptible, but more virulent, community-associated (CA) MRSA strains, could improve public health and infection control efforts to reduce MRSA spread in hospitals and communities, and guide empiric therapy. Published reports suggest that multiple locus variable number tandem repeat assays (MLVA) for *S. aureus* parallel results of pulsed-field gel electrophoresis (PFGE). Our objective was to determine if a simple MLVA protocol, suitable for a clinical laboratory, could differentiate known HA- and CA-MRSA types and replace the more tedious and costly PFGE typing.

Methods: Eighty well-characterized isolates representing the 12 major lineages of *S. aureus* in the US. were selected from the strain collections of the Centers for Disease Control and Prevention (CDC) and Project ICARE. MLVA was performed using a rapid DNA extraction method and a multiplex polymerase chain reaction assay with primers for sdrCDE, clfA, clfB, sspA, and spa, which generated 6–7 bands following agarose gel electrophoresis. PFGE was performed using SmaI as per CDC protocols. Banding patterns were analysed visually (as would be done in a clinical laboratory) and using BioNumerics software. For BioNumerics, Dice coefficients were used with UPGMA clustering algorithms to generate dendrograms.

Results: All isolates were typeable with MLVA, which appeared initially to differentiate among the 12 PFGE lineages. However, when additional isolates were typed, MLVA results placed isolates of PFGE type USA300-0114, the most commonly isolated CA-MRSA in the US., into several unrelated clusters (<70% similarity). MLVA patterns for 2 USA300 (ST8) isolates also clustered with patterns of USA1000 (ST59) isolates. MLVA did not consistently differentiate CA-MRSA lineages (USA300, USA400, USA1000, and USA1100) from HA-MRSA lineages (USA100, USA200, USA500, or USA600).

Conclusions: Although MLVA is feasible for clinical laboratory use, the results do not parallel those of PFGE for the major lineages of CA-MRSA and HA-MRSA in the US. While MLVA may be useful for rapid analysis of isolates from potential outbreaks, it cannot replace PFGE since strains are clustered differently. The epidemiologic and clinical utility of differences between MLVA and PFGE results requires further study.

O173

Molecular typing of *Mycobacterium tuberculosis* by mycobacterial interspersed repetitive unit – variable-number tandem repeat analysis in Lisbon in 2003

E. Fernandes, R. Macedo, C. Furtado, L. Brum, I. Portugal *(Lisbon, PT)*

Objectives: In 2003, the Portuguese Health Authorities reported a tuberculosis (TB) incidence of 41.1/100,000 population nationwide and that 1.7% of tuberculosis cases were multidrug resistant (MDR-TB). Despite the significant decline of TB incidence over the past decades, Portugal remains the country with the highest rate of notified cases due to *Mycobacterium tuberculosis* in Central and Eastern E.U., corresponding to an incidence of about three and a half times more than the E.U. average. Lisbon was the Portuguese districts with the highest incidence, 58 cases per 100,000 inhabitants. Comparing to the standard method IS6110 restriction fragment length polymorphism (RFLP), typing methods based on mycobacterial interspersed repetitive unit - variable-number tandem repeat (MIRU-VNTR) analysis offer an alternative and more accurate method for identifying epidemiological links between patients with tuberculosis The aims of the present study were to determine the genetic diversity of *M. tuberculosis* strains circulating in Lisbon, to evaluate the possible continuous prevalence of previous detected family Lisboa strains, and to evaluate the proportion of recently transmitted disease between patients.
Methods: Four-hundred clinical *M. tuberculosis* isolates collected in several hospital and public health laboratories in Lisbon area in 2003, were characterised at molecular level using MIRU-VNTR typing, including 125 MDR-TB strains and 89 resistant strains other than MDR-TB.
Results: Among the 400 *M. tuberculosis* isolates in Lisbon, we found 124 different MIRU-VNTR patterns. Fifty-three MIRU-VNTR clusters were found in 265 analysed strains, and ranged in size from two to 26 patients. The largest cluster was cluster Lisboa 3, and included 22 MDR-TB strains. We have found another three significant clusters with 18, 12 and 8 isolates, being the first one cluster Lisboa 2, also majority MDR-TB.
Conclusion: Family Lisboa strains are still responsible for the main tuberculosis in Lisbon area, particularly the MDR-TB cases. Although epidemiological investigation is still going on, we were able to establish epidemiologic relationships in a few clustered patients carrying family Lisboa strains. This finding supports the conclusion that these cases resulted from recent transmission. Nevertheless, we did not detect any significant outbreak, as the clustered strains were mostly collected in several hospital units.

O174

Clinical impact of a rapid PCR-based identification of staphylococci directly from positive blood cultures

P. Agmon, I. Oren, R. Finkelstein, H. Sprecher *(Haifa, IL)*

Objective: Staphylococcal bacteremia is common among hospitalized patients and associated with significant morbidity and mortality. Staphylococci are the major gram positive isolates of blood culture in our medical center and 60% of them are caused by Coagulase Negative Staphylococci (CoNS). Isolation of CoNS, unlike bacteremia from Coagulase Positive Staphylococci (CoPS), is often a result of contamination of the blood culture. Rapid and accurate identification of CoPS vs. CoNS, and determination of susceptibility to methicillin is crucial for instituting timely and appropriate therapy. However, standard identification and susceptibility testing requires on average, three days. The aim of the present study was to evaluate a rapid method for identification of Staphylococci directly from blood culture using a multiplex PCR assay.
Methods: A three-month prospective surveillance of patients with Staphylococcal bacteremia was conducted, in which both a standard microbiological approach and a PCR-based assay were comparatively studied. DNA was extracted from positive blood culture bottles using the benzyl alcohol-guanidine hydrochloride method. Four sets of oligonucleotide primers were used: universal bacterial 16S rRNA, staphylococcal specific sequence in the 16S rRNA gene, the CoPS specific nuc gene and the mecA gene which encodes resistance to methicillin.
Results: During the study 330 Staphylococci were isolated from 217 patients. CoNS were isolated in 85% of patients and 53% of CoPS were methicillin-resistant *Staphylococcus Aureus* (MRSA). The sensitivity of the PCR assay as compared with conventional microbiological testing was 98.7 %. The PCR assay shortened the turnaround time of identification and susceptibility results by 20 hours, which translates in practical terms, to one calendar day. The cost of the PCR assay was found to be lower than the cost of the conventional approach. Application of the PCR assay was found to prevent unnecessary therapy in patients from whom CoNS were isolated ($P = 0.0002$) and to correctly direct treatment choice against CoPS ($P = 0.006$).
Conclusions: PCR-based detection of Staphylococci directly from blood culture bottles is rapid (4 h), and highly sensitive (98.7). Implementation of this assay was shown to significantly improve the quality of patient management. Considering the above results, we recommend application of the molecular assay as the standard assay for identification of Staphylococcal bacteremia.

O175

Clinical evaluation of a molecular-based assay for the detection of methicillin-resistant *Staphylococcus aureus* and *Staphylococcus aureus* in positive blood cultures

D. Fuller, T. Davis, M. Parker, R. Buckner, J. Talbott *(Indianapolis, US)*

Objective: *Staphylococcus aureus* (SA) is a major cause of bloodstream infections accounting for up to 20% of isolates found in positive blood cultures. Methicillin-resistant Staphylococcus aureus (MRSA) accounts for nearly 50% of bacteremia caused by SA. Culture techniques for MRSA require a minimum of 48 hours for confirmation. Development of sensitive molecular amplification techniques now allows for detection of only a few copies of bacterial DNA in clinical samples which could affect patient treatment, prevent transmission and control outbreaks.
Methods: The purpose of this study was to evaluate the IDI-MRSA™ and a prototype MRSA/SA assay (GeneOhm Sciences, Inc., San Diego, CA) for direct detection of MRSA and methicillin-sensitive SA (MSSA) in positive blood cultures using real-time PCR technology. The IDI-MRSA assay provides a positive result only when MRSA is present in the sample; the MRSA/SA combines a target specific to MRSA (the SCCmec/orfX junction) and an SA-specific target which provides a separate result for MRSA and MSSA thus allowing the direct detection of MRSA and MSSA (both assays also include an internal control). Aliquots from positive blood cultures were directly processed with the IDI

lysis components and tested with each assay using the Cepheid SmartCycler® instrument.
Results: Of the 323 blood cultures tested to date, 127 were known to be positive for MRSA, 155 were positive for pathogens other than MRSA (including coagulase negative staphylococci and MSSA), and 80 were known negative blood cultures. The IDI-MRSA™ and MRSA/SA assays showed complete agreement with conventional identification and susceptibility results.
Conclusion: The IDI-MRSA™ and the prototype MRSA/SA assays are an excellent alternative for the detection of MRSA and MSSA (only with the combined assay) directly from positive blood cultures. These assays are rapid (2 hours after positive blood culture, accurate (100% sensitivity & specificity), flexible (single sample or batch testing), and adapt easily to existing laboratory workflow.

The pros and cons of combination antimicrobial treatment

S196
Combination therapy for community-acquired pneumonia

A. Torres *(Barcelona, ES)*

Severe community-acquired pneumonia (SCAP) refers to those patients with community-acquired pneumonia who are admitted to an intensive care unit. The frequency of SCAP ranges between 5 to 35% (1,2), which indicates the lack of uniformity used in the criteria for ICU admission.The definition of SCAP is a matter of interest. The most accurate criteria were described by Ewig et al (3,4). The presence of one of two major criteria (mechanical ventilation or septic shock) or two of the three minor criteria (systolic blood pressure ≤ 90, multilobar involvement, PaO2/FIO2 ratio <250) resulted in 78% sensitivity and 94% specificity (3). These criteria compared favourably to other scores such as the PSI or the BTS rules (4,5) and, in summary, is probably the best rule those patients with CAP who will benefit from ICU admission. Mortality of SCAP ranges between 20 to 50% (6). More than 40 prognostic factors are associated with death from CAP (7). They can be divided into basic (before admission), baseline (at admission) and disease progression factors. The most important, to potentially reduce treatment failure or mortality, are the inadequacy of antimicrobial therapy and the delay in appropriate therapy. Two studies (8,9) showed an increased mortality if antibiotic treatment is started 4 or 8 hours after emergency department arrival, respectively. Adequacy of initial antimicrobial treatment refers to the right antimicrobial coverage (including resistances) of causal microorganisms using correct dosages. In 50% of cases the etiology of SCAP is unknown and experts have given recommendations for empirical treatment based in the % of cases in which definite microbial etiology is found.Microbial etiology of SCAP differs according to age (10), presence of pulmonary commorbidities (11), and HIV (12). Geographical and seasonal variables differences have to be taken into account for presence of atypical microorganisms, Legionella, viruses and resistances to antibiotics. These factors have to be considered when prescribing initial antibiotics in SCAP. Nursing-home patients should be treated for nosocomial pneumonia as recently recommended (13). All the guidelines recently published (14, 15, 16) recommend a antibiotic combination for the empirical treatment of SCAP. A betalactam plus a macrolide (mainly clarithromycin or azithromycin) or a respiratory quinolone (mainly levofloxacin or moxifloxacin) are the two options. If Pseudomonas isa consideration (11) a betalactam with antipseudomonal activity plus ciprofloxacin is the best therapeutic option. Some findings suggest that respiratory quinolones could be a better option than macrolides when using combination therapy. Moxifloxacin had slightly better clinical efficacy when compared to a combination of third generation cephalosporin plus minus a macrolide (17) in one study. In a large series of hospitalized CAP in which treatment failure was investigated (18), respiratory quinolones proved to be protective. None of the guidelines mentioned above have recommended monotherapy for the initial treatment of SCAP. One of the main reasons to avoid monotherapy is the lack of extensive experience in SCAP. Another reason the body of evidence that suggests that a combination of antibiotics may improve mortality in bacteremic pneumococcal pneumonia. Two retrospective studies (19, 20) found that combining at least two antibiotics showed a better outcome compared to monotherapy. This finding is difficult to explain but could be due to the potential hidden presence of atypical pneumonia or Legionella associated with *Streptococcus pneumoniae* and secondly to the anti-inflammatory effects of some types of antibiotics such as macrolides (21). Potential pitfalls of these two studies are the lack of control for confounders and their retrospective design. A recent observational and prospective multinational study, Baddour et al (22) proved the beneficial effect of combination therapy compared to monotherapy in bacteremic pneumococcal pneumonia (when septic shock was present). An open randomized clinical trial comparing levofloxacin in monotherapy to a combination of cefotaxime plus ofloxacin was published in 2005 (23). In this study the clinical success was similar in the two arms (79%). The bacteriological response was also similar in both groups. Importantly, patients with septic shock prior to inclusion were excluded. Patients with treatment failure could be included unless one of the antibiotics under study was previously given. The conclusion of the authors is that levofloxacin was at least as effective as the combination in SCAP, unless patients required vasopressors or mechanical ventilation. However, the antibiotic combination chosen by the authors is probably not the best that we currently have to treat SCAP (14,15). Consequently, we cannot say that monotherapy with levofloxacin is as effective as the standard recommended combination. On the other hand patients with septic shock were excluded. Septic shock may be present in up to 30% of patients with SCAP (2). Third, specific criteria of severity were not prospectively used. In fact the inclusion criteria in the trial were ICU admission. These criteria are subjective and depend on each ICU and hospital availabilities and necessities. New trial should use the specific criteria for SCAP described in the literature (3). This will ensure the inclusion f a more heterogeneous population. Finally, 29 out of 139 from the monotherapy treated group had failure at TOC visit. Similar rates were reported with the combination. However, there were 7 cases of insufficient response (7/29; 24%) during treatment in the monotherapy group vs 1 (1/27; 3%) in the combination group. This finding and needs further evaluation. Fogarty et al (25) investigated the safety and the efficacy of levofloxacin compared to ceftriaxone plus erythromycin, followed by amoxiclavulanate plus clarythromycin in the treatment of serious CAP in adults. In this study, the term ''serious'' was not equivalent to ICU-admitted patients. The overall clinical efficacy was similar in

both arms (close to 85%).The key issue in this topic is whether or not antibiotic monotherapy can be used confidently for the treatment of SCAP. This is impossible to answer at the current moment.It is important to avoid combination in such a small and severe group of CAP patients? The answer is no. Using a combination of antibiotics in SCAP may improve treatment failure and mortality without an important impact in resistances. In addition, cost should not be an issue to consider in these patients at all. In my opinion, we have to continue recommending a combination of antibiotics to treat SCAP and to avoid monotherapy, at least in the first 5 days of treatment. New RC trails should be addressed to find the safety and clinical efficacy de-escalation therapy and to determine which is the best antibiotic combination.

References:

1 Alvarez-Lerma F, Torres A. Severe community-acquired pneumonia. Curr Opin Crit Care. 2004 Oct; 10(5):369–74.
2 Ruiz M, Ewig S, Torres A, et al. Severe community-acquired pneumonia. Risk factors and follow-up epidemiology. Am J Respir Crit Care Med. 1999 Sep;160(3):923–9.
3 Ewig S, Ruiz M, Mensa J, et al. Severe community-acquired pneumonia. Assessment of severity criteria. Am J Respir Crit Care Med. 1998 Oct;158(4):1102–8.
4 Ewig S, de Roux A, Bauer T, et al. Validation of predictive rules and indices of severity for community acquired pneumonia. Thorax. 2004 May;59(5):421–7.
5 Angus DC, Marrie TJ, Obrosky DS, et al. Severe community-acquired pneumonia: use of intensive care services and evaluation of American and British Thoracic Society Diagnostic criteria. Am J Respir Crit Care Med. 2002 Sep 1; 166(5): 717–23.
6 de Castro FR, Torres A. Optimizing treatment outcomes in severe community-acquired pneumonia. Am J Respir Med. 2003;2(1):39–54.
7 Ewig S, Schafer H, Torres A. Severity assessment in community-acquired pneumonia. Eur Respir J. 2000 Dec;16(6):1193–201.
8 Halm EA, Fine MJ, Marrie TJ, et al. Time to clinical stability in patients hospitalized with community-acquired pneumonia: implications for practice guidelines. JAMA. 1998 May 13;279(18):1452–7.
9 Houck PM, Bratzler DW, Nsa W, Ma A, Bartlett JG. Timing of antibiotic administration and outcomes for Medicare patients hospitalized with community-acquired pneumonia. Arch Intern Med. 2004 Mar 22;164(6):637–44.
10 Fernandez-Sabe N, Carratala J, Roson B, et al. Community-acquired pneumonia in very elderly patients: causative organisms, clinical characteristics, and outcomes. Medicine (Baltimore). 2003 May;82(3):159–69.
11 Arancibia F, Bauer TT, Ewig S, et al. Community-acquired pneumonia due to gram-negative bacteria and *pseudomonas aeruginosa*: incidence, risk, and prognosis. Arch Intern Med. 2002 Sep 9;162(16):1849–58.
12 Cordero E, Pachon J, Rivero A, et al. Community-acquired bacterial pneumonia in human immunodeficiency virus-infected patients: validation of severity criteria. The Grupo Andaluz para el Estudio de las Enfermedades Infecciosas. Am J Respir Crit Care Med. 2000 Dec;162(6):2063–8.
13 Bonten M, Chastre J, Craig W, et al. Guidelines For The Management of Adults With Hospital-acquired, Ventilator-associated Pneumonia. The American Thoracic Society and The Infectious Diseases Society of America. IN PRESS.
14 Mandell LA, Bartlett JG, Dowell SF, File TM Jr, Musher DM, Whitney C; Infectious Diseases Society of America. Update of practice guidelines for the management of community-acquired pneumonia in immunocompetent adults. Clin Infect Dis. 2003 Dec 1;37(11):1405–33. Epub 2003 Nov 03.
15 Niederman MS, Mandell LA, Anzueto A, et al. American Thoracic Society. Guidelines for the management of adults with community-acquired pneumonia. Diagnosis, assessment of severity, antimicrobial therapy, and prevention. Am J Respir Crit Care Med. 2001 Jun;163(7):1730–54.
16 British Thoracic Society Standards of Care Committee. BTS Guidelines for the Management of Community Acquired Pneumonia in Adults. Thorax. 2001 Dec;56 Suppl 4:IV1–64.
17 Finch R, Schurmann D, Collins O, et al. Randomized controlled trial of sequential intravenous (i.v.) and oral moxifloxacin compared with sequential i.v. and oral co-amoxiclav with or without clarithromycin in patients with community-acquired pneumonia requiring initial parenteral treatment. Antimicrob Agents Chemother. 2002 Jun;46(6):1746–54.
18 Menendez R, Torres A, Zalacain R, et al. Risk factors of treatment failure in community acquired pneumonia: implications for disease outcome. Thorax. 2004 Nov;59(11):960–5.
19 Waterer GW, Somes GW, Wunderink RG. Monotherapy may be suboptimal for severe bacteremic pneumococcal pneumonia. Arch Intern Med. 2001 Aug 13-27;161(15):1837–42.
20 Martinez JA, Horcajada JP, Almela M, et al. Addition of a macrolide to a beta-lactam-based empirical antibiotic regimen is associated with lower in-hospital mortality for patients with bacteremic pneumococcal pneumonia. Clin Infect Dis. 2003 Feb 15;36(4):389–95. Epub 2003 Jan 31.
21 Macrolides as Biologic Response Modifiers: Immunomodulatory and Anti-inflammatory Effects. Chest. 2004 Feb 125; 2 (SUPPL).
22 Baddour LM, Yu VL, Klugman KP, et al. Combination antibiotic therapy lowers mortality among severely ill patients with pneumococcal bacteremia. Am J Respir Crit Care Med. 2004 Aug 15;170(4):440–4. Epub 2004 Jun 07.
24 Leroy O, Saux P, Bédos JP, Caulin E, for the Levofloxacin Study Group. Comparison of levofloxacin and cefotaxime combined with ofloxacin for ICU patients with community-acquired pneumonia who do not require vasopressors.Chest. In press
25 Fogarty C, Siami G, Kohler R, et al. Multicenter, open-label, randomized study to compare the safety and efficacy of levofloxacin versus ceftriaxone sodium and erythromycin followed by clarithromycin and amoxicillin-clavulanate in the treatment of serious community-acquired pneumonia in adults. Clin Infect Dis 2004;38:S16–23.

S197

Combination antifungals: in dubio agite

M. Akova *(Ankara, TR)*

More than 25 years ago combination of amphotericin B (AmB) and 5-fluorocytosine was shown to be more effective than AmB alone in the treatment of cryptococcal meningitis. Since then, the accumulation of evidence in using antifungal combinations for other invasive fungal infections has been slow. This was initially due to the controversy related to *in vitro* antagonism between azoles and polyenes and the lesser frequency of invasive fungal infections which were, then, treatable with early azoles such as fluconazole in case of candidiasis and amphotericin B that sufficiently provided coverage for most of mold infections. However, the clinical picture has significantly changed during the last two decades. First, the incidence and of invasive fungal infections has sharply increased and their epidemiology changed. Secondly, newer, broader-spectrum antifungals with previously non-existent mechanisms of effect have become commercially available. Disseminated infections caused by non-Candidal yeasts and not only *Aspergillus* spp., but also

previously rarely seen non-*Aspergillus* mold infections have emerged as significant causes of morbidity and mortality among immunosuppressed patients. Since these infections are usually difficult-to-treat and related mortality in infected patients remains to be very high, especially in those who are persistently immunosuppressed, the clinicians are tempted to use various newer antifungals with novel mechanism of action in combination in different clinical settings, especially in invasive aspergillosis. The rationale behind this rekindled interest is to achieve synergy and to a lesser extend, to reduce antifungal resistance. Although, there has been no controlled clinical trials comparing antifungal combinations with monotherapy so far, evidence gathered from small series and anectodal experience clearly indicates that antifungal combination therapy has become a clinical reality, particularly in those patients with severe immunosuppression and disseminated mould infections. The available data from above mentioned observations also pointed out that combinations of newly approved imidazoles (e.g. voriconazole) and echinocandins (e.g. caspofungin) with other agents are very unlikely to be antagonistic. Future studies are definitely required whether combination therapy with these new agents would improve survival and treatment outcome in the seriously hampered patients with life threatening fungal infections.

Emerging viral exotica in Europe (Symposium jointly arranged with the ISID)

S200

Rabies and other lyssaviruses in Europe 2006

M.J. Warrell *(Oxford, UK)*

Rabies and European bat lyssaviruses are enzootic in European mammals. 13 people died of rabies in Europe in 2004. Ideal rabies prophylaxis (pre-exposure immunisation with post-exposure boosting) is 100% effective, so every human death must be regarded as a preventable tragedy. Rabies was diagnosed in 5393 European domestic and wild animals in 2004. Although indigenous rabies infection has been virtually eliminated from terrestrial animals in Western Europe, it will be impossible to control European bat lyssavirus infection, which is widespread across the continent. 3.5%, of a total of 1305 bats tested, proved rabid, but 60% of European countries did not report having tested any bats at all in 2004. In Europe the risk of human infection will therefore persist from two possible sources: indigenous species and imported animals, as shown on 3 occasions in France in 2004. The publicity from one case revealed >300 other illegally-imported cats and dogs. A far greater risk is to travellers to countries where dog rabies is endemic. Unrecognised rabies encephalitis in a woman who had returned from India resulted in the deaths of 3 organ transplant recipients in Germany in 2005.The global incidence of human rabies is unknown. Recent WHO surveillance estimates a mortality rate between 24,000 and 93,000 deaths annually in Asia and Africa. The means are now available to eliminate domestic dog rabies. If this were achieved, >99% of all human disease would be prevented and the need for expensive vaccines and rabies immunoglobulin (RIG) drastically reduced. Meanwhile rabies deaths occur due to ignorance, inaccessibility or unaffordability of specific treatment, or occasionally to other diseases inhibiting the immune response. Primary post-exposure treatment is very effective if wound cleaning, RIG and vaccine are given correctly on the day of the exposure, but this cannot be guaranteed worldwide. Now that there is a global shortage of RIG, it is increasingly important, to encourage the use of pre-exposure vaccine treatment, which avoids the need for passive immunisation should exposure occur. The often prohibitive cost of prophylaxis for travellers and residents of dog rabies endemic areas can be reduced by immunising a group with 0.1 ml vaccine intradermally. Rabies in man remains virtually always fatal, although in 2004 in Wisconsin, intensive care and antiviral therapy has contributed to a remarkable recovery in a girl who had not had any previous vaccine treatment.

S201

Toscana virus: emergence in Southern Europe

R.N. Charrel *(Marseille, FR)*

Toscana virus (TOSV) is an arthropodborne virus first identified in 1971 from the sandfly *Phlebotomus perniciosus* in central Italy. Many case reports in travellers and clinical research and epidemiologic studies conducted around the Mediterranean region have shown that TOSV has a tropism for the central nervous system (CNS) and is a major cause of meningitis and encephalitis in countries in which it circulates. In central Italy, TOSV is the most frequent cause of meningitis from May to October, far exceeding enteroviruses. In other northern Mediterranean countries, TOSV is among the 3 most prevalent viruses associated with meningitis during the warm seasons. Therefore, TOSV must be considered an emerging pathogen. Here, we review the epidemiology of TOSV in Europe and determine questions that should be addressed in future studies. Despite increasing evidence of its major role in medicine as an emerging cause of CNS infections, TOSV remains an unstudied pathogen, and few physicians are aware of its potential to cause CNS infections

S202

Viral haemorrhagic fever: diagnosis and management of highly contagious diseases

C. Hatz *(Basel, CH)*

Four families of viruses are known to cause haemorrhagic fevers in man. Most of them are zoonoses. People get infected while undertaking activities in remote areas where the animal hosts live. In general, tourists are unlikely to become infected with viruses causing haemorrhagic fevers. Awareness of the problem in endemic areas and simple precautions such as avoiding trips to remote areas and to places where epidemics are ongoing, protection against mosquito bites, and avoiding contact with dead animals, further reduce the risk. A vaccination against yellow fever is available. If the suspicion of a VHF is raised, immediate action must be taken. A practical approach includes consulting a specialist in tropical or infectious diseases, an admission to a university hospital, and an immediate notification to the health authorities.

2006 *Clinical Microbiology and Infection*, Volume 12, Supplement 4
ISSN: 1470-9465

S208

Connexion between efflux pump expression and virulence in *Pseudomonas aeruginosa*

J.F. Linares, P. Sanchez, J.L. Martinez *(Madrid, ES; San Antonio, US)*

The ubiquity of multidrug (MDR) pumps supports the idea that their original function is not just antibiotic resistance (AR). In fact, the same MDR pumps are find in *P. aeruginosa* isolates from clinical and non-clinical environments (1). It was shown that MDR pumps, besides contributing to antibiotic resistance, play a role modulating bacterial virulence. *P. aeruginosa* MDR pumps contribute to intrinsic and acquired AR. Since some *P. aeruginosa* MDR pumps extrude quorum sensing (QS) autoinducers, and QS response has a prominent role in *P. aeruginosa* virulence, MDR pumps-overexpressing (multiresistant) bacteria might be less virulent than wild-type ones. It has been established that *P. aeruginosa* mutants overproducing MexAB-OprM or MexCD-OprJ produce less amounts of QS-regulated virulence factors and have reduced virulence (2–4). But the scenario is more complex. Overexpression of either MexCD-OprJ or MexEF-OprN reduces *P. aeruginosa* cytotoxicity and challenges Type III secretion (T3S) via a QS-independent way (5). If MDR bacteria are less virulent than wild-type ones, why they can be selected during infection? Two possibilities are possible: (1) Under antibiotic treatment, resistance can be considered a colonization factor (6). (2) Antibiotic resistance might make bacteria more proficient for producing a specific type of infection. It was found that neither MexAB-OprM nor MexCD-OprJ over expression reduced biofilm formation (2). *P. aeruginosa* is the major cause of chronic infection in cystic fibrosis patients, and biofilm is very important for persistent infections. Epidemiological data have shown that, during chronic infection, *P. aeruginosa* evolves to produce less amounts of QS-regulated virulence factors and tends to be defective in T3S, maintaining biofilm production. Thus, the virulence factors required for either acute or chronic infection might be different. Since overproduction of *P. aeruginosa* MDR pumps is associated to the phenotype of chronic infections' isolates, MDR pumps-overproducing *P. aeruginosa* strains might be impaired for developing acute infections, but not for their maintenance during chronicity.

1. A. Alonso, F. Rojo, J. L. Martinez, Environ Microbiol 1, 421
2. P. Sanchez et al., J Antimicrob Chemother 50, 657
3. B. Ruiz-Diez, P. Sanchez, F. Baquero, J. L. Martinez, A. Navas, J Theor Biol 225, 469
4. P. Cosson et al., J Bacteriol 184, 3027
5. J. F. Linares et al., J Bacteriol 187, 1384
6. J. L. Martinez, F. Baquero, Clin Microbiol Rev 15, 647

S209

Quinolone resistance and virulence in uropathogenic *Escherichia coli*

J. Vila *(Barcelona, ES)*

Escherichia coli are the most common microorganisms causing urinary tract infections. Uropathogenic *E. coli* (UPEC) strains possess several virulence determinants that allow them to colonize the urinary tract, avoid host defenses, and cause damage to the uroepithelium. Biofilm formation can also be considered as a virulence determinant. Quinolone-resistant *E. coli* strains have fewer virulence factors than quinolone-susceptible strains. Several urovirulence genes are located in pathogenicity islands (PAIs). We investigated the capacity of quinolones to induce loss of virulence factors, such as hemolysin, cytotoxic necrotizing factor-1, P-fimbriae and autotransporter Sat, included in PAIs in three uropathogenic *E.coli* strains, as well as the relationship between quinolone-resistance acquisition and biofilm formation. Three quinolone-susceptible uropathogenic E.coli clinical isolates belonging to phylotype B2 were chosen. In a multistep selection all three strains lost hemolytic capacity between 1–4 passages when incubated with subinhibitory concentrations of ciprofloxacin, showing a partial or total loss of the PAI containing the hly (hemolysin) and cnf-1 (cytotoxic necrotizing factor-1) genes. RecA- mutants were obtained from the two *E. coli* strains with partial or total loss of the PAI. The inactivation of the RecA protein only affected the partial loss of PAI induced by quinolones. No spontaneous loss of PAIs was observed on incubation in the absence of quinolone in either the wild-type or mutant *E.coli* strains. Growth of biofilm-producing *E. coli* in the presence of salycilate inhibits the productions of biofilm. A quinolone-resistant UPEC mutant was obtained from a biofilm-producing UPEC strain. The resistant mutant did not produce biofilm, had a mutation in the amino acid codon Ser-83 of the gyrA gene and over expression of an efflux pump. The complementation of this mutant strain with a wild-type GyrA did not revert the production of biofilm suggesting that a mechanism of quinolone resistance rather than mutation in the gyrA gene could be involved in the decrease formation of biofilm. In fact, the quinolone-resistant, non-producing biofilm UPEC mutant showed a decrease in the expression of both fimA and papA genes. In conclusion, quinolones induce partial or total loss of PAIs *in vitro* in uropathogenic *E. coli* by SOS dependent or independent pathways, respectively. Moroever the acquisition of quinolone resistance may decrease the production of biofilm in UPEC.

S210

Efflux pump expression and bacterial survival in *Neisseria gonorrhoeae*

W.M. Shafer *(Atlanta, US)*

Neisseria gonorrhoeae is a strict human pathogen that causes the sexually transmitted disease gonorrhea. Over 62 million cases of gonorrhea occur each year worldwide. While gonorrhea remains treatable with antibiotics, the recommended treatment regimens are expensive compared to the relatively cheap cost of penicillin, which was previously (prior to 1985) the drug of choice. Efflux pumps possessed by *N. gonorrhoeae* contributed to the development of resistance to certain antibiotics, such as penicillin. Efflux pumps were expressed by bacteria, including *N. gonorrhoeae*, long before antibiotics were used to treat bacterial infections. For those bacteria capable of existing outside of a host for extended periods of time, efflux pumps likely provided them with an ability to resist antimicrobials in the soil or water. Why would those bacteria that typically must exist within another living system possess efflux pumps? The answer is that these bacteria must have a rapid means of dealing with host-derived antimicrobials that are either naturally present on surfaces or become available in bodily fluids or they will perish. Efflux pumps have the ability to recognize structurally diverse antimicrobials, which serve as ''host antibiotics'' and are on the frontline of the innate immune response. A specific example of the action of efflux pumps in recognizing and exporting these antimicrobials is that of the MtrC-MtrD-MtrE efflux pump of *N. gonorrhoeae*, which recognizes antibiotics (macrolides and certain beta-lactams) and host antimicrobials such as antibacterial peptides produced by phagocytic and epithelial cells. This pump has been shown to be essential for gonococcal survival on the vaginal mucosal surface in an experimental murine infection

model. The expression of genes encoding efflux pumps is regulated at the transcriptional level by both repressors and activators. In *N. gonorrhoeae*, these DNA-binding proteins also regulate other bacterial genes involved in virulence or bacterial physiology. Thus, it is likely that efflux pumps are integrated into the overall processes of bacterial survival.

Diagnostic and laboratory methods

O211

Impact on mortality and cost-effectiveness of rapid bacterial identification and antimicrobial susceptibility testing in comparison with standard methods

J.J. Kerremans, P. Verboom, W.H.F. Goessens, L. Hakkaart-van Roijen, T. Stijnen, H.A. Verbrugh, M.A. Vos *(Rotterdam, NL)*

Objectives: Rapid and correct identification and susceptibility testing of bacteria is valuable for good patient care. Rapid identification and susceptibility results are shown to be cost-effective and did reduce mortality in the United States. In this study, we examined whether accelerated bacterial diagnostics were cost-effective and if this resulted in a significant difference in mortality in a Dutch setting.
Methods: Inpatients of the Erasmus MC, Rotterdam, the Netherlands, were randomly assigned to either the intervention arm or the control arm, when they had a positive culture from a normally sterile body fluid (excluding urine samples). The intervention was by using a fast automated method for bacterial identification and antimicrobial susceptibility testing (the VITEK 2 system, bioMérieux, Marcy-l'Étoile, France) combined with direct inoculation of blood cultures. In the control arm our standard method was used: the Vitek 1(bioMérieux, Marcy-l'Étoile, France) system inoculated from subculture plates. Patients were prospectively followed up for 4 weeks after inclusion for mortality data and costs of health care. Total costs of hospitalization were calculated by taking true cost if possible, otherwise charges were used as defined by the National Health Tariffs Authority (CTG).
Results: 1465 patients were included and randomized: 730 in the intervention arm, 735 in the control arm. In both arms 70% of patients were included based on a positive blood culture result, the remaining 30% were included based on a positive cultures from an other body fluid. These cultures contained Gram-negative rods in 37% of all cases, coagulase-negative staphylococci in 27%, *S.aureus* in 14% and other organisms in 22%.The difference in time between the two arms from randomization until a result of the laboratory was available, averaged 22 hours for susceptibility results and averaged 13 hours for identification ($P < 0.0001$).The treatment-costs of a patient in the intervention arm were on average € 8.831,-, the costs in the control arm were on average € 8.641,-(difference not significant). The number of deaths in the intervention arm was 117 out of 730 patients (16%), in the control arm 99 out of 735 patients (13,5%) (difference not significant)
Conclusion: In our hospital, rapid bacterial identification and antimicrobial susceptibility testing does not lead to a significant difference in mortality and costs.

O212

Comparison of NucliSens easyMAG, NucliSens miniMAG and Qiagen nucleic acid extraction systems using throat swabs for the detection of *M. pneumoniae* and *C. pneumoniae*

K. Loens, K. Bergs, D. Ursi, H. Goossens, M. Ieven *(Edegem, BE)*

Objectives: To evaluate the performance of the NucliSens easyMAG NA extraction system on throat swabs with the Qiagen extraction and the miniMAG as references for subsequent DNA and RNA amplification, respectively.
Materials and methods: 215 throat swabs from patients with community-acquired pneumonia were included in the study: On arrival in the lab, samples were aliquoted and nucleic acids (NAs) were extracted using the Qiagen blood mini kit. NAs extracts were analysed by real-time PCR for the detection of *M. pneumoniae* DNA and *C. pneumoniae* DNA. NAs were retrospectively extracted from other aliquots of the same specimens using the NucliSens miniMAG and the NucliSens easyMAG off-board protocol and subsequently subjected to real-time PCR and real-time NASBA for the detection of DNA and RNA of both organisms, respectively.
Results: The real-time PCR detected *M. pneumoniae* and *C. pneumoniae* in 10 and 5 throat swabs after Qiagen extraction and in 9 and 5 swabs after easyMAG extraction. Real-time NASBA detected *M. pneumoniae* and *C. pneumoniae* in 9 and 5 throat swabs after both miniMAG and easyMAG extraction. For the detection of the 9 *M. pneumoniae* positive specimens the difference between the Lightcycler Ct values obtained after the NucliSens easyMAG extraction and Qiagen extraction were respectively -3.88, −0.6, −5.77, −2.83, −1.2, −0.46, −2.47, 1.9, and −2.49. For the detection of 5 *C. pneumoniae* positive specimens the Ct values obtained for the NucliSens easyMAG were respectively −2.78, −2.32, −3.14, −2.02, and −6.62 as compared to results obtained after Qiagen extraction. Similar results were obtained after real-time NASBA amplification for both organisms. The number of invalid results in negative specimens, due to inhibition, obtained after real-time NASBA decreased from 3.2% to 0.7% when using the miniMAG and easyMAG, respectively. Furthermore, the same NAs extracts could be used for both RNA and DNA amplification.
Conclusion: In this study the NucliSens easyMAG extracted more efficiently the RNA and DNA of the clinical samples (higher recovery and/or less inhibitors) by showing on average lower ct values in the Lightcycler real-time PCR assays and less invalid results in the real-time NASBA assays. The instrument features user-friendly software, intuitive software, and delivers high throughput capabilities with 40 minutes turn-around-times.

O213

Intelligent sample preparation to improve nucleic acid-based pathogen detection

S. Sachse, K.H. Schmidt, M. Lehmann, S. Russwurm, E. Straube *(Jena, DE)*

Objectives: Culture independent pathogen detection methods become increasingly interesting due to the limitations of the blood culture technique, e.g. negative results after antibiotic pre-treatment, non-cultivable micro organisms and time-to-result delays of more than 4 days. However, existing nucleic acid-based detection (NA) methods, e.g. PCR, have some serious drawbacks if applied to clinical samples like whole blood. However, their sensitivity is not adequate compared to the gold standard blood culture technique. The reduced sensitivity of NA methods for bacterial detection is due to very low amounts of bacterial DNA compared to the human DNA background.

Therefore, an improvement of NA methods can be achieved by an innovative sample preparation procedure, which reduce the human DNA background significantly and enrich the bacterial DNA simultaneously. Here, a sample preparation method is shown, which overcomes the limitations of culture independent NA systems.
Methods: A recombinant protein with specific binding affinity for prokaryotic DNA is the keystone of our innovative and easy-to-use sample preparation method. To validate the properties of this protein, we performed classical gel retardation, radioactive experiment, classical as well as real time PCR experiments using artificial DNA mixtures containing pro- and eukaryotic DNA as well as spiked blood samples.
Results: Our experiments showed that prokaryotic DNA is specifically retained by the described protein. The protein can be immobilised to matrices and transferred to spin columns for enrichment experiments. The DNA preparation procedure using such columns enables an enrichment of prokaryotic DNA in mixtures containing both pro- and eukaryotic DNA. Furthermore, by means of radioactive and real time PCR experiments, it could be shown that the application of this procedure decreases the background of human DNA significantly (up to 90%), leading to an increased sensitivity of the PCR methods for bacterial detection in blood samples.
Conclusion: The proposed unique pre-analytic method improves the sensitivity of PCR or other NA methods for the detection of small amounts of bacterial DNA in complex samples (e.g. human whole blood), by a significant reduction of the eukaryotic DNA background and enrichment of bacterial DNA simultaneously. This might be a milestone allowing ''same day diagnosis'' of sepsis and SIRS as well.

O214

Point-of-care detection of group B Streptococci in labouring women: a new approach to preventing early onset neonatal GBS disease

E.H. Smith, P. Milner, J. Gray, L. Spicer *(Birmingham, UK)*

Objectives: GBS is the main cause of serious early-onset neonatal sepsis in industrialised countries. Intrapartum antibiotic prophylaxis (IAP) can prevent up to 80% of infections. Currently decisions about which women receive IAP are based on either risk factors present during labour, or cultures of vaginal and rectal swabs at 36 weeks gestation. Neither approach allows optimal targeting of IAP. The aims of this project were to determine the accuracy of realtime PCR (Smartcycler, Cepheid) and optical immunoassay (OIA, Thermobiostar) and their feasibility as point of care tests (POCT) on labouring women.
Methods: Healthcare Assistants with no laboratory background were trained to undertake both tests within a competency assessment framework. Test accuracy was determined using enrichment culture as the gold standard. Neonatal ear swabs were cultured for GBS to investigate the relationship between maternal results and neonatal colonisation.
Results: All staff achieved competency in undertaking both tests. However the high turnover of staff of this level created an ongoing training need. Training times for the OIA and PCR were 2 and 7 hours. Minimum hands-on testing time and results turnaround time for the OIA were 20 and 35 min, and 40 and 90 min for the PCR. The greater training need and longer hands-on test time for PCR significantly increased the resource required to provide reliable testing 24/7.The results of tests on vaginal and rectal swabs from 226 women were as follows: 2/14 babies colonised with GBS were born to mothers with only rectal colonisation.

Test	No. (%) swabs +ve for GBS:	
	Vaginal	Rectal
Culture	35 (15.5)	41 (19.5)
PCR	32 (14.2)	44 (20.0)
OIA	15 (6.7)	Not tested

Conclusions: Our early data indicate that PCR can accurately detect GBS carriers in labour. PCR is feasible as a POCT, but considerable staff time would be required to maintain a robust clinical service with the SmartCycler platform. This drawback should be overcome by the new generation of fully integrated PCR systems (e.g. GeneXpert) which, by almost eliminating manual specimen preparation, will reduce training and hands-on test times. Evidence that vertical transmission can occur without detectable vaginal colonisation suggests that an intrapartum screening programme might require testing of rectal and well as vaginal swabs, although whether rectal colonisation alone is risk factor for GBS disease rather than colonisation remains to be determined. Work funded by the NHS HTA Programme (02/38/04).

O215

Sepsis diagnosis by real-time PCR (SeptiFast Kit, Roche Diagnostics): preliminary results and possible application

A. Raglio, M. Rizzi, M. Amer, M. Mangia, M.G. Lucà, A. Goglio *(Bergamo, IT)*

Introduction: Traditional blood cultures (BC) have well known limits: time to first results (48 hours) and sensitivity (only 15–25%). New methods are needed and molecular biology seems to offer interesting opportunities. Our evaluation was a part of an european multicenter study.
Objectives: The aim of the study was to evaluate a new PCR based molecular assay (25 pathogens detected in six hours) and its clinical impact in comparison with BC results.
Methods: BC were performed by BactAlert System (BioMerieux) and incubated for 7 days. The SeptiFast test was performed on LightCycler 2.0 (Roche Diagnostics). The principle of the method will be explained. PCR samples were collected after BC from patients fulfilling the SIRS criteria. For each patient a data set was collected: clinical and laboratory data, microbiological results from all specimens. The results were finally discussed by a group of 4 clinicians.
Results: A total of 114 samples were collected from 74 patients. 73 samples were BC and PCR negative. Positive rate for BC was 11.4% and for PCR 27.2%. 13 samples were BC and PCR positive. 4 were BC positive for coagulase-negative staphylococci (CNS) but PCR negative. 24 were BC negative but PCR positive: 6 of them detected only CNS; 11 were of clinical importance. PCR was able to detect: a) *Streptococcus* viridans group in a patient with endocarditis; b) *Staphylococcus aureus* in a patient with pancreatitis; and c) *S. aureus* in a patient with spondylodiscitis.The PCR results were available after 16–30 hours, the biopsy and valve culture results were reported after 5–7 days. In 7 samples PCR detected a pathogen (*Aspergillus fumigatus, Stenotrophomonas maltophilia, Escherichia coli, S. aureus, Enterococcus faecalis*) but there were no additional data that confirmed these results.
Conclusion: In our study PCR detect all BC positive samples and it was the best test able to help with the right diagnosis for 3 patients. PCR requires a DNA-free workflow and well-trained workers, otherwise results could be misleading by the detection of environmental DNA.PCR is faster and more sensitive than

BC. Further studies will show the best use of this interesting new method.

O216

Evaluation of four selective media for the detection of methicillin-resistant *Staphylococcus aureus* from surveillance specimens

B.M. Willey, N. Kreiswirth, P. Akhavan, A. Tyler, S. Malek, V. Pong-Porter, G. Small, N. Nelson, A. McGeer, S.M. Poutanen, T. Mazzulli, D.E. Low, M. Skulnick *(Toronto, CA)*

Objectives: Rapid, cost effective and practical detection methods from surveillance specimens are integral to continued MRSA control. This blinded study compared a standard-subculture protocol using Oxoid Mannitol Salt Cefoxitin [MSF] agar to direct identification of MRSA from Oxoid Modified-MSF [MOD] agar, Bio-Rad MRSASelect [BR] chromogenic agar, and Becton-Dickinson CHROMagar MRSA [BD].
Methods: 2500 consecutive swabs (178 wound, 777 rectal, 823 nasals, 713 pooled nasal-axilla-groin-perineum, and 9 other) from 1271 patients in 13 facilities were inoculated onto each medium. MRSA yields, relative workloads and costs were determined at 24 h and 48 h incubation at 37 C. MRSA were confirmed using Pastorex Staph Plus (Bio-Rad), tube coagulase, PBP2a agglutination (Denka Seiken) and CLSI oxacillin screen plate (performed directly from the primary media when possible).
Results: 147 of the 2500 specimens (5.8%) grew MRSA from at least one medium. Using this as the gold standard, each medium was compared in terms of 24 h/48 h sensitivity, specificity, positive and negative predictive value, % reported, and material and labour costs (expressed as % increase or decrease compared to the MSF protocol).

Medium	SENS	SPEC	PPV	NPV	Reported	Materials	Labour
BR	91/91	98/48	73/10	99/99	74/91	0/+59	-64/+24
MOD	69/86	90/77	30/19	98/99	58/80	-33/-14	-51/-36
BD	67/81	97/81	60/21	98/99	31/68	+12/+17	-68/-52
MSF*	52/82	91/68	27/14	97/98	0/67	NA	NA

*Standard-subculture protocol: 18 h and 48 h reads only (no 24 h read) and no direct testing from primary medium

Conclusions: The BR read at 24 h performed significantly better ($p < 0.00001$) than all other media with no added cost; direct testing reduced time to reporting for positive cultures. However, when incubation exceeded 24 h, BR specificity was highly compromised. The MOD, read at 48 h, performed the second best overall and was significantly ($p < 0.00001$) more specific than MSF. The BD read at 48 h was comparable to the MOD but was limited by mixed growth preventing direct testing thus increasing turn-around-time.

O217

Diagnostic value of procalcitonin to distinguish monomicrobial blood stream infection with coagulase-negative staphylococci from contamination: a pilot study

P. Schuetz, B. Mueller, A. Trampuz *(Basel, CH)*

Objective: Growth of coagulase-negative staphylococci (CNS) in blood cultures does not always indicate a bloodstream infection (BSI). Serum procalcitonin (ProCT) has been shown a useful marker for bacterial infections and for antimicrobial guidance. Therefore, we evaluated the diagnostic value of ProCT to distinguish BSI from contamination with CNS.
Methods: From April through August 2005 we prospectively evaluated all patients with growth of CNS in their blood cultures. Patients with bacteremia caused by additional microorganisms (polymicrobial infections) were excluded. An infectious disease specialist classified the patients as having BSI or contamination according to standard clinical and laboratory criteria and blinded to ProCT results. Serum ProCT was measured one day before (day −1), at the day of blood culture collection (day 0), and on the following day (day +1) using a ultrasensitive immuno-luminometric (functional assay sensitivity < 0.05 ng/ml). Values were compared using the Mann Whitney U test. A receiver operating characteristic (ROC) curve analysis was performed.
Results: Of the 40 patients, 21 were excluded due to polymicrobial infection. From the remaining patients, 7 had monomicrobial BSI and 12 had contamination with CNS (Table 1).

Table. Median (range) serum ProCT concentrations (in ng/dl) in 19 patients with BSI and contamination with CNS.

	Day -1	Day 0	Day +1
BSI (n = 7)	1.12 (0.06 – 5.27)	1.27 (0.14 – 5.20)	1.02 (0.14 – 2.70)
Contamination (n = 12)	0.09 (0.02 – 0.18)	0.08 (0.01 – 0.23)	0.08 (0.01 – 0.20)
	P = 0.02	P <0.001	P <0.002

Median (range) serum ProCT concentrations (ng/dl) were significantly higher in patients with BSI compared to those with contamination at all three time points. In contrast, CRP values and leukocyte counts were only significantly discriminative at day +1. A ProCT cut-off of 0.1 ng/dl showed a sensitivity of 86%, 100% and 100% and specificity of 60%, 84% and 80% at day −1, day 0 and day +1, respectively.
Conclusion: In this pilot study, ProCT was an early, accurate biomarker to distinguish BSI from contamination with CNS. If confirmed in a larger trial, ProCT may prevent unnecessary treatment courses for suspected CNS BSI and guide rapid treatment for those, not yet meeting all criteria for CNS BSI.

O218

Borrelia burgdorferi serodiagnosis: evaluation of a novel Luminex bead-based recombinant-antigen assay

D. Burki, S. Reymann, M. Toelge, J. Schipper, L. Matter *(Basel, CH; Neuried, DE)*

Introduction: Current screening for *B. burgdorferi*-infections relies on a two-tier strategy combining sensitive EIA screening with specific immunoblot confirmation.
Objectives: To compare the performance of a single step bead-based system with the conventional two-tier approach.
Materials and methods: Unselected sera which were sent to a private commercial laboratory for primary serological screening were tested for IgG and IgM by recombinant antigen EIA; reactive samples were confirmed by recombinant immunoblot (recomWell and recomBlot Borrelia, Mikrogen, D-Neuried). Sera were considered positive if reactive for at least one specific plus one non-specific band and indeterminate if reactive for just one specific band. Following storage at −20°C samples were batch-analysed by Luminex-bead-based assays (recomBead, Microbionix/Mikrogen). For IgG, seven antigens (p100, VlsE, p39, OspC from three strains, and p18) were tested, for IgM, three OspC-antigens from three strains were tested. An internal standard curve allowed for normalization of individual immunoglobulin concentrations. For IgG, samples were considered positive if positive for at least two bead-regions and indeterminate if positive for one bead-region.For both

systems, positive interpretation was based on presence of IgG (± IgM) and negative interpretation was based on absence of both IgG and IgM. Other situations were considered indeterminate.
Results: 667 sera were analysed. Agreeing interpretations for conventional and Luminex testing were found for 514 (77.1%) samples: 65 positive, 104 indeterminate, 345 negative.3 sera were positive in conventional and negative in Luminex testing, 9 were negative in conventional and positive in Luminex testing.56 were indeterminate in conventional and negative in Luminex testing. 61 were negative in conventional and indeterminate in Luminex testing. Positive vs. indeterminate discrepancies were considered minor and found in 24 sera (3.6%).

		Luminex		
		positive	indeterminate	negative
conventional	positive	65	7	3
	inde-terminate	17	104	56
	negative	9	61	345

Conclusions: Overall, good qualitative agreement was observed. Serious discrepancies, i.e. leading to potentially false negative reports, were found in 17.5%. False-negative conventional testing may be due to the introduction of VlsE in the Luminex assay. Potentially false-positive conventional testing may be attributed to improved IgM-specificity in the Luminex assay. We conclude that single-step Luminex testing may be suitable to replace conventional two-tier strategies.

O219

Development of a panfungal real-time PCR

H.P. Hinrikson, K. Jaton, D. Ciardo, M. Altwegg, J. Bille, P.M. Hauser *(Lausanne, Zurich, Lucerne, CH)*

Objectives: To develop and evaluate a panfungal real-time PCR for early detection and presumptive identification of pathogenic fungi directly in clinical specimens.
Methods: Based on a comprehensive sequence alignment, we designed a panfungal PCR targeting the variable region 5 of the small-subunit (18S) ribosomal gene. The assay consisted of 4 forward primers, 8 reverse primers and 1 fluorescence-labeled probe to ensure efficient detection of ascomycetes, basidiomycetes and zygomycetes. The sensitivity was assessed by comparison to a quantitative Aspergillus fumigatus-specific PCR. The specificity was tested using reference DNAs of fungal, bacterial and human origins. A retrospective clinical evaluation was conducted using DNA extracts from clinical specimens of both patients with proven invasive fungal infections [N = 15] and negative controls [N = 18]. The identity of amplicons was determined by comparative sequence analysis.
Results: The analytical sensitivity of the panfungal PCR was found comparable to that of the A. fumigatus-specific approach, i.e. one target copy per reaction. However, in order to avoid false positive reactions due to minute fungal contaminations introduced occasionally while sampling or processing specimens, the diagnostic detection limit was considered 5 target copies per reaction. The specificity for fungal DNA was confirmed by amplification of all fungal reference DNAs investigated and by the absence of positive reactions with reference DNAs of bacterial and human origins. The clinical sensitivity and specificity were 87 and 100%, respectively, when considering a particular specimen as PCR positive if it exhibited at least 2 positive reactions per triplicate testing. False negative results were obtained with 2 specimens previously shown to contain only trace amounts of target DNA. Sequencing of the clinical amplicons allowed presumptive identification of yeasts (*Candida* [N = 5], *Cryptococcus* [N = 1]), molds (*Absidia* [N = 1], *Aspergillus* [N = 2], *Fusarium* [N = 1]), *Rhizomucor* [N = 2] and *Pneumocystis* [N = 1] to at least the species group level. For instance, the usually more susceptible Candida species (e.g. *C. albicans*, *C. parapsilosis*, and *C. tropicalis*) were clearly distinguished from the usually less susceptible species such as *C. glabrata* and *C. krusei*.
Conclusion: The present panfungal real-time PCR represents a promising tool for improved diagnosis and empirical treatment of invasive fungal infections thus warranting further clinical validation.

Pneumonia and Pneumococci

O220

The International Circumpolar Surveillance System for population-based surveillance of invasive Pneumococcal disease, 1999–2004

M. Bruce, T. Cottle, S. Deeks, M. Lovgren, L. Jette, T. Hennessy, D. Parks, K. Kristinsson, K. Brinklov Jensen, O. Lovoll, P. Nuorti, A. Nystedt, E. Herva, A. Koch, A. Parkinson *(Anchorage, US; Ottawa, Edmonton, Montreal, CA; Reykjavik, IS; Nuuk, GL; Oslo, NO; Helsinki, FI; Lulea, SE; Oulu, FI; Copenhagen, DK)*

Background: The International Circumpolar Surveillance (ICS) Project is a population-based surveillance network for invasive bacterial disease in the US Arctic, Alaska (AK), Northern Canada (N Can), Greenland (GN), Iceland (IC), Norway (Nor), Northern Sweden (N Sweden), and Finland (Fin). Among circumpolar countries, the 7-valent conjugate vaccine (pcv7) has been used for routine infant immunization in AK since 2001 and in selected areas in N Can since 2002.
Methods: We defined a case of invasive pneumococcal disease (IPD) as illness in a surveillance area resident with isolation of *Streptococcus pneumoniae* from a normally-sterile site. We analysed data on IPD from AK and N Can (Jan 1999–Dec 2004), and from GN, IC, Nor, Fin (Jan 2000–Dec 2004) and N Sweden (2003–2004) to determine: 1) Common clinical syndromes, 2) Rates of disease by country, 3) Serotype distribution and 4) Antimicrobial susceptibility patterns.
Results: A total of 9,251 cases of laboratory-confirmed IPD were reported from AK (647), N Can (226), GN (51), IC (236), Nor (4,712), N Sweden (65) and Fin (3,314). Case-fatality ratios varied from 5.0–27.0%. Pneumonia (46%), septicemia (26%), and

meningitis (8%) were the most common clinical presentations. Rates of IPD in aboriginals in AK and N Can were 43 and 38 cases per 100,000 persons, respectively. Rates of IPD in children < 2 years of age and persons > 2 years of age ranged from 21–137 and 9–24 cases per 100,000 persons, respectively. In AK, the rate of IPD in children < 2 with pcv7 serotypes declined by >85% after routine vaccination; from 137 in 1999–2000 to 18 in 2001–2004 ($p < 0.001$). Rates of non-pcv7 serotypes in AK increased from 26 in 1999–2000 to 56 in 2001–2004 in children < 2 years of age ($p = 0.02$). Overall, 89% of isolates from persons > 2 years of age were serotypes contained in the 23-valent polysaccharide vaccine. The proportion of isolates fully-resistant to penicillin varied by country from <1% in Fin to 5.6% in AK.

Conclusions: Rates of IPD are high in aboriginals and children < 2 years of age residing in Arctic countries. After introduction of pcv7 in AK, rates of disease in children < 2 years of age with pcv7 serotypes rapidly declined; however, increasing rates of non pcv7 serotypes are concerning and merit further surveillance. Continued surveillance is needed to determine the impact of pcv7 in AK and areas of N Can. High IPD rates in children < 2 warrant consideration of pcv7 use in other circumpolar countries.

O221

Invasive pneumococcal disease in the era of human immunodeficiency virus infection

O. Perovic, A. von Gottberg, C. Feldman, O. Perovic
(Johannesburg, ZA)

Objectives: The main aims of the study were to describe patients with invasive pneumococcal disease (IPD) in the era of HIV infection and to determine factors associated with increased mortality.

Methods: This was a retrospective record review of all cases with IPD admitted to the Johannesburg Hospital between December 1999 and November 2001. Demographic, clinical, laboratory, microbiology and outcome data were recorded and risk factors possibly associated with poor outcome were compared in survivors and non-survivors.

Results: 211 cases were enrolled (103 male and 108 female). The mean age was 35.7 yrs (range 17–87 yrs). 165 (78%) were HIV-seropositive. Eleven patients had other underlying co-morbid conditions (malignancy in 6, diabetes mellitus in 2, chronic renal failure in 2, and renal transplant in 1). The source of bacteraemia was lower respiratory tract in 193 (multilobar pneumonia in 114), meningitis in 11, unknown in 3, acute otitis media in 2, and septic arthritis and skin soft tissue infection in 1 each. Complications of infection were recorded in 70 cases (sometimes multiple); pleural effusion in 36, significant haemoptysis in 12, renal failure in 7, pericardial effusion in 4, suppurative in 4, jaundice, empyema, gastroenteritis and ARDS in 2 cases each and haematemesis and CVA in 1 case each. 44 cases died (21%); 35 of the patients with pneumonia (1 each with acute exacerbation of COPD, associated gastroenteritis, associated empyema), and 9 with meningitis. HIV status, underlying co-morbid illness, age, or complications had no impact on outcome. Patients with meningitis had a significantly higher mortality than cases with pneumonia (mortality 81.8% versus 18.1%; $p < 0.0001$). Overall 37 isolates (17.5%) showed decreased susceptibility to penicillin (intermediate 15%, fully resistant 2.5%) and 7 (3.3%) resistance to erythromycin.

Conclusion: Pneumococcal bacteraemia is a common infectious complication in HIV-seropositive patients with a clinical course and outcome in patients with pneumonia similar to that in HIV–seronegative cases. However the outcome of cases in which the primary diagnosis is meningitis is particularly poor.

O222

Are Pneumonia Severity Index and CURB-65 useful for prediction of 30-day mortality in community-acquired pneumonia?

K. Strålin, H. Holmberg *(Örebro, SE)*

Objectives: Two different indexes have been developed for prediction of the 30-day mortality in community-acquired pneumonia (CAP). The pneumonia severity index (PSI) score is calculated from 19 parameters, and divides the patients into severity risk classes I-V, while the CURB-65 score is based on 5 parameters (confusion, urea value, respiration rate, blood pressure, and age) and yields a score of 0–5. We aimed to compare the two scores for identification of CAP patients with low and high risk of death.

Methods: The records of all patients hospitalized at our clinic with a discharge code of CAP during a 4-year period were retrospectively screened. Patients with chest X-ray infiltrates and two symptoms indicating CAP were included in the study. The PSI and CURB-65 scores were calculated, and data on survival at day 30 from admission was collected from the population register. Clinical features not specified were considered normal.

Results: Among 705 identified episodes of CAP an initial serum-urea value was available in 612. Thirty-four of these episodes (5.6%) were followed by death within 30 days. The table shows the distribution of the PSI risk classes and the CURB-65 score correlated to the 30-day mortality.

Index	Index score	Pneumonia episodes (No.)	30-day mortality (No. (%))
PSI	I-II	192	0
	III	154	1 (0.6)
	IV	194	12 (6.2)
	V	72	21 (29)
CURB-65	0-1	283	1 (0.3)
	2	182	9 (4.9)
	3	118	16 (14)
	4-5	29	8 (28)

Conclusion: Both the PSI and CURB-65 scores identified CAP patients with low-risk and high-risk of 30-day mortality. They can support clinical judgment in decisions regarding outpatient treatment or level of hospital supervision. CURB-65 is more easily handled and can be preferable to PSI in clinical practice.

O223

Tigecycline is as safe and effective as levofloxacin in treating patients with community-acquired pneumonia

N. Dartois, H. Gandjini, E.J. Ellis-Grosse *on behalf of the Tigecycline 313 Study Group*

Objectives: Tigecycline (TGC), a first-in-class glycylcycline that has been approved for treating complicated skin and skin structure infections and complicated intra-abdominal infections, has an expanded spectrum of activity against Gram-positive, Gram-negative, anaerobic, and atypical bacteria including resistant strains. Primary objective was to compare TGC efficacy and safety with that of levofloxacin (LEV) in patients with community-acquired pneumonia (CAP).

Methods: In this phase 3, multicentre, randomised, double-blind study, hospitalized patients with CAP received 7–14 days of IV TGC (100 mg loading dose followed by 50 mg every

12 hours) or IV LEV (500 mg once or twice daily). Co-primary efficacy endpoints were clinical response in clinically evaluable (CE) and clinical modified intent-to-treat (c-mITT) populations at test-of-cure (TOC). Secondary endpoints were microbiological efficacy and susceptibility data on TGC for a range of bacteria that cause CAP. Safety evaluations included vital signs, laboratory tests, chest radiographs, electrocardiograms, and record of adverse events (AEs).
Results: 449 patients were screened, 428 were in mITT (TGC 216, LEV 212), 280 were in CE (TGC 144, LEV 136). Demographics were similar in both groups and majority of patients had a Fine Pneumonia Severity Index of 2 to 4 (84.8% TGC, 79.0% LEV, mITT). At TOC (CE), TGC cured 128/144 patients (88.9%; 95% CI 82.6, 93.5) and LEV cured 116/136 patients (85.3%; 95% CI 78.2, 90.8), absolute difference of TGC-LEV 3.6% (95% CI –5.0, 12.2, test for noninferiority $p < 0.001$). For those CE patients with a Fine score of <3 or 3/4, TGC cured 89.6% and 88.0%, and LEV cured 87.3% and 83.3%, respectively. In c-mITT, TGC cured 170/203 patients (83.7%; 95% CI 77.9, 88.5) and LEV cured 163/200 patients (81.5%; 95% CI 75.4, 86.6), absolute difference of TGC-LEV 2.2% (95% CI –5.6, 10.1, test for noninferiority $p < 0.001$). In c-mITT patients with a Fine score of <3 or 3/4, TGC cured 85.4% and 81.6%, and LEV cured 80.8% and 82.8%, respectively. Nausea (26.9% TGC vs 8.5% LEV, $p < 0.001$), vomiting (16.7% vs 6.6%, $p = 0.001$) and leucocytosis (6.9% vs 0.9%, $p = 0.002$) were statistically higher in TGC where hypokalaemia was significantly higher in LEV (0.5% TGC vs 3.8% LEV, $p = 0.019$). Overall treatment discontinuations due to AE was low (TGC: 14 patients [6.5%], LEV 16 patients [7.5%]).
Conclusion: TGC was safe and efficacious against the most common respiratory pathogens observed in patients with CAP, with TGC achieving similar efficacy to that of the comparator.

O224

Risk factors for *Pseudomonas aeruginosa* isolation in sputum at hospital admission in patients hospitalised for acute COPD exacerbation

C. Garcia Vidal, P. Almagro, V. Romaní, H. Monzón, L. Cuadra, M. Rodriguez, E. Cuchí, L. Canales, J.L. Heredia, J. Garau *(Terrassa, ES)*

Background: The presence of *Pseudomonas aeruginosa* (PA) in the sputum has been associated with severity of disease in ambulatory COPD patients, but its clinical significance in hospitalized patients remains unclear. A better knowledge of the clinical meaning of presence of PA in sputum in these patients is important to choose an appropriate antimicrobial treatment in the Emergency room.
Methods: We prospectively studied all exacerbated COPD patients that were hospitalized between June 2003 to September 2004 and we followed their readmissions during one year. Sputum was collected at admission. Spirometry was performed one month after discharge. Thoracic scanning was done in a randomized manner (2:1) to determine the presence of bronchiectasis, quantified following a validated scale expressed in %. Data were analysed with t-test or Mann-Whitney test, and χ^2 for qualitative data.
Results: 188 patients were included, with a mean age of 72 years (SD11). 96 (52%) patients had a hospital admission in the previous year, 32 (17%) in the previous month, and 116 (62%) had been exposed to antimicrobials in the previous 3 months. Bronchiectasis was documented in 50 patients (56.2%); the mean bronchiectasis score was 13.8% (SD16.9). 106 patients required further readmissions for another episode(s) of acute exacerbation during the study, so a total of 469 episodes were analysed. A valid sputum was collected in 220 episodes; two microorganisms were present in 93 episodes and three microorganisms were isolated in 10 episodes. PA was present in 51 (23%), *H. influenzae* in 24 (12%), *S. pneumoniae* in 21 (10.5%), *Enterobacteriaceae* in 8 (4%) and normal flora in 159 (79%). Patients with bacterial infection had a FEV1 lower than those with negative sputum cultures ($p < 0.003$). The presence of PA in sputum was strongly associated with low FEV1 ($p < 0.02$), worst walking test ($p < 0.01$) and number packs/year ($p < 0.01$).
Conclusions: The presence of PA in sputum at entry in patients hospitalized for an exacerbation of COPD is higher than previously reported and is associated to smoking history and functional impairment. No relationship between the presence of bronchiectasis and PA in the sputum could be demonstrated.

O225

Does low antibiotic consumption influence microbiological findings in patients with COPD exacerbations?

Z.B. Harboe, J.H. Wandall, J.D. Knudsen *(Frederiksberg, Hvidovre, DK)*

Objectives: Chronic Obstructive Pulmonary Disease (COPD) is a frequent cause of hospital admission. Antibiotic use is being recognized as the main factor for development of resistance. The Danish outpatient antibiotic consumption is low (14 DDD/1000 inh.-days) compared to most countries in Europe (20–30 DDD/1000 inh.-days). The objective was to describe the epidemiology of microbiological findings from COPD patients admitted with exacerbations (COPD-ptt), and relate that to the efficacy of empiric first-line treatments.
Methods: Retrospective study of all COPD-ptt admitted to three Internal Medicine wards and an ICU in 2003, identified through a hospital database. Sputum samples were spontaneously expectorated or sucked for standard microbiological analysis. Data were analysed by SPSS software. Survival was assessed by Kaplan-Meier test. P-values < 0.05 were considered significant.
Results: 340 COPD-ptt in 560 admissions were included, from a population of app. 90,000 inhabitants. Sputum samples from 176 ptt (52 %) were obtained. The three months-mortality was 16 %; 19 % for ptt with findings in sputum, 9 % for ptt with negative samples ($p = 0.11$). Potentially pathogenic microorganism (PPM) grew in 113 samples (51 %) from 99 ptt (56 %), 88 samples (40 %) did not have PPM growth and 19 samples (9 %) were not suitable for analysis. The findings for COPD-ptt were *S. pneumoniae* 18%; *H. influenzae* 13 %; *P. aureuginosa* 13 %; *Candida* spp. 14% and others PPM penicillin-resistent in 32% of ptt. This is similar to findings reported in the rest of Europe. Administration of i.v. penicillin was sufficient in only 49 % of admissions with positive findings.
Conclusions: Low antibiotic consumption does not influence microbiological findings in COPD-ptt, compared to high-consumptions settings. Penicillin is not sufficient as first-line treatment, as it is recommended.

O226

Management of severe community-acquired pneumonia

C. Brun-Buisson, A.T. Kouatchet *for the French Severe Pneumonia Study Group*

Background: Severe community-acquired pneumonia (SCAP) is the major source of infection and sepsis in the ICU. Early therapy has been associated with improved outcome of patients in retrospective studies.

Objective: To identify characteristics and management of patients with SCAP admitted to the ICU and analyse antibiotic therapy (Rx) and its influence on outcome of patients.
Methods: Patients with SCAP admitted to 20 ICUs in a 1-yr period were prospectively followed-up. Clinical features, comorbidities, severity scores (incl. the Pneumonia Severity score [PSI] and CURB-65 at hospital presentation, SAPS II and LOD on ICU admission), aetiologies, and time to and selection of Rx were recorded, and variables associated with mortality identified.
Results: We included 235 pts aged 58 ± 16 yrs, 47 (20%) of whom had ultimately or rapidly fatal underlying disease. Their mean PSI score was 103 ± 35 (40% class I-III and 60% class IV–V), mean CURB-65 2 ± 1 (41% > 2), and median SAPS II 39 [14–78]; 97 (41%) had hypotension and 81 (35%) required vasopressors on admission; 40 (17%) and 164 (70%) received mechanical ventilation (MV) on and after ICU admission. There were 190 (81%) and 186 (79%) survivors to ICU and hospital discharge, respectively. Infection was microbiologically documented in 64%. Thirty-nine (17%) had received antibiotic Rx for >24 h before admission. In others (N = 196), the median lag between hospital admission and antibiotic Rx was 4 [0–28] hours; Rx was delayed for more than 4 h and 8 h after hosp. admission in 21% and 19% pts, respectively. Pts were started on Rx in the ICU (n = 100) within 1 [0–14] h; therapy was delayed for more than 4 h and 8 h after ICU admission in 21 and 13 pts, respectively. Initial empiric therapy included mostly co-amoxiclav (45%) or a 3rd generation cephalosporin (38%), combined with a macrolide or quinolone in 25% and 24%, respectively. After adjustment for the PSI score, other general severity scores, bacteremia, and GNB infection, the timing of antibiotic therapy or use of a combination did not influence outcome, although inappropriate Rx did in documented SCAP.
Conclusion: Antibiotic Rx in the hospital and ICU was delayed by >8 h in 20% and 13% of pts, respectively. Although early antibiotic Rx is desirable in with severe infection, its appropriateness is likely more important for outcome of patients.

O227

Treatment and mortality from bacteraemic pneumococccal disease in Toronto, Canada

K. Green, A. Shigayeva for the Toronto Invasive Bacterial Diseases Network *TIBDN*

Background: Several prospective studies suggest that choice of antibiotic regimen influences mortality in patients with severe pneumococcal infection. We examined the impact of treatment choices on mortality from pneumococcal bacteremia (PB) in Toronto/Peel, Canada.
Methods: Population based surveillance for PB in residents of Toronto/Peel region, Ontario, Canada (pop = 4M) has been on-going since 1995. Demographic and medical data are collected from patients and their physicians. Since 2000, these data include antibiotic therapy.
Results: From 1/1/2000 to 31/12/2004, 1581 episodes of PB have been identified. Median age of patients was 58y (range 1st day of life to 108y); 825 (54%) patients were male, 964 patients had chronic underlying illness, including: cardiac disease (389, 26%), lung disease (279, 18%), diabetes mellitus (225, 15%), cancer (231, 15%), liver disease (88, 6%), kidney disease (87, 6%), HIV infection (79, 5%), asplenia/sickle cell disease (37, 2.5%). 205 of 1463 (14%) episodes for which isolates were available were serotypes included in the 23-valent vaccine. 74 isolates (5.1%) were resistant to penicillin, 206 (14%) to erythromycin, 30 (2.1%) to ceftriaxone, 18 (1.2%) to levofloxacin, and 5 (0.3%) to moxifloxacin. Overall, 390 (26%) patients were admitted to the ICU, and 273 (18%) required mechanical ventilation. 299 (20%) patients died, including 9/335 (3%) children, and 290/1181 (25%) adults. 13 children and 10 adults received discordant antibiotic therapy: no children, but 5/10 adults receiving discordant therapy died (P = 0.05 vs other adults). Factors associated with 30 day mortality in multivariable (MV) analysis were: older age (MV odds ratio (OR)/decade, 1.36, 95%CI 1.23–1.47), chronic organ system disease (MV OR 2.3, 95%CI 1.5–3.4), nursing home acquisition (MV OR 3.8, 95%CI 2.6–6.1), hospital acquisition (MV OR 2.1, 95%CI 1.2–3.8), and requirement for mechanical ventilation (MV OR 7.1, 95%CI 4.9–10). Patients treated with respiratory fluoroquinolone monotherapy (MV OR 0.63, 95% CI 0.42–0.96) or with a macrolide in combination with any other antibiotic (MV OR 0.65, 95% CI 0.42–1.0) were less likely to die. Results were not different when all patients or adults only were included in analysis.
Conclusion: BP disease in adults is associated with high mortality (25%). Therapy with a respiratory fluoroquinolone alone, or combination therapy with a macrolide and any other antibiotic was associated with a significant reduction in mortality.

O228

Antimicrobial susceptibility of pneumococcal isolates causing bacteraemic community-acquired pneumonia in the public and private sectors in Gauteng, South Africa

C. Feldman, A. Brink, A. von Gottberg, L. de Gouviea, O. Perovic, K. Klugman *(Johannesburg, ZA)*

Objectives: Contradictory and very high rates of pneumococcal resistance have been reported previously from different surveillance studies conducted in South Africa. The aim of this study was to review susceptibility data of isolates from adult patients presenting with bacteremic pneumococcal community-acquired pneumonia (CAP), comparing cases in the public and private sectors in Gauteng, South Africa.
Methods: We wished to recruit 100 consecutive adult patients with pneumococcal bacteraemia and CAP. Patients without radiographic evidence of pneumonia, or with other clinical diagnoses (e.g. meningitis) were excluded. Repeat isolates were excluded. Antimicrobial susceptibility testing was done according to CLSI guidelines, using disc screening and MIC determination with agar dilution and E-tests.
Results: 149 cases were recruited, 97 in the public and 52 in the private sector. Public sector patients were younger (median age 33 years vs. 45 years; $p < 0.001$), with more HIV infection and less co-morbid illness. Cases in the private sector were more likely to have received antibiotics in the past 3 months. The overall rate of antimicrobial resistance was considerably lower than reported previously in South Africa. Resistance to penicillin was 16% in the public and 23% in the private sector (not significantly different). There were also no significant differences in the susceptibility of the isolates to ceftriaxone, chloramphenicol, tetracycline, rifampicin or ofloxacin. However significantly more resistance was seen in the private sector isolates to erythromycin (28% vs. 4%; $P < 0.001$) and clindamycin (21% vs. 3%; $p = 0.001$).
Conclusion: A number of clinical differences were seen in public vs. private sector patients with bacteraemic pneumococcal pneumonia in Gauteng, South Africa. Rates of antibiotic resistance were much lower than previously reported from South Africa. The only differences noted were higher rates of resistance to erythromycin and clindamycin in the private sector (possible erm gene mutation as mechanism of macrolide resistance).

O229

Outcome of adults with penicillin high-resistant and susceptible invasive *Streptococcus pneumoniae* community-acquired pneumonia

M. Mufson, G. Chan, R. Stanek *(Huntington, US)*

Introduction: The emergence of *Streptococcus pneumoniae* exhibiting high resistance to penicillin (PRSP) (MIC = 2.0–4.0 μg/ml) evoked concerns that these infections would respond poorly to the antibiotics usually used in the treatment of community-acquired *S. pneumoniae* pneumonia due to penicillin intermediate (PISP) (MIC = 0.1–1 μg/ml) isolates. We investigated adults with invasive *S. pneumoniae* pneumonia infected with PRSP or PISP and matched controls with susceptible infections (PSSP) (MIC ≤ 0.06 μg/ml) to determine whether resistance to penicillin was a key factor in clinical outcome.
Methods: We identified 52 adults with invasive *S. pneumoniae* pneumonia due to PNSP – 19 PRSP and 33 PISP - admitted to hospitals in Huntington, WV, between 1983 and 2003 and matched them by age and admission time period to a group of 133 patients with PSSP pneumonia. All isolates were capsular types 6, 9, 14, 19 and 23. Deaths were counted if they occurred before the seventh hospital day. MIC was determined by E-test and capsular type by Quellung procedures.
Results: There were no significant differences between the PNSP and PSSP groups in mean age, the severity of admission vital signs, days of fever, mean total leukocyte count, number of lobes involved, pre-existing underlying diseases and antibiotic treatment regimens. Both groups had tachypnea, modest tachycardia, a moderate leukocytosis and in about 60% of cases only one lobe of the lungs involved. Case-fatality rates (CFRs) during the first seven days of hospitalization did not differ significantly between invasive PRSP and PSSP pneumonia cases, 10.5% and 6.8%, respectively ($p = 0.63$, Fisher's exact test, two-tailed). Similarly, CFRs were not significantly different between invasive PISP and PSSP pneumonia cases 15.2% and 6.8%, respectively ($p = 0.16$, Fisher's exact test, two-tailed). The PRSP, PISP and PSSP groups often received antibiotic regimens comprised of a cephalosporin and a macrolide, and occasionally a fluoroquinolone, but there were no significant differences in CFRs between them when compared by antibiotic regimens.
Conclusion: These findings suggest that antibiotic regimens effective in the treatment of invasive PSSP pneumonia are no less effective in the treatment of PRSP with MIC's between 2 and 4 μg/ml and PISP, obviating the need for more complex antibiotic regimens in their treatment.

Keynote lectures I

K243

Antimicrobial intelligence: how eukaryotes combat the quorum sensing strategy

A. Sbarbati *(Verona, IT)*

Microorganisms easily form biofilms on medical devices but the development of similar microfilms on mucosal surfaces may be hampered by the presence of several lines of defence operated by the epithelial cells. As an example, the biological interfaces are electrically charged, secrete antibiotic compounds and can modulate their hydrophobicity mainly by the production of surfactant material. In addition, recent data demonstrated that diffuse chemosensory systems continuously monitor the presence of microorganisms on mucosal surfaces. Bacteria can communicate with one another via signal molecules: this process is generally called quorum sensing. Quorum sensing signals, not only act on different bacteria species but also influence the behaviour and metabolism of plants and animals suggesting that they are involved in interspecies and interkingdom communication. The eukaryotes susceptible to infection by bacteria that use quorum sensing have evolved natural therapies designed to impede bacterial colonization by inhibiting quorum sensing mediated processes. The detection of quorum sensing signals would permits the host organism to mount an early and localized response to bacterial invasion. New data have demonstrate the expression of chemosensory receptors (T2R and V1R), G proteins and other molecules of the chemoreceptorial cascade (PLCbeta2, IP3R3) in secretory elements in direct contact with bacteria. This expression suggests that secretory processes could be controlled by chemoreceptor mechanisms. Methods of functional magnetic resonance imaging allow an *in vivo* evaluation of secretory responses expanding the knowledge obtained by *in vitro* approach. The work reviews studies, which suggest that eukaryotic cells sense QS signals of bacteria and describe a response to these signals. The work also proposes models on how mammalians perceive bacterial signals based on the idea that chemoreceptors may be involved in perception of these signals and initiation of responses to them in addition to the responses mediated by the immune system.

K244

Clean care is safer care: a WHO initiative to improve patient safety worldwide

D. Pittet *(Geneva, CH)*

Health care-associated infection affects hundreds of millions of people worldwide in developed, transitional, and developing countries and is a major, global issue for patient safety. The World Health Organization (WHO) supported the creation of an international alliance to improve patient safety as a global initiative and the World Alliance for Patient Safety was launched in October 2004. The six actions areas of the Alliance are: Patients for Patient Safety; Taxonomy; Research; Solutions for Patient Safety; Reporting and Learning; and a biennial Global Patient Safety Challenge. The topic chosen for the first Challenge, covering 2005–2006, is health care-associated infection. The Global Patient Safety Challenge, ''Clean Care is Safer Care'', was launched in October 2005. It embraces existing WHO strategies to reduce health care-associated infection and also creates the momentum for new actions to improve hand hygiene during patient care. The major objectives of ''Clean Care is Safer Care'' are: to raise awareness of the impact of health care-associated infection on patient safety and promote preventive strategies within countries; to build commitment from countries to prioritise reducing health care-associated infection; and to test the implementation of the new WHO Guidelines on Hand Hygiene in Health Care in specific districts worldwide as part of an integrated package of actions derived from existing WHO strategies in the fields of blood safety, injection and immunisation safety, clinical procedure safety, and water, basic sanitation and waste management.

K245

Human papillomaviruses: impact of disease and vaccine intervention

M. Poljak *(Ljubljana, SI)*

Papillomaviruses are remarkably heterogeneous group of DNA viruses that are causally involved in the etiology of different benign and malignant neoplastic lesions of the anogenital, oropharyngeal and cutaneous epithelium. They are classified into 16 genera, and five of them (alpha, beta, gamma, mu, and nu) are composed exclusively of human papillomaviruses (HPV). Currently, more than 90 distinct HPV genotypes have been fully characterized, and a substantial number of putative novel HPV genotypes are predicted from amplified subgenomic DNA fragments. Clinically, the most important are alpha-HPV, which comprise HPV genotypes originally referred to as ''genital'' or ''mucosal'' HPV. These can be further subdivided into ''low-risk'' and ''high-risk'' genotypes according to the risk of malignant progression. Thus, the ''low-risk'' genotypes HPV-6 and HPV-11 are etiologically associated with the development of virtually all genital warts and laryngeal squamous cell papillomas. In contrast, persistent infection with a subgroup of at least 15 ''high risk'' HPV genotypes is considered as a necessary, although insufficient etiological factor in the development of cervical carcinoma. As a consequence, HPV testing has recently become an important part of the cervical carcinoma screening and detection algorithms. Thus, several consensus guidelines recommend the HPV testing: (i) for appropriate triage of women with a borderline cytology results, (ii) as a test of cure of treatment of high-grade cervical lesions, and (iii) as a primary screening test for cervical carcinoma (in conjunction with cytology) of women aged 30 years or more. The prevention of HPV infection can be achieved by the induction of genotype-specific neutralizing antibodies triggered by recombinant L1 viral-like particle (VLP) vaccines. VLP are attractive candidates for prophylactic vaccines as they are empty, non-infectious, DNA-free viral capsids morphologically indistinguishable from the native viruses. Several multivalent VLP-based vaccines are now being clinically developed. Phase I and II trials have shown that the VLP-based HPV vaccines are well tolerated, that they induce high titers of neutralizing antibodies (50–100 fold greater than during natural infection), and protect with a very high efficacy against a persistent HPV infection and HPV-related clinical disease. Phase III trials of HPV vaccines are currently underway, and the public health benefits associated with vaccination may soon be realized.

Emergence of transferable quinolone resistance (Symposium jointly arranged with the ICAAC Programme Committee)

S247

World emergence of qnr, a novel family of quinolone resistance genes

D. Hooper *(Boston, US)*

Transferable plasmid-mediated quinolone resistance was first found in strains of *Klebsiella pneumoniae* in one region of the United States in 1998 and shown to be due to qnr, which encodes a member of the pentapeptide repeat (PPR) family of proteins. Qnr protein causes low-level quinolone resistance and binds and protects gyrase and topoisomerase IV from quinolone action. Another PPR protein, MfpA, from *Mycobacterium tuberculosis* has a structure that mimics DNA and may bind gyrase in its DNA-binding domain. Although qnr was not identified again in early follow-up surveys, it was identified in 2003 in ciprofloxacin- and ceftazidime-resistant clinical isolates of *Escherichia coli* from Shanghai, China and subsequently in similar isolates of *K. pneumoniae* and *Enterobacter* spp. from throughout the US. qnr was also found in several different species of *Enterobacteriaceae* in Europe and Thailand. qnr was located in class 1 integrons on plasmids but was also found on the chromosome of an environmental water bacterium, Shewanella algae, suggesting that this organism may have been the source of the qnr gene. Two other members of the qnr family, qnrS and qnrB, have been found, and the original qnr is now termed qnrA. Other qnr homologs have found in the genome sequences of several *Vibrio* spp. and *Photobacterium profundum*. qnrS has been identified on plasmids in *Shigella* and *Salmonella* spp., and qnrB has been found in a variety of enteric bacteria from the US and India. Notably all three qnr genes have been found in ciprofloxacin-susceptible isolates as well as quinolone-resistant isolates, suggesting that their presence promotes higher level resistance due to chromosomal mutation, as has been shown in the laboratory. In the US, ceftazidime-resistant isolates of *Enterobacter* spp. have a notably high prevalence of qnr genes, with 17% having qnrA and another 26% having qnrB, and the prevalence has increased over time. Unexpectedly in studies to evaluate the fourfold differences in the MICs of ciprofloxacin between some qnrA transconjugants, another plasmid-encoded quinolone resistance determinant was identified, a variant of the aac(6′)Ib gene encoding an aminoglycoside acetyltransferase that had acquired the ability to acetylate ciprofloxacin and reduce its activity fourfold. This aac(6′)Ib variant occurs independently of and at a higher prevalence than qnrA in some strain sets. When together with qnrA on the same plasmid, it causes additive transferable resistance that approaches the clinical breakpoint for ciprofloxacin susceptibility. It is likely that other qnr variants and possibly other variant modifying enzymes may be identified as quinolone selective pressures continue.

S248

The toxin-antitoxin systems: function and distribution in the bacterial world

M. Wilbaux, N. Mine, L. Van Melderen *(Gosselies, BE)*

Toxin-antitoxin systems (TA) are small genetic elements composed of two genes organised in an operon and encoding a stable toxin and its unstable cognate antitoxin. ATP-dependent proteases are responsible for antitoxin degradation. The antitoxin antagonizes the toxin by forming a tight protein-protein complex. This complex is also responsible for the autoregulation of the operon. TA were originally discovered on low copy-number plasmids. In that genetic context, TA contribute to plasmid stability in growing bacterial population by killing selectively bacteria that have lost the plasmid.Database searching revealed that plasmidic TA have many homologues in bacterial chromosomes. Although it has been shown that some chromosomal TA are activated during starvation or other types of stress, their biological role as well as the selective advantage

they confer to bacteria are still under investigation. The current hypotheses are that TA serve as growth modulators or programmed cell death systems in response to stress. We have compared a well-studied plasmidic TA, the ccdF from the *E. coli* F plasmid with one of its homologues, the ccdO157 located in the chromosome of the pathogenic *E. coli* O157:H7. Despite a low level of identity between the CcdF and the CcdO157 proteins, the CcdO157 proteins have conserved essential motifs for their function as TA i.e. the toxin targets the DNA-gyrase and the antitoxin is degraded by the Lon protease. However, the toxin-antitoxin interaction domains of the two homologues have diverged. This indicates that the plasmidic ccdF system is functional despite the presence of its chromosomal homologue. We propose that chromosomal TA might serve as 'exclusion' systems protecting bacteria from exogenous DNA carrying identical TA. Therefore, only plasmidic TA able to evade cross-interaction might co-exist with homologous chromosomal TA. Although we showed that the chromosomal ccdO157 system is expressed in its host, it is unable to mediate plasmid stabilization when cloned in an unstable plasmid. This supports the idea that plasmidic and chromosomal TA play different role(s). We propose that homologous TA have derived from a common ancestor and have reached different bacterial species and/or location by horizontal transfer and that their function has evolved according to their new location.

Management of endocarditis

S253

Antibiotic treatment of infective endocarditis due to antibiotic resistant bacteria

J. Miro *(Barcelona, ES)*

Currently there are four clinical types of infective endocarditis (IE): native valve IE in the general population (left-sided IE) or in i.v. drug abusers (usually right-sided IE), prosthetic valve IE and IE on pacemakers or defibrillators. These types of IE have a very different clinical characteristics, etiology and prognosis. Overall mortality remains high (25%) despite the important advances in the early diagnosis (transesophageal echocardiogram) and in early cardiac surgery. The etiology has changed, being staphylococcal IE more frequent than streptococcal IE and nosocomial IE caused by highly aminoglycoside resistant *Enterococcus* spp. and methicillin-resistant *Staphylococcus aureus* or *S. epidermidis* has been increasing during the last decade. From the therapeutical point of view, the antibiotic therapy of viridans group streptococci IE and *S. aureus* right-sided IE in drug abusers have been shortened and simplified. These types of IE can be treated with ceftriaxone plus gentamicin or cloxacillin plus gentamicin during two weeks respectively. Furthemore, the introduction of ceftriaxone and teicoplanin have allowed the administration of a single daily dose of these drugs by i.v. or i.m. routes and they make possible the out-patient antibiotic therapy of IE by specialized home-care units. On the other hand, multiresistant staphylococci (MRSA, GISA) and enterococci IE have high mortality ratios and few antibiotic alternatives. In this way, the preliminary *in vitro*, *in vivo* and clinical data with new antibiotic combinations (e.g. ampicillin plus third generation cephalosporins or fosfomycin plus beta-lactam antibiotics) or new antibiotics classes (e.g. linezolid, quinupristin-dalfopristin, daptomycin and telavancin) open new therapeutical alternatives to treat these IE caused by multirresistant bacteria.

Infectivity and emergence of resistance in animal models

O257

Infectivity of probiotic *Lactobacillus rhamnosus* and *Lactobacillus paracasei* in a rat model of experimental endocarditis

V. Vankerckhoven, S. Piu, P. Moreillon, M. Vancanneyt, H. Goossens, J.M. Entenza *(Wilrijk, BE; Lausanne, CH; Ghent, BE)*

Objectives: The aim of this study was to evaluate the potential pathogenicity of different probiotic strains of *Lactobacillus rhamnosus* and *L. paracasei* in a rat model of experimental endocarditis.
Methods: A total of 10 *L. rhamnosus* (6 probiotic and 4 endocarditis) and 7 *L. paracasei* (4 probiotic, 1 faecal and 2 endocarditis) were tested. Rats with catheter-induced aortic vegetations were inoculated i.v. with increasing numbers of organisms (1E04 to 1E08 CFU/ml). After 72 h they were sacrificed, and both the lowest inoculum size infecting 90% of vegetations (ID90) and colony counts were determined. Bacterial densities in infected vegetations were analysed using Mann-Whitney U test. Adhesion properties of the lactobacilli were revealed using crystal violet and measured with an ELISA reader at 570 nm. Collagen type IX, fibrinogen, fibronectin and laminin were used as substrates in twofold dilutions in microtiter plates. Strains were classified as adherent (A570 nm > 0.3), weakly adherent (0.1 ≤ A570 nm ≥ 0.3), or non-adherent (A570 nm> 0.1). Platelet-induced killing was assessed for fibrinopeptide A (FPA).
Results: Four of the six probiotic *L. rhamnosus* strains showed an ID90 which was at least 10 times higher (1E08 CFU/ml) than that of the clinical isolates, while the two other probiotic *L. rhamnosus* demonstrated an ID90 in line with the higher range (1E07 CFU/ml) of the clinical isolates. Importantly, these two strains shared the same genotype (type I) as the clinical isolate showing the lowest ID90 (1E06 CFU/ml). *L. paracasei* tended to have a lower infectivity than *L. rhamnosus*, with an ID90 of 1E07 to ≥1E08 CFU/ml for endocarditis as well as probiotic and faecal isolates. All strains had comparable bacterial counts in vegetations (P > 0.05). Except for one strain which adhered to fibronectin (A570 nm = 0.406) and laminin (A570 nm = 0.425), all tested lactobacilli adhered only weakly or not at all (A570 nm= 0–0.205). The platelet peptide FPA did not show microbicidal activity against any of the lactobacilli.
Conclusions: Overall, these results indicate that probiotic lactobacilli were, in general, less able to cause experimental endocarditis than isolates from human endocarditis. Although, a difference in infectivity between *L. rhamnosus* endocarditis and probiotic isolates was seen, this could not be explained by differences in adherence or platelet-microbicidal protein

susceptibility. Other disease-promoting factors might exist in these organisms and warrant further investigation.

O258

Dissemination of *Borrelia burgdorferi* sensu lato in mice: influence of the species and of the primary human isolation site

S.J. De Martino, C. Sordet, Y. Piemont, C. Barthel, E. Collin, J. Sibilia, B. Jaulhac *(Strasbourg, FR)*

Objective: To study the influence of the *Borrelia* (B.) species and of the primary human isolation site of the bacteria on the dissemination of strains from the *B. burgdorferi* group in the C3H/HeN murine model of Lyme borreliosis.
Methods: Seventeen human european and north american isolates of the *B. burgdorferi* group (6 *B. burgdorferi* sensu stricto, 6 *B. garinii*, and 5 *B. afzelii*) were used. These strains have been isolated from erythema migrans (EM) (n = 7), borrelial lymphocytoma (BL) (n = 1), acrodermatitis chronicum atrophicans (ACA) (n = 1), neuroborreliosis (NB) (n = 6) and Lyme arthritis (LA) (n = 2). Spirochetes (10^5/100 μl) of each strain were inoculated by intradermal route into five mice. Development of arthritis was sought by measurement of tibio-tarsal joint. Four weeks later, mice were euthanased. Murine infection was checked by seroconversion. Spirochetes dissemination was investigated by culture and PCR of murine target organs (skin, heart, joint).
Results: All mice were seropositive for *B. burgdorferi* at day 28 post infection. A clinical murine arthritis (redness, swelling) was significantly correlated with a joint diameter ≥ 3 mm ($p \leq 0.0005$). Low rate of discrepancies between *B. burgdorferi* culture and PCR results were observed in skin and heart whereas 40% of additional joints were found positive by PCR only ($p \leq 0.0005$). Regarding the Borrelia species used for murine infection, *B. burgdorferi* sensu stricto and *B. garinii* strains disseminated more frequently in mouse skin than *B. afzelii* strains. *B. afzelii* strains disseminated more frequently in joints than *B. burgdorferi* sensu stricto and *B. garinii* strains ($p < 0.005$). Regarding their primary human isolation site, strains from secondary and late cutaneous lesions (BL, ACA) were significantly more often detected in skin ($p < 0.005$). Strains involved in human NB or LA, were more frequently detected in murine joints ($p < 0.005$). Strains from EM disseminated in all murine organs.
Conclusion: Dissemination of *B. burgdorferi* sensu lato in the mouse model of Lyme borreliosis varied according to the *Borrelia* species and the primary human isolation site. Human strains isolated from secondary and late cutaneous manifestations were preferentially detected in murine skin whereas strains able to disseminate in humans were preferentially detected in murine joints. EM strains for which human dissemination capability is unknown had a wide dissemination in mice.

O259

Subinhibitory concentrations of phenyl lactic acid produced by *Lactobacillus* probiotic strains attenuate the virulence of *Peudomonas aeruginosa* and *Staphylococcus aureus* in mouse infection model

M.C. Balotescu, V. Lazar, A. Israil, L. Ditu, C. Larion, I. Alexandru, C. Bleotu, R. Cernat *(Bucharest, RO)*

Introduction: The discovery of communication systems (quorum sensing systems) regulating bacterial virulence has afforded a novel opportunity to control infectious bacteria without interfering with growth. Compounds that can override communication signals have been found in the cell-free *Lactobacillus* probiotic strains cultures. During a previous *in vitro* screening of antimicrobial activities of soluble products from *Lactobacillus* cultures, phenyl lactic acid (PLA) was found to exhibit QS inhibitory (QSI) activity.
Purpose: In this study we try to demonstrate the role of PLA in the bacterial virulence attenuation using an *in vivo*, mouse infection model.
Study design: From overnight cultures of *Ps. aeruginosa* and *S. aureus* grown with and without subinhibitory concentrations of PLA, bacterial suspensions of ~107 UFC/ml were prepared in PBS and used for mice inoculation in one single dose given by intravenous (IV), intranasal (IN) and intraperitoneal (IP) route. The mice were followed up during one week after infection and the body weight, mortality and morbidity rate were measured every day. The microbial charge was studied by viable cell counts in lungs, spleen, intestinal mucosa and blood. The mice lots infected with wild *Ps. aeruginosa* bacterial cultures exhibited 29% mortality rates after IV and IP route, exhibiting very high cell counts (>1011 UFC/g or ml) in blood, lungs, intestine and spleen. In exchange, the lots infected with PLA treated bacterial cultures exhibited good survival rates (0 mortality), even though, after euthanisation, high viable cell counts were found in their intestine and blood (~109 CFU/ml or g). As concerning the *S. aureus* infections, the experimental lots exhibited comparable survival rates, but significant differences were noticed concerning the clearance of bacterial cells from the infected mice, which was total in the experimental lots infected with PLA treated bacterial cells, comparatively with those infected with untreated cultures showing high microbial charge in their intestine.
Conclusion: In this study, using a mouse infection model we show that PLA can act as a potent antagonist of bacterial quorum sensing regulated virulence factors in *P. aeruginosa* and *S. aureus*, improving the survival rates as well as the clearance of bacterial strains from the body, in a specific manner, dependent on the infection route and the microbial strain.

O260

Rifampin induction of resistance in *Acinetobacter baumannii, in vitro* and in an experimental pneumonia murine model

M.E. Pachón-Ibáñez, F. Fernández-Cuenca, F. Docobo-Pérez, J. Pachón, A. Pascual *(Seville, ES)*

Objective: The aim of the present study was to determinate the frequency of resistance to rifampin (RIF) in multiresistant *Acinetobacter baumannii* (Ab) exposed to RIF, *in vitro* and *in vivo*, and the prevention of the development of resistance when RIF is used in association with other antimicrobials.
Methods: *In vitro* studies: a) *in vitro* selection of RIF-resistant variants, using time-kill curves, at concentrations of 1, 2, and 4 times the MIC of RIF and all the possible combinations of RIF with imipenem (IMP) or sulbactam (SB), at the same concentrations (1, 2, and 4 times the MIC, respectively); b) controls experiments with IMP and SB alone were performed; c) MIC of RIF of single colonies growth after 24, 48, and 72 h of incubation were determined; d) stability of RIF-resistant variants: the stability of variants of Ab resistant to RIF was tested with six daily passages in RIF-free agar plates. *In vivo* studies: a) a pneumonia experimental model in C57BL/6 mice was used; b) treatment groups (72 h): CON (without treatment), RIF (100 mg/kg/ip/d), IMP (120 mg/kg/im/d),

SB (240 mg/kg/im/d), IMP + SB, IMP + RIF, y SB + RIF; c) the MIC of RIF for the isolates obtained from the mice was performed; d) Frequency of RIF-resistant variants: the experimental pneumonia model was used with two treatment groups: CON (without treatment), and RIF (100 mg/kg/ip/d).
Results: *In vitro* results: a) the MIC of RIF increased from 4 mg/L to 128 mg/L for variants selected after 48 and 72 h of incubation of Ab in presence of RIF alone; b) the susceptibility to RIF did not change in the experiments with IMP or SB alone; c) with the different combinations of RIF plus IMP or SB, the MIC of RIF did not change; d) RIF-resistant variants maintained its resistance (MIC 128 mg/L) over six daily passages in fresh RIF-free agar plates. *In vivo* results: a) in mice treated with RIF alone, the MIC of RIF changed to >128 mg/L; in mice treated with RIF and IMP or SB the MIC of RIF remained at 4 mg/L; b) The frequency of RIF variants obtained was 3×10^{-6}.
Conclusions: These results suggest that rifampin must not be used alone in the treatment of infections caused by multiresistant *A. baumannii* due to the induction of RIF-resistance at high frequency. In these cases, rifampin may be used in combination with imipenem or sulbactam, which prevent the development of resistance.

O261

Experimental dose regimen modelling of intestinal emergence of *Enterococcus faecalis* resistant to linezolid in gnotobiotic mice

N. Bourgeois-Nicolaos, B. Couson, L. Massias, A. Andremont, F. Doucet-Populaire *(Paris, Le Chesnay, FR)*

Objectives: Linezolid (LNZ) is the first of a new class of antibiotics, the oxazolidinones. LNZ is approved for the treatment of Gram-positive bacterial infections. Selection of LNZ resistant (R) mutants during treatment has been reported. The clinical resistance to LNZ has been associated with mutation in the 23S rRNA domain V region. The aim of our study was to develop an experimental model of emergence of LNZ-resistant strains in the digestive tract.
Methods: We studied the rate of emergence of LNZ-R *E. faecalis* mutants in the digestive tract of gnotobiotic mice mono-associated with LNZ-susceptible *E. faecalis* and fed with water containing 0.5, 0.05 or 0.005 g/L of Li (6 mice/group). Fecal S and R *E. faecalis* were counted daily during 21 days on agar containing or not 4 mg/L of LNZ. 23S rRNA mutations were characterized by sequencing individually each of the 4 copies of the rRNA operons. LNZ concentrations in mouse pellets were measured by HPLC.
Results: In the 0.5 g/L group, a LNZ-R mutant (MIC 8 mg/L) was isolated in one mouse once at day one of treatment and R mutants (MICs 16 mg/L) were isolated in two mice after 21 days of treatment. In the 0.05 g/L treatment group, R mutants (MICs 32 mg/L) were isolated in all mice after 5 days of treatment and persisted at 9 log10 UFC/g of feces. In the 0.005 g/L treatment group, R mutants (MICs 8 mg/L) appeared after 4 days of treatment but persisted at 3 log10 UFC/g. Fecal concentrations of LNZ were 9.9, 0.6, 0.1 μg/g of feces for the 3 regimens, respectively. The R mutants selected in the 0.05 or 0.005 g/L groups had a G2576U mutation. By contrast, that from the 0.5 g/L treatment group had a G2505A.We observed a correlation between the number of 23S rRNA copies with the G2576U or G2505A mutation and the level of resistance to LNZ expressed by the mutants obtained *in vivo*.
Conclusions: Intestinal emergence of LNZ-R mutants could be readily obtained *in vivo* using a wide range of treatment doses. However striking differences were observed between dose regimens in terms of rate of emergence and intestinal concentrations of the mutants and type of associated mutations. Infection in the immunocompromised host (except HIV)

O262

Epidemiology of fungal infections in haematological stem cells transplanted patients: SEIFEM 2004 study

L Pagano, M. Caira, M.T. Van Lint, G. Irrera, A Bonini, A. Candoni, C. Caramatti, M. Picardi, R. Invernizzi, D. Mattei, L. Fianchi, P. Falcucci, G. Reddiconto, C.G. Valentini, L. Melillo, M.R. Fadda, A. Nosari, C. Girmenia, F. Aversa on behalf of SEIFEM *(Sorveglianza Epidemiologica Infezioni Fungine Nelle Emopatie Maligne)*

Objectives: To evaluate epidemiology and outcome of invasive fungal infections (IFI) in patients (pts) who underwent autologous (auto) or allogeneic (allo) hemopoietic stem cells transplantation (HSCT).
Methods: A cohort-retrospective study, conducted over 1999–2003, in HSCT patients admitted in 11 hematology divisions in tertiary cares or university hospitals, who developed IFI (proven or probable).
Results: We evaluated 3,228 pts who underwent HSCT: 1,249 (38.7%) allo HSCT and 1,979 (61.3%) auto HSCT. IFI occurred in 121 pts, with an overall incidence of 3.8%; we registered 91 episodes sustained by moulds (incidence 2.8%) and 30 by yeasts (incidence 0.9%). The incidence rate depended upon the type of transplant: 99 episodes occurred in allo HSCT (incidence 7.9%) and 22 in auto HSCT (incidence 1.1%). [Table I]Among moulds, the detected etiological agent was *Aspergillus* spp in 88 episodes (incidence 2.7%). *Candida* spp. was responsible of all the 30 yeast infections we registered (incidence 0.9%). As for aspergillosis, the intraspecies characterization was possible only in 33 cases (38% of total aspergillosis); *A.fumigatus* was identified in 19 cases (58%), *A. flavus* in 4 (12%), *A. terreus* in 5 (15%), *A.niger* in 5 (15%). Diagnosis was proven in 23 of 79 cases (29%), in the other 56 cases aspergillosis was probable. Overall mortality rate was 5.6% in allo and 0.5% in auto HSCT. The case fatality rate (CFR) registered in our population was 65% (79 deaths), with differences between allo HSCT (71%) and auto HSCT (41%). Etiology influenced the pts outcome: aspergillosis CFR was 72% (75% in allo HSCT and 29% in auto HSCT), while that one due to *Candida* spp. was 50% (53% in allo HSCT and 47% in auto HSCT). Interestingly aspergillosis CFR varied during the study period, decreasing from 93% in 1999 to 64% in 2003 (RR 0.69; IC95% 0.51–0.95; p-value = 0.047).

	Allo-HSCT		Auto-HSCT	
	N° cases	Incidence	N° cases	Incidence
Moulds	84	6.7%	7	0.3%
➢ *Aspergillus spp*	79	6.3%	7	0.3%
➢ Zygomycetes	1	0.1%	0	
➢ *Scedosporium spp*	3	0.2%	0	
➢ *Fusarium spp*	1	0.1%	0	
Yeasts	15	1.2%	15	0.8%
➢ *Candida spp*	15	1.2%	15	0.8%

Conclusion: IFI represent a frequent complication particularly for pts undergoing allo-HSCT. *Aspergillus* spp is the most frequently detected agent in these pts and it is characterized by a high mortality rate. Rarely auto HSCT pts develop aspergillosis.

Conversely we did not observe any difference in candidemia incidence and in the CRF between the two groups.

O263

Clustering of *Pneumocystis jirovecii* pneumonia cases in renal transplant recipients: coincidence or a clue for patient-to-patient transmission?

M.G.J. de Boer, L.E.S. Bruijnesteijn van Coppenraet, A. Gaasbeek, S.P. Berger, L.B.S. Gelinck, E.J. Kuijper, F.P. Kroon, J.P. Vandenbroucke *(Leiden, NL)*

Objective: Between the first of March and the first of October 2005 a sudden rise in the incidence of *Pneumocystis jirovecii* pneumonia (PCP) in renal transplant recipients was observed in the Leiden University Medical Center. To investigate the possibility of transmission from patient-to-patient or from a common environmental source, clinical, epidemiological and molecular characteristics of this cluster were analysed.
Methods: All renal transplant recipients in the Leiden University Medical Center diagnosed with PCP between the first of January and the first of October 2005 were included. The diagnosis had to be confirmed by direct microscopy or PCR of the dihydropteroate synthase (DHPS) gene in bronchial alveolar lavage fluid. Data concerning underlying disease, medication, co-morbidity, outpatient department visits and demographical data were obtained from the files. A transmission card was constructed to detect any contact between patients. Genotyping of *Pneumocystis* strains was performed by sequence analysis of the internal transcribed spacer number 1 (ITS1) and ITS2 gene regions.
Results: Out of 20 suspected cases, 16 were identified as confirmed PCP cases; 10 with microscopy and PCR, 6 with PCR only. All patients had clinical symptoms and radiological signs compatible with PCP; none died. The background rate is approximately 2 PCP cases per year. Risk factors for development of PCP e.g. active cytomegalovirus replication and treatment for rejection were present in 8/16 and 4/16 patients. No alterations in immune-suppressive regimens had been implemented. The transmission card showed that patient-to-patient transmission of *Pneumocystis* had been possible on multiple occasions. Sequence analysis of the ITS1 and ITS2 gene regions showed type 'Ne' in 9/16 samples and type 'Bi' in 1 sample. In 3 samples only the ITS2 genotypes could be determined (type 'e' twice and 'g' once). Determination of ITS genotypes failed in 3/16 samples.
Conclusions: In this cluster of PCP, clinical data as well as molecular typing are compatible with patient-to-patient transmission or with a common source of infection. However, definitive proof of transmission cannot be given on these data.

O264

Nosocomial legionellosis outbreak occurring in immunosuppressed patients in a cancer centre

C. Gudiol, J. Carratala, M.A. Dominguez, R. Verdaguer, A. Fernandez-Sevilla, F. Gudiol *(Barcelona, ES)*

Objective: There is scarce information regarding the impact of early diagnosis of nosocomial legionellosis by urine antigen testing and levofloxacin therapy on outcomes of immunosuppressed patients with this infection. We report a legionellosis outbreak due to *Legionella pneumophila* serogroup 1 occurring in a cancer center, in which these modalities of rapid diagnosis and treatment were used.
Methods: The outbreak involved 12 patients. Legionnairés disease was detected in the first patient, on Feb 24th 2005, and the outbreak lasted 4 weeks. After two more cases were diagnosed within 48 h, universal urine antigen testing (n = 155 patients) was indicated. Pulsed-field gel electrophoresis (PFGE) typing was performed in all isolates from sputum and water system samples. Superheating and flushing of water system were undertaken to control the outbreak.
Results: There were 6 males and 6 females, aged between 41 and 74 yrs. Seven patients had underlying hematologic disorders (lymphoma 4, acute leukemia 2, aplastic anemia 1) and 5 patients had solid tumors (lung cancer 3, disseminated cancer 1, pancreas cancer 1). All but one patients were receiving steroids and 6 patients other immunosuppressive drugs at the time or few days before the infection. Three patients had profound neutropenia and one of them had also invasive aspergillosis requiring ICU admission. Ten patients presented with pneumonia (multilobar 3, cavitated 1) while two patients were asymptomatic at the time when the urine antigen test was performed. Urine antigen test was positive in all 12 cases. *L. pneumophila* serogroup 1 was isolated from the sputum of 5 patients, and from water system samples. Only the first patient who was treated with clarithromycin died, whereas the remaining 11 patients who received levofloxacin survived. Overall mortality (<28 days) was 8.3%. PFGE typing demonstrated identical clonal patterns among isolates recovered from sputum and water samples. Superheating and flushing of water system successfully terminated the outbreak.
Conclusions: Early diagnosis of cases by universal urine antigen testing and early levofloxacin therapy were associated with outcomes much better than those classically reported in severely immunosupressed patients with legionellosis.

O265

Implications of the evidence for antibiotic prophylaxis for cancer patients with neutropenia: updated meta-analysis

L. Leibovici, A. Gafter-Gvili, M. Paul, A. Fraser, M. Cullen *(Petah Tikva, IL; Birmingham, UK)*

Objectives: To define groups of neutropenic patients likely to benefit from antibiotic prophylaxis, we updated a previous meta-analysis assessing antibiotic prophylaxis for afebrile neutropenia. We assessed outcomes for patients with solid tumors or lymphoma (SO/LY) and for patients with acute leukemia or bone-marrow transplantation (AL/ BMT).
Methods: Systematic review and meta-analysis including randomized controlled trials comparing any antibiotic treatment vs. placebo or no treatment (control) for afebrile cancer patients following chemotherapy. The last search was conducted in September 2005. The primary outcome assessed was all-cause mortality. Relative risks (RR, 95% confidence intervals) were calculated using the fixed effect model. Numbers needed to treat (NNT) were calculated using mortality rates observed in the placebo group of the new trials.
Results: Two large trials including 760 patients with AL/BMT and 1565 patients with SO/LY were added to the meta-analysis, altogether comprising 56 trials. Quinolones were assessed in 22 trials. Compared to control, any antibiotic prophylaxis significantly reduced all-cause mortality, RR 0.66 (0.55–0.79). The reduction was greater when limited to studies assessing quinolones, RR 0.55 (0.40–0.75). Among AL/BMT patients, significant reductions in all cause mortality were observed for any prophylaxis, RR 0.67 (0.55–0.83) and for quinolone prophylaxis, RR 0.58 (0.40–0.84). The NNT to prevent one death was 48 patients. Significant reductions in mortality were observed also in studies assessing SO/LY patients during the

first chemotherapy course: RR 0.58 (0.33–1.00) for any prophylaxis and RR 0.48 (0.26–0.88) for quinolones, NNT 84 patients. All secondary outcomes were reduced both in AL/BMT and SO/LY patients, including febrile neutropenia, bacteremia, clinically documented infections and infection-related mortality (Table). Both Gram-negative and Gram-positive infection rates were reduced. Fungal infections did not increase. Prophylaxis was not associated with serious adverse events. No significant difference in quinolone-resistant infections between study arms was observed in studies comparing quinolones to control.

Outcome	Acute leukemia/ BMT		Solid organ cancer/ lymphoma	
	Any AB	Quinolone	Any AB	Quinolone
All cause mortality	0.67 (0.55-0.83)	0.58 (0.40-0.84)	0.58 (0.33-1.00)	0.48 (0.26-0.88)
Febrile neutropenia	0.80 (0.77-0.84)	0.77 (0.73-0.82)	0.65 (0.55-0.77)	0.67 (0.57-0.80)
Clinically documented infections	0.76 (0.69-0.84)	0.75 (0.64-0.89)	0.59 (0.47-0.73)	0.61 (0.48-0.76)
Any bacteremia	0.55 (0.49-0.62)	0.62 (0.53-0.73)	0.32 (0.17-0.61)	0.36 (0.18-0.70)
Gram-negative bacteremia	0.44 (0.35-0.56)	0.36 (0.25-0.50)	0.29 (0.10-0.88)	0.32 (0.09-1.18)
Gram-positive bacteremia	0.71 (0.59-0.86)	0.81 (0.65-1.01)	0.32 (0.12-0.89)	0.35 (0.12-1.04)
Infection-related mortality	0.62 (0.48-0.80)	0.51 (0.31-0.84)	0.65 (0.29-1.46)	0.41 (0.14-1.14)

Relative risks with 95% confidence intervals; AB - antibiotic

Conclusions: Given the low NNTs for all-cause mortality, antibiotic prophylaxis using quinolones should be considered for all patients with AL/BMT. Among SO/LY patients, prophylaxis may be warranted during the first chemotherapy course.

O266

Bacteraemia in Danish patients with haematological malignancies: coverage and impact of inappropriate empirical antibiotic treatment on 7-day and 30-day mortality

M. Nørgaard, H. Larsson, G. Pedersen, H.C. Schønheyder, H.T. Sørensen *(Aalborg, Aarhus, DK)*

Objectives: To examine the coverage of the empirical antibiotic treatment in first episode of bacteraemia in patients with haematological malignancies and to determine the impact of inappropriate treatment on mortality.

Methods: We conducted this population-based cohort study in North Jutland County, Denmark from 1992–2002. We included all patients ≥15 years with a new haematological malignancy registered in both the Hospital Discharge Registry and the Danish Cancer Registry and a successive episode of bacteraemia registered in the County Bacteraemia Registry. Empirical antibiotic therapy was classified inappropriate if isolates were found to be resistant to all administered antibiotics, or if the doses or route of administration were insufficient. Mortality within 30 days was determined through the Civil Registration System. Cox's regression analysis was used to compare mortality rates with adjustment for age, gender, and co-morbidity. The analysis was stratified according to neutropenia (neutrophile count $<1.0 \times 10^9$/l).

Results: In total 358 patients were included. At first notification of positive blood culture 19 patients had already died or a decision to withhold therapy had been made because the patients were terminally ill. Further 8 patients were excluded due to missing information. In the remaining 331 patients empirical antibiotic treatment was found to be appropriate in 225 (68%) patients and inappropriate in 106 (32%) patients. The cumulative 7-day mortality in patients who were given inappropriate empirical antibiotic treatment was 15% vs. 11% in the appropriate group, yielding an adjusted mortality rate ratio (MRR) on 1.2 (95% CI: 0.6–2.4). The corresponding cumulative 30-day mortality was 35% vs. 25%, yielding an adjusted 30-day MRR on 1.3 (95% CI: 0.8–2.0). For the 134 neutropenic patients adjusted MRRs for 7 days and 30 days of follow up were 0.6 (95% CI: 0.2–1.7) and 0.7 (95% CI: 0.3–1.5), respectively. The corresponding MRRs for non-neutropenic patients were 2.1 (95%CI: 0.8–5.3) and 1.7 (95%CI: 1.0–3.0).

Conclusion: One third of haematological patients with bacteraemia were given inappropriate empirical antibiotic treatment, which is associated with increased mortality. Data do not support that neutropenic patients with bacteraemia are in particular sensitive to inappropriate empirical antibiotic treatment.

Multidrug-resistant Gram-negative organisms in the hospital and community

O267

Risk factors for faecal carriage of extended-spectrum beta-lactamase producing *Escherichia coli* and *Klebsiella* spp. in the community

Ö.K. Azap, H. Arslan, S.Ö. Karaman, T. Togan *(Ankara, TR)*

Objectives: Community acquired infections caused by ESBL-producing bacteria is an emerging problem. Digestive tract colonization is a prerequisite for infections by ESBL-producing microorganisms. The aim of this study is to determine the prevalence of and risk factors for fecal carriage of ESBL-producing *Escherichia coli* or *Klebsiella* spp. in the community.

Methods: A total of 928 stool samples admitted to the laboratory during the 4-month period were included in the study. Samples were diluted in saline and cultured in two EMB agar plates supplemented with 1 mg/mL cefotaxime and 1 mg/mL ceftazidime, respectively. All isolates that grew were identified to the species level. *E.coli* and *Klebsiella* spp. strains were tested for ESBL production with ceftazidime and ceftazidime-clavulonate discs acccording to the Clinical and Laboratory Standards Institute (CLSI) Guideline.

Results: Of the 928 stool samples included 133 (14%) were isolated from inpatients and 795 (86%) were isolated from outpatients. Sixty-three (47.3%) of 133 hospitalized and 121 (15.2%) of 795 outpatients harbored ESBL-producing *E. coli*,

Klebsiella spp. (p = 0.000). Chronic hepatic failure (OR: 8.7; CI: 1.65–46.12; p = 0.011) and recent antibiotic use (OR: 4.4; CI: 1.76–11.16; p = 0.002) were found to be associated with ESBL positivity for the hospitalized patients. Recent antibiotic use (OR: 2.8; CI: 1.61–5.12; p = 0.000) was found to be the only independent variable associated with ESBL positivity for the outpatients.
Conclusion: The high prevalence (15.2%) of fecal carriage of ESBL-producing bacteria in the community warrants further study in this field including the consequences of this colonization in the hospital setting.

O268

Differences in the epidemiology of extended-spectrum beta-lactamase producing *Escherichia coli* according to the types of enzymes

J. Rodríguez-Bano, M.D. Navarro, L. Romero, M.A. Muniain, J.R. Hernández-Bello, A. Pascual *(Seville, ES)*

Objectives: Extended-spectrum beta-lactamase-producing *Escherichia coli* (ESBLEC) are an emergent cause of community and nosocomial infections. We analyse the epidemiology of ESBLEC in our area according to the type of ESBL produced.
Methods: All patients from whom an ESBLEC was isolated in our area (550,000 population) from January 2001 to May 2002 were included. The following data were collected: age, sex, chronic underlying diseases, previous health-care relation (HCR), invasive procedures, previous antimicrobial use, and types of infection. ESBL production and antimicrobial susceptibility were studied by microdilution (NCCLS guidelines). ESBLs were characterized by isoelectric focusing, PCR, and sequencing. Clonal relationships among the isolates were determined by REP-PCR.
Results: Ninety-six cases were included; 77% of them had previous HCR. Forty-nine (51%) were outpatients. Among these, 65% were being attended in primary care, 20% in the emergency service and 14% in the outpatient's clinic. The other 47 patients (49%) were hospitalized. Seventy-four (77%) were considered to have an infection: UTI, 66%; skin and soft tissues, 18%; primary bacteraemia, 10%; and respiratory tract, 6%. Seventy-two percent of the isolates produced one ESBL, 26% produced 2, and 2% produced 3; 63% of the isolates produced CTX-M enzymes (mainly CTX-M-14), 47% produced SHV (mainly SHV-12), and 21% TEM. Two homogeneous groups were identified for comparison: patients with isolates producing only CTX-M (n = 47) and those producing TEM + SHV but not CTX-M (n = 14). Significant differences were found in nosocomial acquisition (36% vs 100%, p < 0.001), non-fatal disease (70% vs 36%, p = 0.05), diabetes (49% vs 21%, p = 0.06), urinary catheter (32% vs 64%, p = 0.03), and fluoroquinolone use (30% vs 64%, p = 0.01). Isolates producing only CTX-M enzymes were more frequently susceptible to gentamicin (83% vs 43%, p = 0.01) and ciprofloxacin (24% vs 0, p = 0.05). There was no clonal relationship among the CTX-M-producing isolates, while those producing TEM + SHV belonged to 2 clonal groups.
Conclusions: There are significant differences in the epidemiology of ESBLEC according to the types of enzyme produced. Isolates producing CTX-M enzymes are most commonly community-acquired, are less frequently resistant to non-betalactams, and are not clonally related, while those producing TEM + SHV cause nosocomial outbreaks. These differences should be taken into account for the design of control measures.

O269

Frequency and predictors of colonization of the respiratory tract by Gram-negative bacteria with the blaVIM gene in patients of a newly established intensive care unit

M. Horianopoulou, N. Legakis, M. Kanellopoulou, S. Lambropoulos, A. Tsakris, M. Falagas *(Athens, GR)*

Objectives: Our aim was to examine the frequency and predictors of the colonization of the respiratory tract by Gram-negative bacterial with the blaVIM gene in patients admitted to a newly established intensive care unit (ICU) of a tertiary care hospital.
Methods: Specimens of tracheobronchial aspirates were obtained every day during the first three days of the ICU stay and subsequently every third day during the rest of the ICU stay for microbiological studies. Also, cultures of specimens from the axillary and the inguinal area of the patients were taken during the fifth day of the ICU stay. In addition, cultures of specimens from several environmental sites as well as from the oropharynx and the hands of the staff were taken. Molecular studies were performed to identify bacteria with the blaVIM gene. Also, an analysis of potential risk factors for colonization with Gram-negative bacteria with the blaVIM gene was done.
Results: Thirty-five patients (20 male, 15 female) were hospitalized during the initial three-month period of the function of the ICU. Colonization of the lower respiratory tract by Gram-negative bacteria was found in 29 of 35 patients (83%) during the first 6 to 20 days (median 13 days) following admission to the ICU (*Acinetobacter baumannii* 13 patients, *Pseudomonas aeruginosa* 10, *Enterobacter aerogenes* 3, *Klebsiella pneumoniae* 2, and *Stenotrophomonas maltophilia* 1). *A. baumannii* and *P. aeruginosa* were isolated from 6 and 4 out of 80 cultures of specimens from patient bed bars, respectively. Six of 29 (21%) of patients with colonization with Gram-negative bacteria had microorganisms with the blaVIM gene. Previous use of carbapenems (p = 0.01) or other b-lactams (p = 0.03) as well stay in the ICU more than 20 days (p = 0.01) were associated with colonization with a bacterium with the blaVIM gene.
Conclusion: Colonization by Gram-negative bacteria of the respiratory tract of patients in this newly established ICU was common (83%). Use of b-lactams including carbapenems was associated with the subsequent colonization of the respiratory tract with Gram-negative bacteria with the blaVIM gene.

O270

Epidemiological investigation of quinolone resistance in infections due to extended-spectrum beta-lactamase-producing *Enterobacteriaceae* in a tertiary hospital

A. Katsandri, A. Vasilakopoulou, V. Mela, G.L. Petrikkos, C. Kosmidis, J. Papaparaskevas, A. Avlamis *(Athens, GR)*

Objectives: The incidence of infections due to extended-spectrum β-lactamase (ESBL)- producing *Enterobacteriaceae* has increased markedly in recent years. Although treatment is difficult because of frequent multidrug resistance, quinolones offer an effective alternative therapy. Aim of this study was the identification of risk factors for quinolone resistance in ESBL infections.
Materials and methods: A prospective study of 84 consecutive ESBL hospital infections in a tertiary care hospital during one year period (15/1/2004–15/1/2005) was conducted. Data collected included gender, age, ward type, duration of

hospitalization, number of hospital days before infection, infection site, invasive procedures, underlying diseases and outcome. The antimicrobial profile of the infecting organism, as well as all antimicrobial therapy prior to recovery of an isolate were documented. Infections by quinolone resistant ESBL producing bacteria (QR-ESBL) were used as cases and those by quinolone sensitive ESBL (QS-ESBL) producing isolates were used as controls.

Results: 84 ESBL isolates were identified in clinical specimens from 77 patients. The prevalence of QR-ESBL bacteria was 59.52%. The median age of patients was 60 years and the percentage of females was 58.4%. The distribution of ESBL isolates was: *E. coli* 51%, *Klebsiella* spp. 17%, *Enterobacter* spp. 9%, *Proteus* spp. 3%, *Serratia* spp. 2% and others 2%. The site of infection varied, the most frequent sites being: the urinary tract (65.5%), wound or surgical site (14.3%), bacteraemia (10.7%), the respiratory tract (7.1%) and other (2%). The overall mortality was 26%. Isolates from kidney transplant patients were significantly more likely to be quinolone resistant ($p = 0.047$; OR = 2.56; 95% CI, 0.99–6.58). Infection from QR-ESBL bacteria was associated with increased hospital stay (mean days 46.28 vs 35.28, $p = 0.028$). On the average, QR-ESBL bacteria were isolated from cultures later during hospitalization than QS-ESBL bacteria (mean days 30.64 vs 19.44, $p = 0.089$). Factors associated with quinolone resistance were the previous use of carbapenems ($p = 0.063$) and quinolones ($p = 0.058$). QR-ESBL bacteria were significantly more likely to be resistant to co-trimoxazole ($p = 0.002$), gentamicin ($p = 0.047$) and tobramycin ($p = 0.006$).

Conclusions: Quinolone resistance is widespread among ESBL producing bacteria and the risk factors associated were increased hospital stay and the previous use of carbapenems and quinolones.

O271

The changing epidemiology of sequential outbreaks of multiresistant *Acinetobacter baumannii* bacteraemia: a ten-year study in an intensive care unit

J.J. Camarena, R. Gonzalez, R. Zaragoza, J.C. Navarro, S. Sancho, A. Artero, J.M. Nogueira *(Valencia, ES)*

Objective: During the last ten years, multiresistant *Acinetobacter baumannii* (MRAb) bacteremia remains like the most important problem in ICU-nosocomial acquired infections in our teaching hospital. The goals of this study were to describe the pheno-genotypic characteristics of the MRAb bloodstream infections, the temporal evolution of the clones associated to outbreaks or endemic infections and the influence of adequate empiric treatment (AET) or control measures.

Methods: Prospective study of all strains of *A. baumannii* isolated in bacteremic patients of an ICU during the last 10 years. Genotypic analysis of DNA by PFGE and REP-PCR. Susceptibility study of the isolates performed by disk-diffusion and E-TestR. Clustering of the phenol-genotypic patterns and analysis of their temporal evolution. Analysis of AET and preventive control measures.

Results: A total of 507 isolates from 137 significant Ab bacteremia cases in ICU were analysed. The impact of Ab bacteremia on ICU- acquired infections was differed in two study periods. In the first one (1995–2001) seventy-two episodes (52.5%) were classified in six mainly clusters. The multiresistant, including carbapenems, outbreaks was caused in this period by two clusters which grouped the 66.6% of the cases: R3/97/C3 (96.5% of isolates in 1996–98), and R3B/99/C3B (58% of isolates in 1999–2000). Since February 2001, with the description of the R5C5 cluster carbapenem sensible, the MRAb problem seemed to be eradicated and Ab remained endemic in the ICU until 2002. In the second period (2002–2005) MRAb problem reappears with the detection of 65 news cases (47.4%) with 3 mainly clusters all of then carbapenems and doxicicline resistant (R7/03/C7, 40.2% of isolates in 2003–04; R8/04/C8, 60.3% of isolates in 2004–05; and R3/05/C10, only colistin sensible, emergent in 2005 with 38.2% of the isolates, which is the actual cause of the MR-Ab outbreak in the ICU.

Conclusions: Since the detection of the MRAb bacteremic cases in 1995, an endemo-epidemic situation, with nine sequential outbreaks pheno-genotipically characterized, has been described in the ICU in our teaching hospital. After ten years, MRAb bacteremia in our ICU continues to be, despite the AET guidelines and preventive measures for the control of this endemo-epidemic situation, a serious ICU-problem.

Infection control

O272

New World Health Organization alcohol-based formulations to implement handrubbing as the gold standard for hand hygiene worldwide

B. Allegranzi, A. Voss, M. Rotter, S. Sattar, W. Griffiths, S. Dharan, L. Donaldson, D. Pittet *(Verona, IT; Nijmegen, NL; Vienna, AT; Ottawa, CA; Geneva, CH)*

Objective: The advanced draft of the WHO ''Guidelines on Hand Hygiene (HH) in Health Care'' was recently issued. Although all means to ensure HH should be promoted, the Guidelines recommend alcohol-based hand rubs (AHRs) at the point of care as the gold standard for HH and provide instructions for in-house local production of suitable, effective, and low-cost formulations.

Methods: A group of international experts in microbiology and infection control were consulted to identify the ingredients and related proportions of a WHO alcohol-based formulation for handrubbing. Issues related to the procurement of raw materials, safety, distribution and storage of the finished product were also discussed and solutions proposed.

Results: The two proposed formulations have different alcohol content (ethanol 80% V/V and isopropyl alcohol 75% V/V) and include H_2O_2 (final concentration, 0.125%) as a preservative against spores and the same humectant-like substance to increase user acceptance. WHO-designated,

independent laboratories are testing the formulations for efficacy according to ASTM International and the European Committee for Standardization methods. Based on expert opinion, these formulations, preferably in liquid form rather than gel, are suitable for both hygienic hand antisepsis and surgical hand preparation. For the latter, substances with sustained antimicrobial activity could be added, but are not considered necessary for routine hand antisepsis. Other alcohol-based handrubs with equivalent safety and efficacy profiles may also be considered. The issue of alcohol content should be considered in settings where its use is prohibited for religious or cultural reasons. In case of reuse, containers should be cleaned and decontaminated by autoclaving, boiling or chemical disinfection with chlorine. Reusable bottles should be refilled only when empty and cleaned. In tropical climates, in particular, additional care is needed in storage of alcohol-containing materials.

Conclusions: Governmental authorities and healthcare administrators should make every possible effort to provide healthcare workers with AHRs at the point of care to achieve maximum benefit and optimal compliance with HH. The proposed formulations are designed to be suitable for worldwide use based on cost, efficacy, procurement and cultural factors. Within the pilot phase of the WHO Guidelines implementation strategy the proposed formulations are being evaluated for feasibility and acceptability.

O273

Decrease of external ventricular or lumbar drain related infections by a multidisciplinary approach

M.A. Leverstein-van Hall, T.E.M. Hopmans, H.E.M. Blok, J.W. Berkelbach van der Sprenkel, M.J.M. Bonten *on behalf of the UMCU Working Party Neurosurgical Drain infections*

Introduction: Extraventricular (EVD) and lumbar drains (LD) are important temporary measures for patients requiring continuous cerebrospinal fluid (CSF) drainage. Reported incidences of drain related meningitis have varied from 2.4 to 15%. In the UMCU, microbiologically confirmed CSF infections in patients with EVD/LD increased from 28% in 2001 to 47% in 2003. The aim of this prospective study was to reduce the incidence of drain-related meningitis to less than 10% in 2005.

Patients: All patients who received EVD or LD from Jan–April 2004 (period I), Aug–Dec 2004 (period II), and Jan–May 2005 (period III).

Interventions: A multidisciplinary team (neurosurgery, clinical microbiology, and hospital hygiene) designed a strategy based on 4 pillars: (1) implementation of drain management protocols for medical and nursing staff based on the ''no-touch'' concept, optimal hygiene and strict criteria for placement and removal of drains, (2) implementation of an algorithm on diagnostic and therapeutic management of patients suspect of drain-related meningitis, (3) implementation of a new protocol on pre-operative prophylaxis, (4) introduction of a closed drain system. Results are depicted in the table. The decrease of the infection rate in period III versus I was more pronounced for ELD than for EVD. The RR to acquire an infection per 100 days at risk decreased for the ELD with 0.2 (95%-CI: 0.03–1.7) and for the EVD with 0.7 (95%-CI: 0.2–2.2). From period I to III a sharp shift was observed in the kind of micro-organisms isolated from the CSF; in period III the typical nosocomial pathogens had disappeared.

Results:

Period	Baseline[1]	I	II	III
Interventions				
1) - Awareness	-	+	+	+
- Protocol development	-	+	+	+
- Implementation of protocol	-	-	+	+
- Enforcement of protocol	-	-	-	+
- Insertion LD in special room	-	-	+	+
2) Diagnostic and therapeutic management	-	-	+	+
3) Protocol on surgical prophylaxis	-	-	+	+
4) Introduction of closed drain-system	-	-	-	- / +
Number of patients with EVD or LD	115[2]	67	64	53[3]
Number of CFS sample per 100 drain-days	-	27.5	34.2	27.8
Patients with drain-related infections (%)	45 (39.1)[2]	12 (17.9)	9 (14.1)	5 (9.4)
Drain-related infections per drain-episode (%)	-	12 (14.6)	9 (13.4)	5 (7.6)
EVD infections per 100 days at risk	-	1.8	2.1	0.9
LD infections per 100 days at risk	-	4.5	1.3	1.8
Multiple drain infection episodes	-	4	0	0
Total number of nosocomial infections	-	46	28	18
% patients with nosocomial infections	-	40.3	28.1	28.3
Incidence per 1000 days at risk	-	40.1	31.4	18.8
Adequate antibiotic prophylaxis % EVD	-	37	38	35
No prophylaxis EVD %	-	25	19	28

[1] baseline period January 2002-June 2003; [2] only EVD; [3] incomplete data

Conclusions: The incidence of drain-related meningitis has decreased from 39% in 2003 to less than 10% in 2005. Awareness of the problem and the interventions performed in 2004 were probably important causative factors in this reduction. Adequate prophylaxis remains a point of concern and new strategy seems needed for improvement.

O274

Evidence for the effectiveness of 'Search and destroy' of methicillin-resistant *Staphylococcus aureus* in the Netherlands

M.J.C.A. van Trijp, D.C. Melles, A. Ott, W.D.H. Hendriks *(Rotterdam, NL)*

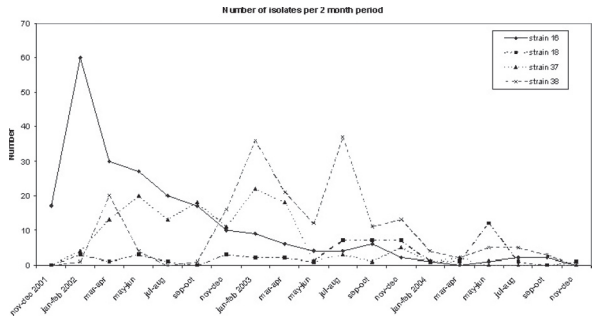
Objectives: Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important pathogen causing nosocomial infections. Prevalence of MRSA varies widely among countries. In the Netherlands MRSA prevalence in clinical isolates is below 1%, due to a national 'Search and destroy' policy. The efficacy of this policy in endemic situations is debated. In this study we describe a large MRSA outbreak with a highly transmissible strain in a Dutch hospital, and the measures that were taken to control the epidemic.

Methods: MRSA was isolated using conventional culturing, including a selective broth. MRSA-isolates were typed using Pulsed Field Gel Electrophoresis. Measures used to control the epidemic included: screening of contacts (patients and hospital staff), screening at re-admission and in some departments at regular intervals and at discharge. In addition: strict isolation of colonized or infected patients, decolonisation of patient and hospital staff MRSA carriers using topical agents. Also an electronic patient signalling, and personnel culture-information system was developed.

Results: The epidemic started around November 2001. As the involved strain had a low oxacillin MIC, it was not immediately recognized as MRSA. In January, when gradually more contacts were screened, it appeared that MRSA had spread to many departments, and that members of staff were also colonized. During Nov 2001 – Dec 2004 722 new isolates were detected among 559 patients and 129 staff members respectively; some persons were infected more than once. Of these isolates 87% belonged to four epidemic clones, the remainder being sporadic types. Since May–June 2004, when a last upsurge of one of these clones occurred, only sporadic cases have been detected.

Conclusion: This study shows that with application of a strict 'Search and destroy' policy even endemic MRSA can be controlled.

ISSN: 1470-9465



O275

Reducing the impact of methicillin-resistant *Staphylococcus aureus* infection in an intensive care unit: a nine-year analysis

A. Pan, E. Raineri, P. Mondello, L. Ferrari, G. Carnevale, A. Acquarolo, S. Lorenzotti, S. Magri, L. Soavi, L. Crema *(Brescia, Cremona, IT)*

Objective: To evaluate the effectiveness of a control system for nosocomial transmission of methicillin-resistant *Staphylococcus aureus* (MRSA) in an Intensive Care Unit (ICU) in a hospital with hyperendemic MRSA.
Methods: We retrospectively analysed prospectively collected data. This study was performed in a 10 bed general ICU in Italy, with high rates of MRSA transmission and infection rates: 6.0 and 2.8 cases per 100 admissions respectively. Three phases were identified over a 9-year time period. Period 1 (p1), 1996–1997, prior to the introduction of the hospital MRSA control program. Period 2 (p2), 1998–2002, after the introduction of the MRSA control program guidelines. Period 3 (p3), 2003–2004, after the ICU moved into a new ward, when MRSA modified guidelines, including isolation or cohorting, were implemented. All patients admitted to ICU were included in the analysis. MRSA guidelines included active surveillance, contact precautions and topical treatment of carriers. In 2003 modified MRSA guidelines included isolation/cohorting of MRSA positive patients.
Results: The rate of MRSA infected patients during p1, p2, and p3 was: 2.8, 1.2, and 0.6 cases per 100 admissions, respectively. A significant reduction of the infection rate was observed comparing p1 both with p2 ($P = 0.002$; RR: 0.4 CI95%: 0.22–0.73) and p3 ($P = 0.0008$; RR: 0.22 CI95%: 0.08–0.56). No significant reduction of the infection rate was observed between p2 to p3, ($P = 0.2$; RR: 0.54 CI95%: 0.21–1.37). A significant reduction of the methicillin resistance rate of *Staphylococcus aureus* isolates was observed: 51%, 32%, and 16% during p1, p2, and p3 respectively ($P < 0.0001$ for trend).
Conclusions: The ''search and destroy'' strategy, based upon a combination of active surveillance, contact precautions and topical treatment might be effective in significantly reducing MRSA acquisition rate even in an ICU with high rates of MRSA colonization / infection. Patients' isolation or cohorting did not further improve the results obtained with the ''search and destroy'' strategy.

O276

Comparison of the bacterial efficacy and acceptability of an alcohol-based hand rinse with 2 alcohol-based hand gels during routine patient care

F. Barbut, E. Maury, L. Goldwirt, D. Neyme, R. Aman, B. Rossi, P. Boelle, G. Offenstadt *(Paris, FR)*

Most alcohol-based gels used for handrubbing have been reported less efficacious than alcohol based rinses using the standard method EN1500, but usually better accepted by health care workers (HCWs). We compared the bacterial efficacy of handrubbing with an alcoholic rinse with two different alcoholic gels in reducing hand contamination under practical use.
Material and methods: A prospective crossover clinical trial was performed in a 14-bed medical intensive care unit in a French university-affiliated hospital during 3 consecutive 6-weeks periods. For each period, only one product was available for handrubbing: HCW used either Sterillium* rinse (45% 2-propanol; 30% 1-propanol; 0.2% mecetronium ethyl-sulfate) or Sterillium* gel (85% ethanol) or Manugel Plus* (Ethanol 53%, isopropanol 17%). Immediately after a direct contact with a patient, pre- and post-handrubbing hand contamination was measured using the glove juice technique. Bacterial counts were evaluated blindly on trypticase soy agar plates. At the end of each period, a self-administered questionnaire was completed by the HCWs. Skin tolerance and acceptability were assessed using a visual analogical scale (VAS). Data were entered and analysed using EpiInfo software 6.04d (CDC, Atlanta).
Results: We studied 242 hand-rubbing opportunities. Median duration of handrubbing was 30, 27, and 35 sec. for Sterillium* rinse, Sterillium* gel and Manugel Plus*, respectively. The mean reduction factor (expressed as the log10 CFU/mL) of the Sterillium* rinse, Sterillium* gel and Manugel Plu* were 1.28 ± 0.95, 1.29 ± 0.84 and 0.51 ± 0.73, respectively. Sterillium* rinse and Sterillium* gel were both significantly more effective in reducing bacterial counts from the hands than Manugel Plus* ($p < 0.001$). Sterillium* rinse and Sterillium* gel were also significantly better tolerated (VAS for dryness: 3.56 and 2.5 versus 7.5, $p < 0.05$), and better accepted (VAS 6.8 and 7.9 versus 4.9, $p < 0.05$) than Manugel Plus*.
Conclusion: When testing under practical use conditions, Sterillium* gel was found similar to Sterillium* rinse but they were both more efficacious and better tolerated than Manugel Plus*.

Galactomannan antigen detection in invasive aspergillosis: test optimisation and impact on patient management (Symposium organised by Bio Rad)

S277

Optimisation of the cut-off value of the Platelia Aspergillus ELISA

P.E. Verweij, C. Masson, R. Klont, C. Heinen, B. Crepin, J. Maertens *(Nijmegen, NL; Marnes-La-Coquette, FR; Leuven, BE)*

Objectives: The Platelia Aspergillus (PA)-ELISA (BioRad, France) is routinely used in many institutes for the early diagnosis of invasive aspergillosis (IA). There is considerable variability in the cut-off values used. Although a cut-off of 1.5 is recommended by the manufacturer, most institutes use a lower cut-off value varying between 1.0 and 0.5. In the USA the same kit is used with an approved cut-off value of 0.5. The aim of the current study was to re-evaluate the cut-off level of the PA-ELISA.
Methods: A retrospective study was performed using prospectively collected and analysed serum samples from adult patients with hematological disorders at high risk for developing IA. The data were collected between 2001 and 2005 in 2 different sites (site 1, University hospital Gasthuisberg, Leuven, Belgium and site 2, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands). Patients were classified according to the EORTC/MSG consensus definitions and a ROC analysis was performed in order to determine the optimal cut-off value.
Results: 123 treatment episodes from 88 patients were enrolled from site 1 (3929 serum samples) and 116 episodes from 115 patients from site 2 (944 serum samples). Among these patients, 19 were diagnosed with a proven IA and 19 with a probable IA. The analysis on the cumulated data of proven and probable cases, showed that a decrease of the positivity index from 1.5 to 0.5 corresponded with an increase of sensitivity of 21% (76.3% to 97.4%) and a decrease of specificity of 7 % (97.5% to 90.5%). The performance at a cut-off value of 0.5 was similar at both sites: sensitvity and specificity was 100% and 92.2% respectively at site 1 and 94.4% and 88.8%, respecitvely, at site 2. Combined, at a cut-off of 0.5 the highest sensitivity (97.4%) was achieved as well as an acceptable specificity (90.5%) and a positive and negative predictive value of 66.1% and 99.4%, respectively.
Conclusion: At a cut-off value of 0.5 the most favourable performance characteristics were found with the PA-ELISA. This cut-off value is identical to that approved in the USA.

S278

Galactomannan and computed tomography-based preemptive antifungal therapy in neutropenic patients at high risk for invasive fungal infections

J. Maertens *(Leuven, BE)*

A timely and reliable diagnosis of invasive aspergillosis in patients with an underlying hematological disorder remains frustratingly difficult and is seldom-achieved ante mortem. Conventional laboratory diagnostic methods are insensitive and time consuming, resulting in late diagnosis and treatment and contributing to unacceptably high mortality. In response to these diagnostic obstacles, prophylactic and empiric strategies have been recommended, especially in prolonged neutropenic patients and hematopoietic stem cell transplant recipients. However, over treatment associated with these strategies results in increased toxicity and cost. The use of sensitive and rapid non-culture based diagnostic assays, such as the detection of Aspergillus antigens (galactomannan, beta-D-glucan) or the detection of genomic DNA sequences may allow a shift in emphasis from empirical to pre-emptive therapy, especially when substantiated by suggestive radiological findings. These tools may be used to confirm a presumed diagnosis of invasive aspergillosis, or, when used to screen high-risk patients, may identify an infection at the early stage of disease. The excellent negative predictive value of these new tools may convince clinicians to withhold antifungal therapy in persistently febrile neutropenic patients with no other signs of fungal infection. On the other hand, consecutive positive results in a high-risk population should at least trigger a complete diagnostic work-up.Whether pre-emptive strategies will finally improve patient outcome and whether they will prove to be cost-effective remains to be investigated in carefully designed randomized clinical trials.

How to detect the next emerging pathogen

S282

How to detect new viral agents and what Europe should do

X. de Lamballerie *(Marseilles, FR)*

Prediction of the emergence of viral pathogens is intrinsically difficult and highly pretentious. However, assessment of our current knowledge and of the present time organisation of diagnosis & research laboratories allows to propose a few modest but practical guidelines.1. Is it possible to elaborate a specification sheet of good candidates for viral emergence?There are ~20viruses that meet the 1992 WHO definition of emerging pathogens and are responsible for several million of deaths each year. They are not associated with specific clinical presentations (hepatitis, immunosuppression, haemorrhagic fever, encephalitis, respiratory syndrome etc. are observed) and transmitted by various routes (aerosols, oral-fecal, bite of arthropods, sexual relations etc.). However, strikingly, 90% of these viruses have an RNA genome and 90% have a zoonotic origin.2. Is it possible to identify common features in previously observed scenarii of emergence?In most of the cases, 3 steps can be reported: (i) the transmission of an animal virus to humans: this step is poorly understood but human activity (& not plasticity of virus genomes) seem to be a crucial determinant. (ii) the inter-human transmission: selection of virus mutants is a key factor that allows the emergence of an "humanised" virus. (iii) the massive spread of the disease in humans is, again, primarily linked with human behaviour.Since parameters in relation with

ISSN: 1470-9465

viral genetics will not to change in the future while risks in relation with human behaviour (travels, deforestation, overpopulation etc.) increase constantly, it is not expected that viral emergence will decrease in the future. 3. Do we have the appropriate laboratory capacity for diagnosis?Previous bioterrorist alerts have shown that centralised diagnostic structures would be poorly adapted to the diagnosis of emerging pathogens in case of outbreak. The elaboration of standardized diagnostic kits (especially real-time PCR assays) and their availability in a large number of hospitals is necessary but requires a large effort at organising things.4. Do we have the appropriate laboratory capacity for research?(i) Efforts are necessary to bring together medical & veterinary researchers within common research and survey programmes. (ii) The presence of European research institutions in Developing Countries is important for studying emerging pathogens. It is to date poorly organised, expensive and merits reassessment. (iii) The study of animal viruses related to human pathogens should be promoted and (iv) a tremendous effort is needed to identify antivirals effective against RNA viruses.The role of the EC is potentially of major importance. The EC can support large research programmes aiming at promoting the diagnosis of emerging viruses, studying virus groups likely to include emerging viruses and identifying new antivirals.

Paediatric viral respiratory tract infections: the major killers

S287

The link between asthma and viral respiratory infections

N. Papadopoulos *(Athens, GR)*

Viral respiratory infections have been related to asthma in several ways. Severe bronchiolitis in early life is related to subsequent wheezing and therefore may represent a marker of susceptibility to asthma; alternatively it could be involved in the initiation of the disease. It has also been proposed that some infections may protect from the development of asthma and allergies by promoting a type-1 host response. However, whether respiratory or other viruses could mediate such a protective effect is debated. Nevertheless, it is well established that viral common colds are the major precipitants of asthma exacerbations. Using sensitive diagnostic methodologies, epidemiological studies during the last decade have allowed the identification of human rhinoviruses (RVs), generally recognized as "common cold viruses", as major asthma precipitants. This association was further established by evaluating the impact of RV infection in airway obstruction and inflammation during naturally acquired or experimentally induced RV colds. There is now strong evidence that RVs are able to infect and propagate not only in the upper but also in the lower airways. Bronchial and pulmonary epithelia infected by RVs are rich sources of inflammatory mediators, which may initiate and/or augment airway inflammation and obstruction. Furthermore, in an atopic environment, responses to the virus are skewed by and towards an "atopic", Th-2 like, balance, which may further enhance inflammation and exacerbate asthma. Recent evidence indicate that such an immune environment may also affect the epithelial inflammatory response to the virus: a reduced production of proinflammatory mediators is observed, correlating with increased viral proliferation and increased virus-induced cytotoxicity. This is also associated with increased production of growth factors such as TGF-b and VEGF which affect with fibrosis and angiogenesis respectively, suggesting that viral infections may also be implicated with airway remodelling in asthma.

S288

Paediatric influenza: severity and need for protection

A. Linde *(Solna, SE)*

Children are the engines of influenza epidemics in interpandemic years. They have no or limited immunity to circulating virus strains, and therefore the age-specific attack rate is highest among children. Twenty to twenty-five per cent of young children may contract the disease yearly. The main complication is otitis media, and 5–8 children per100 are prescribed antibiotics yearly indirectly due to influenza. The risk for hospitalisation is highest in children below 2 years of age, and has been estimated to be 103/105 person months. Main reasons for hospitalisation are suspected septicaemia, lower respiratory tract infection, asthma/bronchitis and febrile seizures. Influenza infection is normally self-limiting in children, but fatalities occur. Children with severe heart and/or lung disease or other severely debilitating disease belong to risk groups of severe influenza like adults. During the influenza season 2003–2004 more than 140 children were reported dead due to influenza in the US, and in Japan there has been several cases of lethal encphalopathia in influenza-infected children during recent years. Most of the dead children did not belong to any known risk group. Influenza may thus be a severe disease among children, and children below three years of age are most vulnerable. The ordinary influenza vaccine is more reactogenic in children that adults, and vaccination of children demands two doses. With a good strain match, vaccination has a protective efficacy of 70–90%, and it may reduce the incidence of otitis media with around 30%. In the US vaccination of children of 0.6 to 23 months has been recommended since 1993. The measure is regarded cost-effective, and it may also be diminish the spread of influenza to the elderly. Despite this, no European country recommends general influenza vaccination of children, and even the rate of vaccination of vulnerable children at risk of complicated disease is low. One reason may be an unproven fear that vaccination may prevent the development of basic immunity against influenza. Intranasal vaccination with live, cold-adapted virus has proven to induce similar heterolgous immunity to drifted influenza strains as wild-type infection in children, and a large study of the vaccine has recently been conducted in Europe. The outcome has not been published (Jan 2006), but if successful it may be an attractive option for prevention of the consequences of influenza in children.

S289

Human metapneumovirus infections in children; approaches for prevention

B. van den Hoogen *(Rotterdam, NL)*

Acute respiratory tract infections (RTI) are a major cause of morbidity and mortality worldwide. In 2001, we detected a previously unknown virus in samples from hospitalised

children suffering from RTI. Classical virology and genetic analyses revealed that this "novel" virus was the first human member of the genus Metapneumovirus in the Pneumovirinae subfamily of the Paramyxoviridae family. This human metapneumovirus (hMPV) is related to RSV, which belongs to the Pneumovirus genus within the same subfamily. After the identification of hMPV researchers around the world have begun to analyse the epidemiology and clinical presentation of hMPV related disease, demonstrating that the virus circulates world wide, primarily in the winter months. hMPV infections account for approximately 5–25% of the reported RTIs in hospitalised children, primarily between 6 and 12 months. RSV often is found in children that are slightly younger. hMPV infections in patients older than 5 years do occur, and many of these patients have impaired immunity or underlying disease. In the general community, hMPV accounts for 1–9% of RTI-related visits to physicians. A wide spectrum of clinical symptoms associated with hMPV infection has been reported in patients of all ages, ranging from mild RTI to severe disease requiring hospitalisation. In comparative studies, hMPV infections tend to be slightly milder than RSV infections, although in many studies the differences are not statistically significant. Recombinant hMPV has been produced by using reverse genetics techniques. These techniques are now used to develop live attenuated vaccines, based on deletion of genes or insertion of heterologous genes. In animal studies these viruses have shown to be attenuated but are able to produce virus neutralising antibodies and protect against infection, and therefore could be future vaccine candidates. For RSV the prophylactic and therapeutic use of a virus neutralising monoclonal antibodies directed against the fusion protein has been reported to decrease the severity of illness. Similar reagents are currently being developed for hMPV.

Limiting antibiotic usage in the community: benefits and collateral effects

S296

What is the impact of reductions in antibiotic use on antibiotic resistance in the community?

P. Huovinen *(Turku, FI)*

The goal of rational use of antimicrobial agents is effective treatment with minimal amount of harmful side-effects. To deal with bacterial infections we have to use antimicrobial agents, but we also know that there is a substantial overuse of these drugs in most parts of the world. The side-effects include not only allergic reactions, antibiotic diarrhoea, costs and development of bacterial resistance but also disturbance of the normal human microbiota (normal flora) that may be more important than we have previously thought. Bacterial resistance is linked with consumption of antimicrobial agents both in hospitals and in the community. On the country level, less antimicrobial agents is linked with less bacterial resistance, but resistance bacteria may spread despite of low level of antibiotic use. Several articles show that use of certain antibiotics has lead to increased resistance against these drugs. However, there are only few studies that show effect of reduction of antibiotic use on high level of resistance. Due to multiresistance, reduction of antibiotic use may not be the best way to decrease antibiotic resistance. The most rational way to tackle antibiotic resistance is to try to prevent development and spread of resistant bacteria. This includes optimal antimicrobial prescribing but also improved hygienic measures especially in child day care centres. Optimal antimicrobial prescribing needs national current care guidelines that are uniformly or widely accepted. The term 'optimal or rational use' includes careful clinical and laboratory diagnostics. Automatic computer notifications may be the best way to guide prudent antimicrobial prescribing. Current care guidelines have also to be flexible to react when bacterial resistance situation is changing. Economical approaches, pricing and especially reimbursement policy, may also be reasonable approaches. There is a need to increase public awareness on antibiotic use and harmful effects linked to improper use. For this purpose, several interesting publications have recently been published. These reports show that healthy human microbiota seems to protect use against allergy, obesity and cancer and antibiotic use may greatly disturb this balance. Paradoxically, antibiotic use may also increase risk of bacterial infections. Once more data is accumulated, we may better understand the value of our microbiota, and, hopefully, patients may not anymore be so eager to ask for antimicrobial prescriptions. Bacterial resistance is emerging, and we have to protect our normal microbiota. Therefore, new therapeutic options should be developed to treat and prevent bacterial infections. There are already interesting new study fields, like bacteriotherapy and therapy with bacteriophages or their enzymes. Future will show the importance of these approaches. Finally, I will also put my hope on unexpected discoveries and totally new solutions that medical technology may create. We need creative innovations.

S297

Have reductions in antibiotic use in general practice led to re-emergence of infections?

R. Wise *(Birmingham, UK)*

The tools available to reduce the impact of antimicrobial resistance are very limited and consist of infection control, new agents including vaccines) becoming available and more prudent (that is, in the main part, less) antimicrobial prescribing. It is well known that there will be few agents coming on stream in the near future and infection control, in the community setting, is extremely difficult. Therefore the main approach by most countries is to control antibiotic use. However, are there any risks associated with this approach?There is some evidence, much of which is weak, that those countries which have either low prescribing patterns or have recently reduced their prescribing, have an increased incidence of adverse effects, such as ''breakthrough'' infections or other problems. These studies will be examined critically and advice on how such events could be approached by those seeking a more prudent approach to antimicrobial prescribing.In most counties, the amount of antibiotic prescribing is so high that any risks are low: there are, however some suggestions that low prescribing countries may be seeing some effects, but it must be stated that the risks

ISSN: 1470-9465

are outweighed by the potential benefits. There has to be a limit in the ability of reducing antibiotic prescribing as a strategy for preserving antibiotic efficacy. However, most developed countries have yet to approach this limit.

S298

Clinical and public health impact of antibiotic resistance

B. Dunais *(Nice, FR)*

Clinically, antibiotic resistance is responsible for delayed diagnosis, prolonged disease and eventual disability through chronic infection, exposure to a succession of anti-microbial agents, with potentially serious adverse events, hospitalisation with its double risk of dissemination of patients' resistant microbes and acquisition of new resistant nosocomial pathogens, which may then be introduced in the community.From the angle of public health, resistant pathogens circulating in the community represent a threat to its more susceptible population, i.e. children and the elderly, individuals with chronic disease and/or depressed immune responses. Exchange of genetic information with commensal flora can result in dissemination of resistance traits and thus widen the reservoir. More patients are hospitalised due to ineffective first line treatments, entailing high costs for the community and loss of revenue for the patients. Escalation of antimicrobial use draws on the effectiveness of compounds previously kept as a last resort and leads to vanishing resources in antibiotic therapy. Increasingly rapid resistance to new antibiotics discourage the industry from conducting new research. Reinforced surveillance and attempts to reverse the situation have become necessary, at considerable cost. Specific examples will be briefly examined.

Antibacterial susceptibility studies: activities, mechanisms and drug interactions

O299

Evaluation of drug interactions and CYP450 inhibition with Faropenem medoxomil

A. Beaudry, F. Dean, S.C. Gill, C. Black, K. Khan *(Louisville, Austin, US)*

Objectives: Faropenem medoxomil is a novel prodrug antibiotic that is currently being developed as an orally administered drug for treatment of community infections. Faropenem medoxomil is rapidly converted to faropenem, the active drug, following oral absorption by plasma esterases with a high degree of bioavailablity of 72–84%. Faropenem is cleared from the plasma by glomerular filtration and active renal secretion. Several clinical drug-drug interaction studies were performed, where only one study, between faropenem and probenicid, resulted in modest increases in both C_{max} and AUC values for faropenem. The objective of this study was to evaluate the ability of faropenem to inhibit CYP450 enzymes *in vitro* and evaluate the corresponding potential for clinically relevant drug-drug interactions.
Methods: Faropenem was evaluated for potential to inhibit human CYP450 enzymes in pooled human liver microsomes. The isoforms tested were: CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, and CYP3A4. In addition, the stability of faropenem in pooled human liver microsomes was evaluated.
Results: Stability analysis of faropenem (10 and 100 μM) in human liver microsomes (37° C) showed no substantial degradation over the time course required for CYP450 inhibition analysis. For the human CYP450 inhibition studies, none of the CYP450 enzymes evaluated were inhibited by faropenem at concentrations up to 100 μM (30 mg/L).
Conclusions: The absence of inhibition observed for faropenem with all eight CYP450 enzymes evaluated indicates a low potential for drug-drug interactions with faropenem. Previous drug-drug interaction studies with Faropenem medoxomil and the following drugs:theophylline, warfarin, digoxin, furosemide, probenicid, ranitidine, Maalox, cholestyramine, and Microgynon (oral contraceptive) have shown minimal effects with the exception of probenicid, where faropenem C_{max} and AUC were increased by 1.26- and 2.44-fold, respectively. This increase in total exposure of faropenem was expected due to the effect of probenicid on blocking active renal secretion. The overall observations from the nine drug-drug interaction studies and the *in vitro* CYP450 inhibition study indicate a low potential for interactions with Faropenem medoxomil and other drugs that may be co-administered in the clinic.

O300

Bactericidal activities of daptomycin and vancomycin in vancomycin-susceptible *Staphylococcus aureus* and heterogeneously vancomycin intermediate *S. aureus* strains

M. Wootton, T.R. Walsh, A.P. MacGowan *(Bristol, UK)*

Background: *Staphylococcus aureus* is an important pathogen in both the hospital and the community. Glycopeptides are considered the drugs of choice when treating methicillin resistant *S. aureus* (MRSA) infections however the emergence of hVISA isolates has caused concern. Prospective new treatments for MRSA, and especially VISA/hVISA include daptomycin (D), an acidic cyclic lipopeptide that is cidally active against Gram-positive bacteria. This study aims to determine the bactericidal activity of D compared to vancomycin (V) using time-kill curve analysis.
Methods: Time-Kill curves using V and D at 2x and 4x the MIC was performed on 10 vancomycin-susceptible *S. aureus* (VSSA) and 10 hetero-vancomycin intermediate *S. aureus* (hVISA) strains. Viable counts were taken at 0, 1, 2, 4, 6 and 24 h and plotted against antimicrobial concentration. Log reduction in viable count (LRVC), area under bacterial kill curve (AUBKC) and area under curve/MIC were calculated.
Results: At 6 h D was bactericidal (> than 3-log reduction) against all VSSA and hVISA strains at both 2x and 4x the MIC while V was not bactericidal. On average, D was 2-fold more potent than V against VSSA strains at both 6 and 24 h. In addition, only D was bactericidal at both 6 and 24 h against all hVISA strains. At 2x the MIC V was not bactericidal against either VSSA or hVISA strains at 6 or 24 h; however V was bactericidal at 24 h with 4x MIC against some hVISA strains.

Table 1: Mean values for vancomycin and daptomycin at 2x and 4x MIC

VSSA	Log drop in viable count		AUBKC		Log AUC / MIC	
	0-6h	0-24h	0-6h	0-24h	0-6h	0-24h
Mean 2x V MIC	1.07	1.48	21.37	70.43	1.46	2.03
Mean 4x V MIC	1.54	2.29	19.78	57.27	1.42	1.93
Mean 2x D MIC	3.13	3.77	14.74	31.17	3.91	4.96
Mean 4x D MIC	3.69	4.92	11.72	17.75	3.57	3.98

hVISA	Log drop in viable count		AUBKC		Log AUC / MIC	
	0-6h	0-24h	0-6h	0-24h	0-6h	0-24h
Mean 2x V MIC	1.27	2.23	22.31	61.82	0.67	0.87
Mean 4x V MIC	1.39	3.41	19.97	48.99	0.64	0.80
Mean 2x D MIC	3.18	4.29	14.87	27.90	3.26	3.94
Mean 4x D MIC	3.52	5.70	13.26	15.61	3.12	3.27

Conclusions: D has considerably greater bactericidal activity against VSSA and especially hVISA than V. This new agent is a promising alternative for treatment of infections caused hVISA.

O301

The high activity of meropenem against Gram-negative bacteria from a paediatric intensive care unit in Poland (1997–2005)

J.A. Patzer, D. Dzierzanowska, A. Pawinska, P.J. Turner *(Warsaw, PL; Macclesfield, UK)*

Objectives: MYSTIC (Meropenem Yearly Susceptibility Test Information Collection) is a longitudinal surveillance study comparing the *in vitro* activity of meropenem (MEM) and other antibiotics against bacterial strains from clinical units that use MEM for treatment. The aim of this analysis was to assess the *in vitro* activity of MEM and eight other antibiotics against Gram-negative isolates from a paediatric ICU.
Methods: 1020 Gram-negative isolates were obtained from clinical specimens of children hospitalized in the ICU in 1997–2005. Duplicates from the same patients were discarded. The isolates were identified using conventional methods. MICs of MEM, imipenem (IPM), piperacillin/tazobactam (TAZ), cefotaxime (CTX), cefepime (FEP), gentamicin (GM), tobramycin (TM) and ciprofloxacin (CIP) were determined using the NCCLS agar dilution method.
Results: The collection of Gram-negative isolates included Escherichia coli (n = 147), *Enterobacter cloacae* (n = 193), *Klebsiella oxytoca* (n = 87), *Klebsiella pneumoniae* (n = 171), *Serratia marcescens* (n = 41), *Acinetobacter baumanii* (n = 99), *Pseudomonas aeruginosa* (n = 203) and other species (n = 79). MEM, IPM, and CIP were the most active against 93.9%, 91.9% and 94.6% of Gram-negative isolates, respectively. The MIC90 (mg/L) of MEM was nearly identical in 1997 and 2005. It was equal to 0.06 for *Enterobacteriaceae*, 1.0 for *A. baumanii*. However, the MIC90 of MEM for P. aeruginosa increased from 8 in 2003, to 16 in 2004 and to 32 in 2005. The MIC90 of IPM was equal to 0.25 for *Enterobacteriaceae*, 1.0 for *A. baumanii* and 16 for *P. aeruginosa*. From 1997 to 2005 among *Enterobacteriaceae* were only found three isolates (two *E. cloacae* and one *E. amnigenus*) resistant to carbapenems. Among IPM-resistant *P. aeruginosa* isolates no metallo-beta-lactamase producing strains were found. The overall order of susceptibility of tested isolates to other beta-lactams was FEP (84.6%) > TAZ (71.8%) > CAZ (67.8%) > CTX (50.8%). Susceptibility to aminoglycosides, GM and TM, characterized 65% and 64.5% of Gram-negative isolates, respectively. The incidence of AmpC beta-lactamase and extended spectrum beta-lactamases producers among *Enterobacteriaceae* decreased from 68.5% in 1997 to 32.9% in 2005.
Conclusion: MEM, IPM and CIP were the most active antibiotics (> 90% susceptibility) against the tested strains, with no observed reduction in activity over 9 years. Carbapenems, such as MEM, can be used as an option in the first line therapy of nosocomial infections in ICU.

O302

Morphological and bactericidal effects of amoxicillin and clarithromycin on *Helicobacter pylori*

F. Can, M. Demirbilek, G. Karabay, H. Arslan *(Ankara, TR)*

Background: Coccoid forms of *Helicobacter pylori* are suspected to play an important role on transmission and relapsing of infection. Amoxicillin and clarithromycin are used for *H. pylori* eradication therapy. This study focused on the growth kinetics of *H. pylori* after being exposed to amoxicillin, clarithromycin and different amoxicillin-clarithromycin combinations with the conjunction of cell morphology.
Methods: *H. pylori* NCTC 11637 inoculated into Brucella Broth with 2.5% fetal calf serum was exposed to -10xMIC, -1xMIC, 1xMIC, 10xMIC concentrations of antibiotics either alone or in combination. Growth characteristics were determined by the time kill assay. TEM was used for ultra structural morphology.
Results: Amoxicillin and clarithromycin alone decreased the viable counts of *H. pylori*, depending on the antibiotic concentrations. Exposure to these antimicrobials resulted in morphological changes of cell shape, cell-wall disintegration and cell lyses. Amoxycillin showed the most potent effect on viability and morphology of *H. pylori*, which was accompanied with the increase of coccoid forms at a concentration 10 fold higher than the MIC, as rapidly as 6 hours inoculation. The effects of clarithromycin were observed after 12 hours of inoculation. Amoxicillin plus clarithromycin combinations showed additive effect on viable counts and caused a decrease in morphological changes.
Conclusion: Our results showed that amoxicillin-clarithromycin combination caused a decreased frequency in transformation to coccoid forms and higher growth inhibition than amoxicillin exposure alone. Although the clinical importance of coccoid forms is unknown; these forms should be take in to consideration when anti-*Helicobacter pylori* agents are tested *in vitro*.

O303

A comparison of susceptibility to antibiotics in nosocomial and non-nosocomial infections in a hospital in Israel

A. Zalounina, S. Andreassen, M. Paul, L. Leibovici *(Aalborg, DK; Tel-Aviv, IL)*

Objectives: The purpose of this study was to compare the susceptibility to antibiotics in nosocomial and non-nosocomial infections from microbiological data collected at Rabin Medical Center in Israel.
Methods: The retrospective study was based on a bacteraemia database of clinically significant blood isolates from 11520 bacteraemic patients collected in 1996–2001. The database included the name of the pathogen, whether the infection is nosocomial, and *in vitro* susceptibility to a set of antibiotics. For each pathogen in the database the probability of susceptibility to all antibiotics was determined in all nosocomial and all non-nosocomial cases with exact two-sided confidence intervals based on the binomial distribution. Odds Ratios (OR) with 95% confidence intervals (CI) were calculated for the 14 pathogens represented by 50 or more cases in the database by the Mantel-

Haenszel method a) per pathogen and per antibiotic, b) per antibiotic, c) per pathogen and d) across all pathogens and antibiotics.
Results: The nosocomial and non-nosocomial infections are represented by 60% and 40% of all cases in the database, respectively. For the most prevalent pathogen *Escherichia coli* (15%) the probability of susceptibility to ampicillin for non-nosocomial infections is 33% (CI 29–36%) and 25% (CI 20–31%) for nosocomial. This gives an OR of 1.4 (CI 1.1–2.0) for non-nosocomial *Escherichia coli* being more susceptible than nosocomial. The OR across all pathogens for the 27 antibiotics for which ORs could be calculated ranged from 1.4 (CI 1.2–1.5) for ofloxacin to 3 (CI 2.5–3.6) for ceftazidime. The OR for *Escherichia coli* (across all antibiotics) is 1.6 (CI 1.5–1.7). Among the microorganisms, the lowest ORs were observed for Proteus (1.1, CI 0.95–1.5) and for *Enterococcus* spp. 1.2 (CI 1.1–1.6). The highest ORs are for *Klebsiella pneumoniae* 2.6 (CI 2.4–2.8) and 4.1 for *Acinetobacter* sp. (CI 3.6–4.7). The average OR obtained across all antibiotics and pathogen is 1.8 (CI 1.76–1.89).
Conclusion: The low ORs for *Proteus* sp. and for *Enterococcus* sp. show that the antibiotic pressure for these two pathogens is equal in the hospital and in the community. The highest OR was for *Acinetobacter* sp. a bacterium that turned pathogen only in the hospital. The high OR for ceftazidime is not surprising, since cephalosporins are only used in hospitals. The ORs for the fluoroquinolones were small, indicating that the antibiotic pressure is similar in the two compartments.

O304

Comparative minimum inhibitory and mutant prevention concentrations of contemporary (2004–2005) urinary tract pathogens tested against ciprofloxacin and levofloxacin

J. Blondeau, S. Borsos, C. Hesje *(Saskatoon, CA)*

Objectives: Urinary tract infection remains amongst the most common clinical complaints in female patients and fluoroquinolones are important agents for treatment. The comparative minimum inhibitory (MIC) approach determines the drug concentration threshold required to block the growth of first-step mutants when > 1 billion cfu are exposed to drug. We are interested in comparing MIC and mutant prevention concentrations (MPC) values for contemporary *E. coli* (Ec), *K. pneumoniae* (Kp) and Enterococcus (En) pathogens recovered from urinary tract specimens in order to determine if urinary drug concentration with ciprofloxacin (CPX) and levofloxacin (LFX) exceeded these values over the recommended dosages and dosing intervals.
Methods: MIC testing was performed in accordance with the recommended procedure of the Clinical and Laboratory Standards Institute utilizing 100,000 cfu/ml exposed to doubling dilution of drug in ambient atmosphere and temperature. For MPC testing, 10 billion·cfu were applied to agar plates containing 2-fold concentration increments of drug. Following incubation in ambient atmosphere and temperature, the lowest drug concentration preventing growth was recorded as either the MIC or MPC.
Results: A total of 135 Ec, 43 Kp and 18 En species that were CPX and LFX susceptible were tested to determine MIC and MPC values. Against Ec, CPX and LFX MIC50, MIC90 values (ug/ml) respectively were 0.004 and 0.016 versus 0.008 and 0.031; MPC values were 0.125 and 2 versus 0.125 and 0.5. Nine strains had CPX MPCs of 16- > 32 ug/ml as compared to 7 strains tested against LFX. For Kp, CPX and LFX MIC50, MIC90 values (ug/ml) respectively were 0.016 and 0.125 versus 0.05 and 4; MPC values were 0.5 and 0.5 versus 0.5 and 4. No strains had MPCs > 4 ug/ml. For En, CPX MIC 50/90 and MPC 50/90 values (ug/ml) were 0.5/1 and 4/4 versus 1/1 and 2/4 respectively for LFX. One isolate had an MPC to LFX of 128 ug/ml. For Ec, 89–95% and for Kp, 93–100% of strains had MPC values that were below the susceptibility breakpoints for CPX and LFX.
Conclusion: MPC values for CPX and LFX against recently collected uropathogens were ≤ 0.5–4 ug/ml and as such were well within achievable and sustainable urine drug concentrations based on conventional dosing. Dosing to ensure drug concentrations in excess of the MPC will minimize resistance selection and represents a rational approach for treating UTIs. Minimizing resistance selection will prolong the clinical utility of these compounds.

O305

Activity of moxifloxacin against community-acquired MRSA and other quinolone-susceptible MRSA isolates

J. von Freyberg, S. Scherpe, M.A. Horstkotte, J.K. Knobloch *(Hamburg, DE)*

Objectives: Current-generation quinolones are potential drugs for the therapy of lower respiratory tract infections (LRTI) and of skin and soft tissues infections (SSTI). In these infections MRSA isolates susceptible to quinolones are observed with increasing frequency. In the presented study we investigated the activity of Moxifloxacin (Mox) against such isolates.
Methods: 50 MRSA isolates classified as Ciprofloxacin (Cip) susceptible by disc diffusion were collected consecutively from the Hamburg area during a time period from February 2001 to April 2005. The clonal relationship of these isolates was determined using pulsed field electrophoresis (PFGE). MIC values of Oxacillin (Oxa), Cip, and Mox were determined for all isolates using the E-test method.
Results: 47 isolates could be characterized by PFGE, whereas for 3 isolates no PFGE-pattern could be determined even in repeated assays. 11 isolates were identified as community-acquired (CA)-MRSA with a homologous PFGE-pattern compared to the CA-MRSA prevalent in France. Quinolones-resistant CA-MRSA isolates were not detected during the study period of more than four years. The Panton-Valentine-Leucocidin genes could be detected in all CA-MRSA by PCR. Additionally, four other clonal groups were observed by PFGE analysis comprising 12, 4, 3, and 3 isolates. The remaining isolates were distributed in 14 sporadic PFGE-patterns. 37 isolates, including all CA-MRSA, displayed a MIC for Oxa of 64 mg/L or lower, whereas only 5 isolates displayed MIC values for Oxa greater than 256 mg/L. The MIC50 values of Cip and Mox were determined as 0.5 mg/L and 0.064 mg/L, respectively. The MIC90 values were determined as 1 mg/L and 0.125 mg/L for Cip and Mox, respectively. In contrast to the initial results of disc diffusion testing two isolates displayed a MIC for Cip of 2 mg/L. These isolates displayed MIC values for Mox of 0.064 mg/L and 0.125 mg/L. The observed MIC data indicate a four to eightfold higher activity of Mox compared to Cip against the investigated MRSA isolates.
Conclusions: All CA-MRSA isolated in the Hamburg area since 2001 were susceptible to quinolones. For the modern quinolone Mox a four to eightfold higher activity was observed compared to Cip. These data indicate that modern quinolones are highly active drugs for the therapy of LRTI and of SSTI even in areas where CA-MRSA are endemic.

O306

Antimicrobial susceptibility patterns correlate with staphylococcal cassette chromosome mec types for hospital- and community-acquired methicillin-resistant *Staphylococcus aureus*

A. Kilic, H.J. Li, S.E. Sefers, C.W. Stratton, Y.W. Tang *(Nashville, US)*

Objectives: MRSA is becoming the most commonly isolated Gram-positive pathogen and can cause both community acquired (CA) and hospital acquired (HA) infections, each with different antimicrobial susceptibility patterns. The aim of this study was to compare antimicrobial susceptibility patterns with Staphylococcal cassette chromosome mec (SCCmec) types.
Methods: Both invasive and non-invasive MRSA isolates recovered at Vanderbilt University Medical Center (VUMC) were collected from October 1, 2004 to September 30, 2005. A real-time PCR assay (TaqMan) was developed to detect and differentiate four SCCmec types, which are associated with HA-MRSA (SCCmec-I, II, and III) and CA-MRSA (SCCmec-IV). Antibiotic susceptibility for amoxicillin-clavulanate, cefazolin, clindamycin, erythromycin, gentamicin, levofloxacin, minocycline, penicillin, rifampin, trimethoprim/sulfamethoxazole (SXT) and vancomycin was determined by a disc diffusion method and inducible clindamycin resistance was determined by a D zone test.
Results: Among the total 1,315 MRSA isolates collected, 448 (34.1%) were SCCmec-II, 847 (64.4%) were SCCmec-IV, 2 (0.2%) were mixed SCCmec-II/IV, and 18 (1.4%) were untypeable. More SCCmec-II was detected in invasive isolates than SCCmec-IV (72.5% vs. 19.3%, $p = 0.00$) and vice versa in non-invasive ones (24.7% vs. 79.6%, $p = 0.00$). CA-MRSA strains, which carry SCCmec-IV, showed greater susceptibility than SCCmec-II-carried HA-MRSA strains to clindamycin (89.6% vs. 4.2%, $p = 0.00$), erythromycin (9.3% vs. 1.8%, $p = 0.00$), levofloxacin (89.6% vs. 5.1%, $p = 0.00$), gentamicin (99.4% vs. 93.5%, $p < 0.01$), rifampin (99.9% vs. 96.2%, $p < 0.01$), minocycline (100.0% vs. 99.3%, $p > 0.05$), and SXT (98.9% vs. 98.0%, $p > 0.05$). Within clindamycin-resistant isolates, 152 (35.4%) of the HA-MRSA strains were erythromycin induced in comparison to 36 (40.9%) of the CA-MRSA strains ($p > 0.05$). Non-invasive strains showed significant greater susceptibility than the invasive ones to clindamycin (73.9% vs. 23.1%, $p = 0.00$) and levofloxacin (75.0% vs. 23.9%, $p = 0.00$). All isolates were resistant to methicillin, amoxicillin-clavulanate, cefazolin and penicillin. No isolates was resistant to vancomycin.
Conclusion: These data indicate that MRSA isolates predominantly recovered at VUMC carried either SCCmec-IV (CA-MRSA, 64.4%) or SCCmec-II (HA-MRSA, 34.1%). The SCCmec typing correlates with major antimicrobial susceptibility patterns. CA-MRSA strains possessed significantly greater susceptibility to several commonly used antibiotics.

O307

Comparative MIC and MPC results for five quinolone compounds repeatedly tested against American type culture collection control strains: suggested MPC quality control value ranges

J. Blondeau, C. Hesje, S. Borsos, L. Blondeau, B. Blondeau *(Saskatoon, CA)*

Objectives: The minimum inhibitory concentration (MIC) determines the minimum drug concentration inhibiting 100,000 cfu/ml of bacteria *in vitro* whereas the mutant prevention concentration (MPC) determines the minimum drug concentration required to block the growth of first-step mutants when ≥ 1 billion cfu are applied to drug containing agar plates. While MIC testing is standardized and controlled using American Type Culture Collection (ATCC) strains, similar standardized protocols are not yet available for MPC testing. We compared MIC and MPC results with 2 ATCC strains repeatedly tested against 5 quinolones to document MIC and MPC QC ranges.
Methods: MIC testing was based on current Clinical and Laboratory Standards Institute procedures using a 100,000 cfu/ml inoculum and for MPC testing ≥ 1 billion cfu was inoculated to drug containing media-each in 2-fold concentration increments. Optimal media, incubation in established atmospheres and temperatures were used. For each organism type, lowest drug concentration preventing growth was recorded as either the MIC or MPC. ATCC strains included *S. aureus* (SA) 29213 and *S. pneumoniae* (SP) 49619 tested against garenoxacin (GAR), gatifloxacin (GAT), gemifloxacin (GEM), levofloxacin (LFX) and moxifloxacin (MFX).
Results: Following 12–42 repeat MIC assays, MICs were within acceptable QC limits with the majority of MICs being at the middle of the QC range (53–87%) for all organisms/compounds tested. By MPC testing, the following QC organism MPC results (ug/ml) were noted against ATCC strains 29213, 49619:GAR 0.063–2, 0.063–0.5; GAT 0.125–0.5, 0.25–2; GEM 0.063–0.5, 0.125–2; LFX 0.5–1, 1–4; MFX 0.063–0.25, 0.125–2. As with MIC results, the QC strains MPC values fell within the middle of the MPC ranges (62–86%) for the majority of the compounds and strains tested.
Conclusion: As with MIC testing, MPC testing provides reproducible QC organism results that are consistent over narrow drug concentration ranges with the majority of MPCs being at the middle of the range. The MPC drug concentration ranges presented may serve as a useful guide to insuring MPC assay accuracy for clinical isolates of SP and SA tested against quinolones.

O308

Investigation of tolerance to triclosan of five international MRSA clones

Z. Al-Doori, D. Morrison, G.F.S. Edwards, C.G. Gemmell *(Glasgow, UK)*

Triclosan is a broad spectrum biocide that has been in use for more than 30 years. It is used in hospitals to decolonise patients with MRSA. The ability of bacteria and especially MRSA to develop resistance to antimicrobials and biocides is well reported. It is important that products should be tested for any resistance arising from repeated and continued exposure. Two hundred thirty two MRSA isolated in 30 Scottish hospitals (1997–2000) were tested for MIC triclosan by NCCLS method. Adaptation/tolerance studies were performed on representatives of the five major international MRSA clones (CC5, CC8, CC22, CC30 and CC45) by growing in BHI broth in the presence of increasing concentrations of triclosan for up to 67 days. Triclosan tolerant isolates were checked for their stability by fifteen serial passages in triclosan free broth. To investigate susceptibility to triclosan, MIC for ''parents'', triclosan tolerant isolates and passaged isolates were determined. The triclosan MIC of 232 MRSA was MIC50 of 0.03 μg/ml and MIC90 of 0.06 μg/ml (range ≤ 0.015–4 μg/ml). The triclosan MIC of the ''parent'' isolates used in the adaptation were CC5 (0.03 μg/ml), CC8 (4 μg/ml), CC22 (0.015 μg/ml), CC30 (0.015 μg/ml) and

ISSN: 1470-9465

CC45 (4 μg/ml). During adaptation studies, MRSA small colony variants (SCV) emerged in different clones and at different times of exposure to triclosan. After up to 67 days triclosan exposure the triclosan MIC of all five adapted/tolerant derivatives was 4 μg/ml. Following serial passage, the MIC remained 4 μg/ml. In a bactericidal assay triclosan was less effective against tolerant isolates (5-Log reduction in 5 min) than the parents (5-Log reduction 0.5 min). In conclusion, MRSA clones responded differently to increasing concentrations of triclosan. Three of the five clones tested [CC22 (EMRSA-15), CC30 (EMRSA-16) and CC5] produced tolerant derivatives with a very high increase in the triclosan MIC when compared to their parents.

Epidemiology of MRSA in Europe

O309

MRSA incidences and proportions: how well do these correlate on a European level?

M.E.A. de Kraker, N. van de Sande, J. Monen, M.A. Borg, G. Kahlmeter, J. Kolman, H. Grundmann and EARSS participants

Objectives: Resistance proportions, as used by the European Antimicrobial Resistance Surveillance System (EARSS), are often presented to indicate the magnitude of the antimicrobial resistance in different countries. Nevertheless, their comparability is biased by variable sampling and ascertainment procedures. Incidence rates are superior and provide patient based risks, but are mostly not available, due to lacking denominator data. EARSS collects resistance data, as well as, denominator information. In this study, the incidence of antimicrobial resistance was calculated and related to resistance proportions. The possible effect of differential sampling of blood cultures was examined as well.
Methods: Since 1999, EARSS collects routine antimicrobial susceptibility test data amongst others from invasive *S. aureus* isolates, tested according to standard protocols. In 2005, a questionnaire was sent out to collect denominator information (number of patient days, blood culturing rates etc.). To calculate MRSA incidence per 1,000 patient days, we linked the information on patient days to resistance results in the database. The Spearman correlation coefficient, Wilcoxon rank-sum test and linear regression were used for statistical analysis.
Results: This year, 735 of 1205 hospitals (61%) and 483 of 758 laboratories (64%) from 28 of 30 EARSS countries responded to the questionnaire and reported of, overall, 18,729 *S. aureus* isolates in 2004. In the different countries, MRSA proportions ranged from < 1% to 40% and incidence rates varied from 0.26×10^{-2} to 19.29×10^{-2}. Overall, the resistance proportions and incidence rates highly correlated (Spearman coefficient : 0.85, $p < 0.05$). In subgroup analysis, separating Eastern and Western European countries, correlation coefficients were even higher, being highest for the latter. Western European countries had a significantly higher culturing rate than Eastern European countries. From regression analysis it became clear that blood culturing rates did influence the relationship between MRSA resistance proportions and incidence rates for Eastern European countries, whereas it did not for Western.
Conclusion: Resistance proportions seem to be very similar to resistance incidence rates, in the case of MRSA. Nevertheless, this relationship appears to be dependent of some preconditions, for example the level of blood culturing.

O310

Statistical analysis of trends in mandatory MRSA bacteraemia in NHS acute hospital trusts

A. Pearson, A. Charlett *(London, UK)*

The paper summarises the 4 years of local, regional and national statistical analyses of the Department of Health's mandatory *Staphylococcus aureus* bacteraemia surveillance scheme required to be done throughout the acute healthcare system in the NHS in England.
Methods: The number of MRSA bacteraemia in each trust in each quarter has been used as the dependent variable in a Negative Binomial regression analysis. The denominator data has been calculated by multiplying the reported KH03 average bed occupancy for the particular ''financial'' year within which each quarter falls by the actual number of days within the quarter. The natural logarithm of this denominator has then been used as an offset within the regression model to allow direct estimation of incidence rate ratios. Semester, type of trust and region have been used as independent factors. When it has been required to assess linear trends, semester has been fitted as a covariate rather than a factor within the regression model.
Results: National: in the first four years, from April 2001 to March 2005 of this mandatory surveillance scheme, a total of 29,479 MRSA bacteraemias were reported from the 173 NHS Trusts within England from an estimated 168,136,172 occupied bed-days giving an estimated incidence density of 1.75 MRSA bacteraemias per 10,000 bed-days.There is no evidence to suggest that the rates differ by quarter. A significance test of heterogeneity of rate using the negative binomial regression model gives a likelihood ratio test statistic of 15.84 on 15 degrees of freedom, $p = 0.39$. Regional: there was evidence of an increasing trend over time in one region. In the other eight regions negative binomial regression models showed little evidence of temporal trends. Trust: strong evidence that the trends are different by trust category. District general hospitals had three times the number of hospitals with increasing trends in MRSA bacteraemia rates as compared to those with decreasing trends in rates. In specialist hospitals there were twice as many with decreasing trends in rates as compared to those with increasing trends.
Conclusion: Mandatory surveillance revealed strong evidence of trends at local level and related to the type of hospital. As yet there was no evidence of reduced rates of MRSA bacteraemia nationally or regionally in the acute sector of NHS hospital practice in England.

O311

Massive spread of the New York/Japan MRSA clone (ST5-SCCmec II) in a European country

T. Conceição, M. Aires de Sousa, M. Füzi, Á. Tóth, J. Pászti, E. Ungvári, W. van Leeuwen, A. van Belkum, H. Grundmann, H. de Lencastre *(Oeiras, PT; Budapest, HU; Rotterdam, Bilthoven, NL; New York, US)*

Objectives: Earlier reports on the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in Hungary showed that a major epidemic MRSA clone designated as the ''Hungarian clone'' [ST239-SCCmec III, spa type t989/389 (Ridom Spa Type/B. Kreiswirth ID) or related] was widely dispersed in Hungarian hospitals between 1993 and 1998. In the present work we provide an update of the MRSA clonal types circulating in Hungary between 2001 and 2004.
Methods: The 159 MRSA isolates included in this study comprise: (i) 24 isolates previously characterized by antibiogram and pulsed-field gel electrophoresis (PFGE), which were selected as representatives of each of the different PFGE types circulating in Hungarian hospitals between 1994 and 1998, and (ii) 135 representatives of a total of 391 invasive isolates recovered between 2001 and 2004 from several hospitals located in 20 Hungarian counties. All isolates recovered between 2001 and 2004 were analyzed by antibiogram, phage typing and PFGE. Representatives of all PFGE profiles were also characterized by spa typing, multilocus sequence typing (MLST) and SCCmec typing.
Results: Although the 135 MRSA recovered between 2001 and 2004 were distributed into 14 PFGE patterns, almost half of the isolates (49%) showed a single PFGE profile A associated with ST5-II and spa type t002/2, which corresponds to the New York/Japan clone. In addition, 19% belonged to the Southern German clone (PFGE B, ST228-I, spa types t001/385 or t041/388), 4% to the Brazilian clone (PFGE C, ST239-III, spa types t030/351 or t984/392), 4% to the Hannover clone (PFGE F, ST254-IV, spa type t139/649) and only 5% of the isolates showed the genetic background of the Hungarian clone (ST239-III but PFGE similarity with the Brazilian clone of 49.7% only), the earlier predominant clone in Hungary. The characterization of the 24 isolates from 1994–1998 by spa typing, SCCmec typing and MLST grouped them into 4 clones and evidenced the absence and the low prevalence of the New York/Japan and Southern German clones, respectively.
Conclusion: This study documents the complete replacement of the Hungarian clone by the New York/Japan and Southern German epidemic clones among the hospital setting in Hungary between 2001 and 2004. Moreover, we described for the first time the dominance and massive spread of the New York/Japan clone in a European country.

O312

Molecular characterisation of MRSA in the South-Eastern part of Norway from 1991 to 2005

A.E. Fossum, K. Alm-Kristiansen, G. Bukholm *(Lørenskog, NO)*

Objectives: The incidence of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates discovered in the South-Eastern part of Norway has increased during the years 1991–2005. In this study we characterised all MRSA isolates from the time period 1991–2005, and compared isolates from 2 groups: the health care institutions and the primary health care.
Methods: The isolates were analyzed using multilocus sequence typing (MLST), staphylococcus cassette chromosome mec typing (SCCmec) and polymerase chain reaction to verify the presence of the Panton-Valentine Leukocidin (PVL) gene loci.
Results: MRSA was isolated from 180 persons. The incidence of MRSA increased from being less than 7 isolates per year during the period 1991 to 1999, to an estimated number of above 40 in 2005. This trend was observed in both groups. Twenty-seven different sequence types (ST) were discovered. Among these 27, ST8-IV and ST80-IV were dominating, representing over 40% of the isolates. Most STs were represented in both groups, but were not evenly distributed between the groups. Of the different SCCmec types discovered in MRSA, type IV is the dominating type in this study. Type IV has increased from being represented in less than 50% of the isolates in 2001 to above 90% in 2005. The increase has been approximately equal for isolates from both groups. Of all SCCmecIV isolates in this study, over 50% contained the PVL gene loci. The percentage of MRSA containing SCCmecIV and the PVL gene loci differed between the 2 groups. Among the primary health care isolates, over 80% of SCCmec type IV contained the PVL gene loci, which were higher than for the health care institutions isolates.
Conclusion: In the South-Eastern part of Norway the incidence of MRSA isolates are increasing in both health care institutions and in the primary health care. The incidence of MRSA isolates containing SCCmec type IV has increased, and today this type is represented in more than 90% of the isolates. The PVL gene loci were found almost exclusively in MRSA having SCCmec type IV, and were markedly more prevalent in the isolates from the primary health care than from the health care institutions.

O313

National Danish case-control study of community onset infections caused by methicillin-resistant *Staphylococcus aureus*

S. Böcher, A. Gervelmeyer, D.L. Monnet, K. Mølbak, R. Skov *(Copenhagen, DK)*

Objectives: Denmark has for more than 25 years been a low prevalence country for MRSA (< 1percnt;). However, during the last 3 years a significant rise in MRSA infections especially with community onset (CO-MRSA) has been observed. In order to characterise patients with staphylococcal infections and to identify possible risk factors for CO-MRSA infection we conducted a nationwide case-control study.
Methods: During 2004 patients with community onset MRSA in Denmark were identified; controls were selected from a random sample of patients with community onset methicillin sensitive *S. aureus* (MSSA). After informed consent the patients were interviewed by telephone regarding type of infection, previous antibiotic treatment, chronic illnesses, household infections, hospitalisation, or contact to health care or other institutions, travel abroad, sports and occupation. The general practitioners of the patients received a questionnaire regarding clinical signs, localisation of infection and chronic illnesses.
Results: 99 CO-MRSA patients were identified, 37 had both GP and patient questionnaires completed. 330 MSSA controls were identified by the laboratories, 84 of these had both questionnaires completed. Skin and soft tissue infections were the predominant manifestation in both cases (68%) and controls (60%). A large proportion of cases (27%) and controls (38%) had underlying skin disease. Skin lesions prior to infection was associated with a reduced risk of MRSA (OR: 0.2, 95% CI 0.1–0.7). Most cases (69%) and controls (59%) had received antibiotics within the last 6 months and 47% resp. 30% had been hospitalized within the last year. More MRSA patients had been hospitalised for > 7 days (OR 4.1, 95% CI 1.2–14.3). Foreign

ethnicity was associated with an increased risk of MRSA (OR 19.1, 95% CI 2.2–872.3).
Conclusion: Prior hospitalization within the last year of > 7 days and other ethnicity than Danish was found to be significant risk factors for CO-MRSA infections. CO-MRSA infections were dominated by skin and soft tissue infections and the majority of MRSA patients had received antibiotics within the last 6 months. The apparent protective effect of skin lesions is not yet fully understood.

O314

EUREGIO MRSA-net Twente/Münsterland: A Dutch-German cross-border network for surveillance and control of methicillin-resistant *S. aureus*

A.W. Friedrich, F. Verhoeven, A. Mellmann, A.K. Sonntag, L.J.E.W.C. Gemert-Pijnen, I. Daniels-Haardt, M.G.R. Hendrix *(Munster, DE; Enschede, NL)*

Objectives: Hospital infections caused by methicillin-resistant *S. aureus* (MRSA) are particularly critical due to limited therapeutic options. During the last years the rate of MRSA increased in Germany from 2% (1990) to 23% (2003). In contrast, in the Netherlands the MRSA prevalence remains under 1% due to a consequent search & destroy policy. This difference becomes a problem at the bordering regions. Furthermore, CA-MRSA has also been noted in Germany and the Netherlands.
Methods: In order to control these problems we established ''EUREGIO MRSA-net'', an EU-Interreg IIIA funded network for cross-border co-operation in the Twente/Münsterland region. The main goal of this network is to understand the epidemiology of MRSA and to co-ordinate the action of health care providers (i.e. hospitals, laboratories, public health services, GPs) fighting MRSA on a local level in order to lower the prevalence of MRSA and CA-MRSA on both sides of the border in and outside hospitals. MRSA-guidelines of both regions must be synchronised and user-centered. The epidemiological backbone of the project is a spa-typing network providing a common nomenclature for the typing of MRSA.
Results: The sequence based typing is performed in the 6 major laboratories. More than 1000 isolates from patients in hospitals and nursing homes of the region have already been typed and the results could be compared within the whole region and across the border. Spa-type t003, t032, t001 are the most frequent clones in the region. The results of a questionnaire for the German hospitals about screening of patients, typing, and eradication policy showed that although 83% of the hospitals say that they screen patients from risk groups on admission, 65% of them screen e.g. patients with chronic ulcers. 53% of the German hospitals type every first MRSA isolate of a patient, while 75% type outbreak associated MRSA. 97% indicate that they isolate patients with MRSA and perform an eradication therapy, but only in 45% of the cases only the eradication therapy is continued after dismissal of the patient from the hospital.
Conclusion: Cross-border transfer of patients and health-care providers becomes more and more important. EUREGIO MRSA-net sets up a network at two bordering regions of very low and medium size endemicity of MRSA. The project wants to elucidate the euregional epidemiology of MRSA and will provide a backbone to diminish the ''MRSA-barriers'' existing for high quality of cross-border health care.

O315

Prospective surveillance and decolonisation of Panton-Valentine leucocidin-positive MRSA among residents and staff of three German nursing homes

F.M.E. Wagenlehner, H.J. Linde, E. Bambl, U. Raab, D. Kahlau, C. Höller, N. Lehn, W. Witte, K.G. Naber *(Straubing, Regensburg, Oberschleissheim, Wernigerode, DE)*

Background: Panton-Valentine leucocidin-positive methicillin resistant *S. aureus* (PVL-MRSA) was first noticed in our hospital in the first half of 2004 by screening of nursing home residents at hospital admission [6 index patients of three nursing homes (A, B and C), 2 of each nursing home]. Systematic screening of all residents and staff for PVL-MRSA was initiated. Subsequent decolonisation and control of carrier status were performed 1 and 12 months later.
Methods: Screening for MRSA and PVL-MRSA of all residents and staff was initiated in nursing homes A, B and C from January to December 2004. Swabs from anterior nares (residents and staff) and wounds were collected. Routine microbiology techniques were used, including MRSA selective screening agar (Mueller-Hinton agar supplemented with 2% of sodium chloride and 5 μg/ml oxacillin). The presence of the genes nuc (*S. aureus* specific), mecA (penicillin-binding protein 2A), lukS/F-PV (Panton-Valentine leucocidin) was determined by block-cycler PCR. Colonized residents and staff were treated for 5 days using mupirocin nasal ointment and antiseptic washings (chlorhexidine in most cases). Decolonization was controlled by follow up swabs after treatment. Systematic screening of all was repeated 6 to 12 months later in 2005. Hygienic training of nursing home staff and presentation of surveillance data was performed in each nursing home.
Results: Screening results of 2004 and 2005 are shown in the table 1. A significant decrease in the rate of PVL-MRSA carriers ($p < 0.001$) was achieved from 2004 (9.1%) to 2005 (3.4%) by implementing systematic screening of nursing home residents and staff and hygienic training. The MRSA rate did not change.

Nursing homes		2004		2005	
		PVL-MRSA	MRSA	PVL-MRSA	MRSA
A	Residents	15/ 197	6/ 197	3/ 193	8/ 193
	Staff	6/ 104	1/ 104	0/ 67	0/ 67
	Total	**21/ 301**	**7/ 301**	**3/ 260**	**8/ 260**
B	Rresidents	18/ 191	4/ 191	11/ 183	2/ 183
	Staff	13/ 88	1/ 88	2/ 70	3/ 70
	Total	**31/ 279**	**5/ 279**	**13/ 253**	**5/ 253**
C	Residents	7/ 70	2/ 70	5/ 78	0/ 78
	Staff	4/ 39	0/ 39	0/ 35	0/ 35
	Total	**11/ 109**	**2/ 109**	**5/ 113**	**0/ 113**
Total of residents and staff of the three nursing homes		**63/ 689 (9.1%)**	**14/ 689 (2.0%)**	**21/ 626 (3.4%)**	**13/ 626 (2.1%)**

Conclusions: PVL-MRSA was highly prevalent in residents and staff of three nursing homes. The carrier rate could be lowered significantly by systematic screening, decontamination efforts and hygienic training of staff. We conclude that outbreaks with PVL-MRSA in nursing homes may happen and possibly could be sensed by screening nursing home residents at hospital admission. Detection of PVL-MRSA in nursing home residents should prompt systematic screening of all residents and staff. Approved methods (screening, decolonisation, training of staff) to control MRSA may be applied to control PVL-MRSA in nursing homes. Table 1: Residents and staff of three nursing homes positive for PVL-MRSA and/ or MRSA of all screened persons (n/n).

O316

The first national methicillin-resistant *Staphylococcus aureus* prevalence study in Belgian nursing homes indicates high carriage rates among residents

B. Jans, C. Suetens, O. Denis, M. Struelens *(Brussels, BE)*

The increasing incidence of imported Methicillin resistant *Staphylococcus aureus* (MRSA) cases in acute-care facilities in Belgium led us to investigate the prevalence of MRSA carriers among nursing home (NH) residents.

Objectives: To study the national prevalence of MRSA colonisation in Belgian NH-residents, to identify risk factors for MRSA carriage.

Methods: In 2005, a prevalence survey was performed in a random stratified sample of 60 NH, representative of the geographic regions of the country and patient care profile. For each NH, maximum 50 residents were randomly selected for screening (nose, throat, skin wound/ urinary meatus) during a single day survey. Risk factors for MRSA carriage were collected by questionnaire, at resident - and at NH level.

Results: The mean size of the NH was 106 beds, 46% of which were high-care beds. Most of these facilities (68%) were private, 36 were located in the Flanders region, 18 in the Walloon region and 6 in the Brussels region. From all screened residents, 50.7% were *S. aureus* carriers and 19.0% (95%CI, 16.5–21.5) were MRSA carriers. There were no statistically significant differences in MRSA prevalence by region, by proportion of high-care beds in the NH's or by NH size. MRSA carriership was more frequent in private (20.9%) than in public NH's (17.6%) ($p = 0.004$). In NH with an infection control protocol the MRSA prevalence is lower (17%) than in NH without such protocol (22.2%) ($p = 0.046$). Among *S. aureus* carriers the mean resistance proportion was 37.8% (95% CI, 33.4–42.1). A linear relation was observed between resistance proportion and prevalence of MRSA carriership (Pearson: 0.88, $p \leq 0.001$).

Conclusions: This first national prevalence survey in Belgian NH's revealed that colonisation with MRSA is affecting one in five residents in all regions of the country. Bad infection control practices are associated with higher MRSA prevalence rates. Analysis of risk factors at resident- and NH level should clarify the determinants of high prevalence of MRSA carriage.

O317

The EPISA study: characterisation of fusidic acid and methicillin-resistant *Staphylococcus aureus* causing skin and soft tissue infections in outpatients in France

A.R. Larsen, A. Santerre Henriksen, V. Jarlier, P. Menday, R.L. Skov *(Copenhagen, Ballerup, DK; Paris, FR)*

Objective: Two *Staphylococcus aureus* (*S. aureus*) clones have recently been associated with skin and soft tissue infections (SSTI) in different European countries. The European community acquired MRSA, Panton-Valentine leukocidin (pvl) positive clone (ST80-IV) and the European epidemic impetigo clone. Both clones are resistant to fusidic acid, caused by the fusB gene. As part of the EPISA study, *S. aureus* causing SSTI in the French community was characterized with respect to resistance to methicillin (MRSA) or fusidic acid (FARSA). The MRSA and FARSA isolates found were typed in order to see if the two mentioned pan- European clones were also disseminated in the French community.

Method: A total of 480 patients with SSTIs were enrolled in the EPISA study. *S. aureus* isolates were grown from lesional swabs from 205 patients. All MRSA and FARSA isolates were subjected to PFGE and Spa typing and representative isolates were typed by MLST. Toxin gene profiles were obtained by PCR for pvl, Toxic Shock Syndrome Toxin (TSST-1), Exfoliative toxinA (etA) and B (etB). SCCmec type I-IV was analysed for all MRSA. Fusidic acid resistance mechanism; FusB was analysed by PCR using fusB specific primers.

Results: A total of twelve (5.9%) MRSA and nine (4.4%) FARSA were found. One isolate was resistant to both antibiotics. Sequence type (ST) 8 was the dominating genetic lineage (12/21). However, all ST8 isolates displayed different PFGE patterns. The remaining 9 isolates belonged to ST1 (2/21), ST5 (2/21), ST15 (1/21) ST59 (1/21), ST80 (1/21) and ST45 (2/21). All MRSA carried SCCmecIV. No isolates harboured any toxin genes, except for the ST80-IV, which were pvl positive. Resistance against fusidic acid was caused by fusB for the ST80-IV and one of the ST8 isolates. None of the fusB positive, European epidemic impetigo clone was found.

Conclusion: MRSA isolates comprised 6% of the total *S. aureus* isolates causing SSTI in out patients in France. ST8 isolates dominated among the MRSA/FARSA isolates. Surprisingly, no ST8 isolates harboured the pvl or other toxin genes. The study identified only one isolate of the European community acquired MRSA pvl positive clone (ST80-IV) known to cause SSTIs in the community. None of the fusB positive, European epidemic impetigo clone was found. The results indicate that the epidemiology of *S. aureus* causing SSTI is different in France than in other European countries as the UK, Ireland, Germany and the Scandinavian countries.

O318

Methicillin-resistant *Staphylococcus aureus* in children with allergy

I. Kuznecovs *(Riga, LV)*

Objectives: *Staphylococcus aureus* (SA) enterotoxins have been suggested to have an impact on specific local IgE production in nasal polyps in patients with allergy. Hypothetically high level of the local IgE during long period maybe associated with methicillin-resistant *Staphylococcus aureus* (MRSA) formation in the upper respiratory tract in this enlarging group of children with allergy. The aim of the present investigation was to detect the amount of MRSA carriers among children with allergy: nasal polyposis (NP) and allergic rhinitis (AR).

Methods: Nasopharyngeal swabs for SA and MRSA and nasal tissue samples were taken from 1020 children (ages 2–3, 4–7, 8–12) with NP (456 persons) and AR (564 persons) and 980 healthy children (ages 2–12) enrolled for long-term study from 1999 through 2004. Patient's age, gender, diagnosis of allergy, therapy, IgE levels, and use of antibiotics was recorded. Antibiotic resistance of the strains was determined with the disc diffusion method. Nasal tissue samples were analyzed for total and specific IgE to SA.

Results: The rates of nasal carriage of SA were found to be 15% (147/980) in the control group and 62% (632/1020) in patients with NP and AR. MRSA was found in 2% of control and in 29% of patients with NP and AR in 1999, 3% and 32% in 2000, 2% and 37% in 2001, 1% and 40% in 2002, 1% and 52% in 2003, and 1% and 66% patients in 2004. B1 MRSA strain was predominant. Children ages 4–7 years ($p = 0.02$), glucocorticoid use in NP ($p = 0.02$), rate of hospitalization ($p = 0.005$), and specific local IgE level ($p = 0.001$) were significantly associated with MRSA colonization.

Conclusion: The nasal carriage of MRSA in patients with nasal polyposis is high, undetectable, and growing. It is possible that allergy maybe the cause of nasal carriage of MRSA. Children with NP and AR are an unidentified and less well studied group at high risk for spreading MRSA in children's hospitals, day-care centers, and schools.

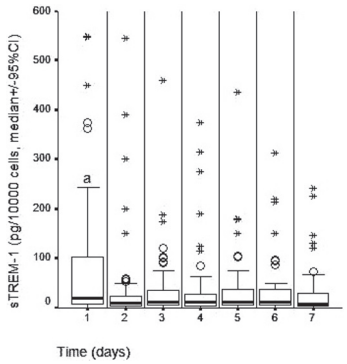
Pathogenesis and therapy of sepsis (Symposium jointly arranged with ISF)

S336

The pathophysiological role of blood monocytes for the release of soluble triggering receptor expressed on myeloid cells – 1 (sTREM-1) in patients with sepsis

E.J. Giamarellos-Bourboulis, C. Routsi, D. Plachouras, I. Andrianakis, M. Raftogiannis, C. Roussos, H. Giamarellou *(Athens, GR)*

Objective: sTREM-1 is the soluble counterpart of the surface receptor TREM-1 that is highly expressed on neutrophils and mature monocytes after bacterial and fungal triggering. The exact site of production of sTREM-1 remains unclarified. The present study aimed to investigate the role of monocytes in the release of sTREM-1 in patients with sepsis and its correlation with monocyte apoptosis.
Methods: Peripheral blood monocytes were isolated from 90 patients with septic syndrome due to ventilator-associated pneumonia on seven consecutive days after presentation of sepsis, by density gradient centrifugation of whole blood, incubation in RPMI and removal of non-adherent cells. Apoptotic rate on the first day was measured by flow cytometry after incubation with FITC-conjugated monoclonal antibodies to Annexin-V and Propidium Iodide. Monocytes were cultured in the absence and presence of LPS and concentrations of sTREM-1 in supernatants were estimated by an enzyme immunoassay.
Results: Concentrations of sTREM-1 in supernatants of isolated monocytes were higher on the first day compared to the next six days (a: $p < 0.05$, see Figure). No increase was observed after triggering of monocytes with LPS. Median sTREM-1 of supernatants of monocytes isolated on the first day from patients sepsis severe sepsis and septic shock were 26.78, 39.13 and 11.79 pg/10000 cells (pNS between sepsis and severe sepsis; p: 0.038 between severe sepsis and septic shock). Negative correlations were observed between sTREM-1 of monocyte supernatants and their apoptotic rate (rs: -0.404, p: 0.0001). Median sTREM-1 of supernatants of monocytes of day 1 of patients who eventually survived was 20.64 pg/10000 cells compared to 17.41 pg/10000 cells of non-survivors (p: 0.048).



Conclusions: sTREM-1 is released from monocytes isolated form patients with septic syndrome. That release is inversely correlated to monocyte apoptosis being lower in the event of septic shock. The above data signify sTREM-1 as an anti-inflammatory mediator in sepsis.

S338

Host defence in pneumococcal pneumonia

T. Van der Poll *(Amsterdam, NL)*

Introduction: The lungs are repeatedly exposed to respiratory pathogens, either by inhalation or (micro-) aspiration of microorganisms that colonize the oropharynx. Infectious agents that have passed the structural defences and have entered the terminal airways may cause pneumonia, one of the most common infectious diseases. *Streptococcus pneumoniae*, the pneumococcus, the most frequently isolated pathogen in patients with community-acquired pneumonia (CAP) [1]. In the United States alone, more than half a million cases of pneumococcal pneumonia are reported each year, with a fatality rate of 5–7%. Bacteremia with *S. pneumonia*e is in almost 90% of cases the consequence of pneumococcal pneumonia. In addition, in recent sepsis trials, *S. pneumonia*e was an important causative pathogen especially in the context of pneumonia. The mortality rate of 40,000 per year caused by *S. pneumonia*e in the United States is larger than the mortality rate caused by any other bacterial pathogen. Infections caused by *S. pneumonia*e are increasingly difficult to treat due to the emergence of antibiotic resistant strains. Altogether it is clear that respiratory tract infection by *S. pneumonia*e represents a major health care problem.
Innate immunity: Three cell types present in the lung are primarily responsible for host defence against pneumonia, i.e. respiratory epithelial cells, macrophages and granulocytes. The respiratory epithelium plays an important role in preventing colonization and infection by inhaled bacteria by physical removal of microorganisms through ciliary clearance and the presence of broad-spectrum antimicrobial agents in the mucus lining the epithelium. The alveolar macrophage is the primary phagocytic cell in the airways, ideally positioned to orchestrate the initial response to an infectious challenge that reaches the lower airways. Likely, if the bacterial challenge is small and/or of low virulence, the macrophage can eliminate the invading bacteria without recruiting granulocytes into the airspaces. If, however, the challenge is more extensive, large numbers of granulocytes are recruited into the alveolar spaces from the marginated pool of granulocytes in the pulmonary vasculature. This process is regulated by a variety of soluble mediators such as cytokines, chemokines and leukotrienes. A few aspects of the innate immune response will be discussed here.
Toll-like receptors: The innate immune system discriminates potential pathogens from self through a series of receptors that recognize conserved motifs on pathogens that are not found in higher eukaryotes. These motifs have been termed "pathogen-associated molecular patterns" or PAMPs, whereas their cognate

binding partners on host cells involved in the innate immune response have been named "pattern-recognition receptors" or PRRs. Examples of PAMPs include lipopolysaccharide (LPS) from the outer membrane of Gram-negative bacteria, peptidoglycan (present in most bacteria), lipoteichoic acid (in many Gram-positive bacteria) and mannans in the yeast cell wall. The Toll family of receptors, which is conserved throughout evolution from flies to humans, has been implicated to play a central role as PRRs in the initiation of cellular innate immune responses. TLR2 is considered the most prominent PRR for the recognition of PAMPs expressed by Gram-positive bacteria. Nonetheless, TLR2 played a limited role during pneumococcal pneumonia [2]. Mice lacking TLR2 had lower chemokine concentrations in lung tissue together with a reduced pulmonary PMN influx, but cytokine levels, bacterial outgrowth and survival did not differ when compared to wild-type animals [2]. This finding can be explained by another report that revealed pneumolysin, one of the crucial virulence factors of *S. pneumoniae*, as a TLR4 ligand [3]. The recognition of pneumolysin by TLR4 has been demonstrated to correlate with the invasiveness of pneumococcal disease *in vivo* [3]. In addition, a moderately impaired survival and increased bacterial outgrowth was found in TLR4-mutant mice with invasive pneumococcal pneumonia [4].

Cytokines: There is ample evidence that underlines the importance of TNF and IL-1β in host defence in bacterial pneumonia: In a murine *S. pneumoniae* pneumonia model, treatment with a neutralizing anti-TNF moAb strongly impaired antibacterial defence [5]. Anti-TNF-treatment resulted in an enhanced outgrowth of *S. pneumoniae* from the lungs, and anti-TNF-treated mice died significantly earlier from pneumococcal pneumonia than control mice. Comparable results were obtained for IL-1. IL-1 receptor type I deficient mice infected with *S. pneumoniae* displayed an increased bacterial outgrowth together with a reduced capacity to form inflammatory infiltrates [6]. Of considerable interest, treating IL-1 receptor deficient mice with a neutralizing anti-TNF antibody made them extremely susceptible to pneumococcal pneumonia - more so than IL-1 receptor knockout mice treated with a control antibody and wild type mice treated with anti-TNF [6]. Thus, whereas excessive production of pro-inflammatory cytokines at the systemic level causes organ failure and death in animal models of fulminant sepsis, the local production of pro-inflammatory cytokines importantly contributes to host defence during pulmonary infection. Coagulation and fibrinolysis: In recent years the interaction between coagulation and inflammation has received much attention. We have demonstrated local activation of coagulation and fibrinolysis, within the bronchoalveolar compartment, of patients with pneumococcal pneumonia [7, 8]. Inhibition of coagulation by recombinant NAPc2, an inhibitor of tissue factor, did not influence the outcome of murine pneumococcal pneumonia [8]. Moreover, mice deficient for plasminogen activator inhibitor type 1 (PAI-1) showed an unremarkable host defence against *S. pneumoniae* pneumonia [7]. Thus, the role for coagulation and fibrinolysis seems limited in pneumococcal pneumonia.

Conclusion: Pneumoccocal pneumonia remains a major challenge for clinicians. Knowledge of host defence mechanisms against *S.pneumoniae* is rapidly increasing. This abstract represents a selection of recently acquired information on the immune response to the pneumococcus in the airways. It is our hope that such knowledge will enlarge our therapeutic arsenal in the near future.

References

1. Bartlett JG, Dowell SF, Mandell LA, File Jr TM, Musher DM and Fine MJ. Practice guidelines for the management of community-acquired pneumonia in adults. Infectious Diseases Society of America. Clin Infect Dis 2000; 31: 347–82.
2. Knapp S, Wieland CW, van 't Veer C, et al. Toll-like receptor 2 plays a role in the early inflammatory response to murine pneumococcal pneumonia but does not contribute to antibacterial defence. J Immunol 2004; 172: 3132–8.
3. Malley R, Henneke P, Morse SC, et al. Recognition of pneumolysin by Toll-like receptor 4 confers resistance to pneumococcal infection. Proc Natl Acad Sci U S A 2003; 100: 1966–71.
4. Branger J, Knapp S, Weijer S, et al. Role of Toll-like receptor 4 in gram-positive and gram-negative pneumonia in mice. Infect Immun 2004; 72: 788–94.
5. van der Poll T, Keogh CV, Buurman WA and Lowry SF. Passive immunization against tumor necrosis factor-alpha impairs host defence during pneumococcal pneumonia in mice. Am J Respir Crit Care Med 1997; 155: 603–8.
6. Rijneveld AW, Florquin S, Branger J, Speelman P, Van Deventer SJ and van der Poll T. TNF-alpha compensates for the impaired host defence of IL-1 type I receptor-deficient mice during pneumococcal pneumonia. J Immunol 2001; 167: 5240–6.
7. Rijneveld AW, Florquin S, Bresser P, et al. Plasminogen activator inhibitor type-1 deficiency does not influence the outcome of murine pneumococcal pneumonia. Blood 2003; 102: 934–9.
8. Rijneveld A.W. WS, Bresser P., Florquin S., Vlasuk G.P., Rote W.E., Spek C.A., Reitsma P.H., van der Zee J.S., Levi M., van der Poll T. Local activation of the tissue factor-factor VIIa pathway in patients with pneumonia and the effect of inhibition of this pathway in murine pneumococcal pneumonia. Crit Care Med 2006; In press.

Non-pneumococcal community-acquired RTIs – current state of the art

S342

Changing drug susceptibility in *H. influenzae* with special reference to macrolides, azalides, ketolides and quinolones

P.C. Appelbaum *(Hershey, US)*

Untypeable *Haemophilus influenzae* strains are important pathogens of community-acquired respiratory tract infections [pneumonia, otitis media, sinusitis and (particularly) acute exacerbations of chronic bronchitis]. In countries without Hib vaccine, systemic bacteremia and meningitis are also important. The main mechanism of resistance to ß-lactamases in *H. influenzae* is production of TEM-1 or (rarely) ROB-1 ß-lactamases. Ampicillin-resistant ß-lactamase resistant (BLNAR) strains (with mutations in PBP3) also occur and have recently been described with increasing frequency in Europe (especially France, Spain, Portugal, the U.K, Ireland and Poland). The incidence of BLNAR strains in Japan approaches 30%. ß-Lactamase producing strains with mutations in PBP3 have been reported in France and Japan. Intrafamilial spread of *H. influenzae* with different genotypes

from pediatric carriers has been described. Although quinolones are active against most *H. influenzae* strains, resistant organisms with mutations in QRDR have been described. A clinical outbreak of quinolone resistant strains from a long-term-care facility associated with levofloxacin use has been described in New York City. A recent fatal case of pneumonia caused by a quinolone resistant *H. influenzae* strain in a patient treated with levofloxacin emphasizes the need for susceptibility testing: the strain was not tested for quinolone susceptibility prior to levofloxacin therapy. Macrolides, azalides and ketolides are recommended for treatment of community-acquired respiratory tract infections including those caused by *H. influenzae*: these drugs yield a unimodal MIC distribution. However, a macrolide, azalide and ketolide efflux mechanism has been detected in strains with ''baseline'' MICs that are susceptible by CLSI. Strains without efflux with significantly lower macrolide, azalide and ketolide MICs occur clinically with an incidence of approximately 2%: these strains are also clindamycin susceptible. Approximately 2% of strains are highly resistant to macrolides by efflux plus one or more ribosomal protein mutations (L4, L22, 23S rRNA). In the light of the finding of an efflux mechanism in ''susceptible'' strains it is suggested that macrolide, azalide and ketolide *H. influenzae* breakpoints are all to high and should be reevaluated. Strains should also be regular monitored for BLNAR and development of quinolone resistance.

Risk assessment in primary care: clairvoyance or evidence-based medicine? (Symposium arranged with ESPRIT)

S346

Cystitis, mostly simple but not always

T. Christiaens *(Ghent, BE)*

We want to discuss the problems general practitioners face when managing the "simple" issue of a potential uncomplicated upper urinary tract infection with a qualitatively high standard. In the field of cystitis there are at least three major diagnostic and two therapeutic problems of interest that concern both clinical microbiologists and general practitioners. The first diagnostic problem deals with the gold standard, the threshold of what is considered a significant infection, or in other words the 20 years old discussion between "Kass and Stamm". Secondly, the basic diagnostic problem is which test can substantially improve the diagnostic accuracy, given that the prior probability of clinical signs is more than 80%. Thirdly, even without such a test, we need to rule out the only frequent serious problem that is misleading, i.e. *Chlamydia trachomatis* infections resulting in potential PID and fertility problems. The choice of therapeutic strategy is still complicated by the multitude of possible treatment regimes. A recent Cochrane review comparing 3-day treatment regimes with 5 days or more offers an interesting point of discussion: a course of 3 days is as effective as a longer one with regard to symptomatic cure and relapse, but less effective for bacteriological eradication. So what should we do?Finally the problem of empirical treatment remains. Already for decades we find the same pathogens in primary care all over the world, with *Eschericia coli* in nearly 80% of the cases. But although the uropathogens are the same, their antimicrobial resistance profiles are different. Periodically alarming resistance data are released by regional bacteriological laboratories. Apparently bacteriologists are convinced that these worrying data will make clinicians use less broad-spectrum antimicrobial agents. But often the effect is the opposite: the growing resistance rather encourages general practitioners to use more and newer broad-spectrum agents, since they fear their patients will not be cured if they carry resistant bacteria. One of the key issues in this debate is the reliability of the resistance data. Even when limited to ambulatory patients the data strongly overestimate the real situation, since general practitioners only send urines for further investigation if they suspect a complicated infection. We investigated this issue in two countries with a very different use of antimicrobial drugs (Belgium and Norway). This difference in use was reflected in the resistance of uropathogens found in the microbiological laboratories, but not in a population of "typical" patients consulting the GP with cystitis (healthy adult women). Only this kind of surveillance can convince clinicians to continue using antimicrobial drugs such as trimethoprim or nitrofurantoin empirically. Perhaps some simple criteria could make the easily accessible laboratory data more reliable and applicable to primary care. We shall discuss this issue. One of the major goals of general practice is to treat simple things with high standards of care. Clinical microbiologists can help us greatly in this task by collaborating in our research.

S348

Bacterial meningitis in adults

D. van de Beek *(Amsterdam, NL)*

Bacterial meningitis is a life-threatening disease. The estimated incidence is 2–5 per 100.000 people per year in developed countries and is up to ten-times higher in less developed countries. Bacterial meningitis is often considered in adults but can be difficult to recognize. Optimal use of the clinical examination aids physicians in identifying patients at sufficient risk for meningitis to require further definitive diagnostic testing with a lumbar puncture. Patients in whom meningitis is suspected require this invasive procedure to effectively establish or refute the diagnosis. Studies show that physical examination lacks adequate sensitivity to accurately identify meningitis. In adults presenting with community-acquired acute bacterial meningitis, the sensitivity of the classic triad of fever, neck stiffness, and altered mental status is low (44%), but almost all such patients present with at least two of four symptoms-headache, fever, neck stiffness, and altered mental status (as defined by a score below 14 on the Glasgow coma scale). Textbooks provide tables with classically described lumbar puncture results for viral and bacterial disease, but the available literature suggests that these findings cannot reliably predict the risk of bacterial meningitis in individual patients. In fact, several published studies report significant numbers of patients with bacterial meningitis who do not have findings on initial lumbar puncture that would traditionally predict bacterial meningitis. As delay in the initiation of antimicrobial therapy can result in poor outcome in this disease, in some countries family doctors are advised to give (parenteral) antibiotics before transferring the patient to hospital if meningococcal meningitis is suspected. 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 when general practitioners decide to treat patients with suspected

bacterial meningitis with parenteral antibiotics, should dexamethasone be given before or with this first dose? New approaches to the treatment of bacterial meningitis are established and recommendations for antimicrobial therapy are changing as a result of emergence of antimicrobial resistance. Furthermore, the management of the critically ill neurologic patient with bacterial meningitis poses important dilemmas. These important and controversial areas will be reviewed and relevant literature will be discussed in the framework of current treatment guidelines.

Update on the influenza pandemic threat

S349

H5N1 and other pandemic threats

R. Fouchier *(Rotterdam, NL)*

In 1997, an outbreak caused by highly pathogenic H5N1 influenza A virus occurred in the live bird markets of Hong Kong, which also resulted in the first reported case of human infection and fatality attributable directly to avian influenza virus. The virus resurfaced again in 2002, 2003 and 2004, devastating the poultry industry in a large part of SE Asia. Subsequently, the virus appeared in Mongolia, Kazakhstan, Russia, Turkey, Romania, and Croatia. During these outbreaks, the virus was transmitted to 147 humans, leading to 78 deaths (as of January 13, 2006) and was isolated from pigs, cats, tigers and leopards in addition to poultry and wild birds. So far, all human cases resulted from bird-to-human transmission. It is still largely unknown why this virus is so fatal to humans. Recent studies suggest that the virus may be replicating beyond the respiratory tract in mammals, as is generally the case only with highly pathogenic viruses in birds. Whether this virus could become transmissible between humans, and start a new pandemic is still one of the key questions. To prepare for a potential H5N1 pandemic, candidate vaccines have been prepared using reverse genetics technology and are currently tested in humans. However, there are several problems related to such vaccines, making it unlikely they could become available for human use in the short term. Drugs such as the neuraminidase inhibitors are another tool to protect against disease from H5N1 infection. However, it is possible that resistance to such drugs could become a problem when they are used on a large scale. Mathematical and epidemiological models suggest that neuraminidase inhibitors could be useful to control outbreaks in humans if they are used for early intervention. Real-time diagnostic methods are available for H5N1 surveillance in humans and animals, facilitating the intensified surveillance studies around the globe.Recent developments in the H5N1 outbreaks will be discussed. Some of the key observations during these H5N1 outbreaks will be discussed in the context of other outbreaks, such as the Dutch H7N7 influenza virus outbreak in the Netherlands in 2003, and in the context of the pandemics of the last century.

Antimicrobial PK/PD

O353

Impact of sample size on the performance of multiple model pharmacokinetic simulations

V.H. Tam, S. Kabbara, R.F. Yeh, R.H. Leary *(Houston, Los Angeles, US)*

Objectives: Stochastic forecasting methods such as Monte Carlo simulations (MCS) have been increasingly used to predict pharmacokinetic (PK) variability of antimicrobials in a population, based on well-characterized data from relatively few subjects. We have previously shown that a multiple-model (MM) approach to stochastic PK simulation is associated with a higher precision than conventional MCS for a well-characterized cohort (Tam, ICAAC 03). However, the ability to extrapolate to other cohorts is dependent on the size of the sample population. We investigated the sample size necessary to provide robust PK predictions using MM simulation.
Methods: A universal population consisting of 500 subjects with a 2-compartment linear model was constructed. Using known model parameter values (Kel = 0.3 h^{-1}, Kcp = 1 h^{-1}, Kpc = 0.3 h^{-1}, V = 20 L) and dispersions (CV = 25% for all parameters), serial concentrations (0.25, 0.5, 1, 2, 4, 8, 12 and 24 h) in the central compartment were simulated after a 1000 mg bolus dose. Normal distribution of parameter values and no correlation among the parameters were assumed. System noise was incorporated as a fixed proportional to the drug concentrations. The best-fit parameter estimates were determined using the Non-Parametric Adaptive Grid program using various sample sizes (5, 10, 25, 50, and 125 PK profiles) randomly picked in quadruplicate. Based on these estimates, the mean ± SD AUC0-inf for each sample population was simulated using the MM approach (weighted average of the simulations from each of the discrete vector support points), and they were compared to those derived from the known values of the universal population.
Results: With increasing size in sample population, a trend towards more accurate and consistent estimation of AUC0-inf of the universal population was observed. A sample population size ≥ 50 was found to be necessary to achieve < 10% and < 25% error in the mean and SD estimates of AUC0-inf, respectively.
Conclusions: Our results suggest that the inferring capability of MM PK simulation is dependent on the sample population size. In order to obtain reasonably robust PK predictions, a non-parametric model derived from a sample population size ≥ 50 may be necessary as the input information.

O354

Continuous versus intermittent intravenous administration of antibiotics with time-dependent antibacterial action: a systematic review of pharmacokinetic and pharmacodynamic parameters

S. Kasiakou, K. Lawrence, N. Choulis, M. Falagas *(Athens, GR)*

Objectives: To compare the pharmacokinetic and pharmacodynamic properties of the continuous versus the

intermittent mode of intravenous administration of various antibiotics.

Methods: Data for this systematic review were identified from Pub Med (01/1950 to 01/2005), Current Contents, Cochrane central register of controlled trials, and references from relevant articles, and reviews. Randomized clinical trials comparing continuous with intermittent intravenous administration of the same antibiotic regimen, and examining the pharmacokinetic and pharmacodynamic properties were included in this systematic review. Two reviewers extracted independently data regarding the clinical setting, number of participants, antimicrobial agents and dosages used, as well as maximum serum concentration (C_{max}), trough serum concentration (C_{min}), steady state or plateau serum concentration (C_{ss}), area under the concentration-time curve (AUC), time above MIC (T > MIC), AUC/MIC, elimination rate constant, elimination half-life, volume of distribution, and systematic clearance.

Results: A total of 17 randomized clinical trials that compared pharmacokinetic and pharmacodynamic data of antibiotics administered by the continuous or intermittent intravenous mode were included in the analysis. The mean Cmax of the intermittently administered antibiotics was higher compared with C_{ss} achieved by the continuous infusion of the same antibiotic in all eligible studies (C_{max} was on average 5.5 times higher than C_{ss}, range: from 1.9 to 11.2). C_{ss} was on average 5.8 times higher than the C_{min} of the intermittently administered antibiotics (range, from 1.2 to 15.6). In 3 out of 6 studies, the length of time that drug concentration was above the MIC of the responsible pathogens was longer in patients receiving the antibiotics continuously.

Conclusion: The mode of intravenous antibiotic administration seems to be of greater importance at least when confronting bacteria with high MICs. Continuous infusion of antibiotics with time-dependent bacterial killing seems to be a superior mode of administration, from a pharmacodynamic point of view, compared to the intermittent in cases of multiresistant bacterial infections.

O355

Testing the mutant selection window hypothesis with *in vitro* dynamic models: pros and cons

A. Firsov, I. Lubenko, S. Zinner *(Moscow, RU; Cambridge, US)*

Objectives: Contradictory reports on the enrichment of resistant mutants in *in vitro* model studies that simulate antibiotic pharmacokinetics probably relate to inappropriate experimental design and data analysis. The mutant selection window (MSW) hypothesis that provides a better understanding of this phenomenon might also be useful to properly relate resistance to simulated ratios of area under the curve (AUC) to MIC. To test this hypothesis, the selection of resistant *Staphylococcus aureus* was studied with lypopeptides, glycopeptides and fluoroquinolones, by simulating their concentrations within and out of the MSW.

Methods: Three to five-day treatments were mimicked with once-daily daptomycin, gatifloxacin, levofloxacin and moxifloxacin, and twice-daily vancomycin and ciprofloxacin over an 8–16-fold range of 24 h AUC/MICs. Peak antibiotic concentrations were simulated to be close to the MIC, between the MIC and the mutant prevention concentration (MPC), and above the MPC. Changes in the susceptibility of *S. aureus* were examined by daily MIC determinations.

Results: Both in terms of susceptibility testing and population analysis, selection of resistant mutants occurred at antibiotic concentrations that fell into the MSW but not at concentrations out of the MSW. Similar bell-shaped AUC/MIC relationships with resistance were established with daptomycin, vancomycin and the four fluoroquinolones. Based on these findings, an AUC/MIC ratio that protects against the selection of resistant mutants was predicted at 125–250 h for the lypopeptide and glycopeptide and at 200–250 h for the fluoroquinolones. These estimates are in concordance with reported data on ciprofloxacin- and garenoxacin-exposed *S. aureus* although relationships of resistance and the time when antibiotic concentrations are within the MSW (TMSW) are controversial.

Conclusions: Overall, the available data are in support of the MSW hypothesis, but additional testing with other groups of antibiotics is needed. Also, further examination of the TMSW relationships to resistance is suggested.

O356

Pharmacodynamic evaluation of the bactericidal activity of telavancin against extracellular and intracellular MSSA and MRSA

M. Barcia-Macay, M. Mingeot-Leclercq, P.M. Tulkens, F. Van Bambeke *(Brussels, BE)*

Objectives: Telavancin (TLV) is a lipoglycopeptide with multiple mechanisms of action that exhibits rapid concentration-dependent bactericidal activity against Gram positive bacteria, including MRSA. We demonstrated previously that TLV is bactericidal against intracellular MSSA (ICAAC 2005, A1831). We compare here the activity of TLV against extracellular and intracellular forms of MSSA and MRSA using a pharmacodynamic model that *analyse*s the time- and dose-responses of bacteria to the drug over a wide range of clinically achievable concentrations.

Methods: MSSA ATCC 25923 and 29213 and MRSA ATCC 33591 and 43300 were used to infect mouse (J774) and human (THP-1) macrophages. Cells were then exposed for up to 24 h to TLV (0.01 to 1,000 x MIC). Activity was assessed by examining the change in CFU recovered from infected cells compared to the initial, post-infection inoculum. Extracellular activity was measured in parallel (using whole culture medium) by CFU counting.

Results: TLV MICs were 0.5 μg/mL for all strains. Extracellular bactericidal activity developed in a bimodal fashion over concentration at 3 h, with zones of concentration-dependency (i) around the MIC and (ii) at concentrations higher than 10 to 100 X MIC. At 24 h, - 5 log (limit of detection) was reached for all strains at C_{max} (90 μg/mL). Intracellular activity developed more slowly and to a lesser extent, but also showed a bimodal response with respect to extracellular concentration. The table describes the bimodal concentration-effect relationships at 3 h (extracellular) and 24 h (intracellular), and the effects observed at C_{max} (with concentration ranges expressed in X MIC, and effects, in change of log CFU from the initial inoculum).

Strains	Condition	First zone of marked conc.-dependency	Zone of low conc.-dependency	Second zone of marked conc.-dependency	Δ log at Cmax (180 X MIC)
extracell. (3 h)	ATCC25923	0.3-1 X MIC (+0.1 to -2.0 log)	1-10 X MIC (-2.0 to -2.6 log)	10-1000 X MIC (-2.6 to -4.8 log)	-4.5 log
	Other strains	0.3-30 X MIC (0 to -1.4 log)	30-100 X MIC (–1.4 to -1.8 log)	100-1000 X MIC (- 1.8 to -3.9 log)	-2.0 log
J774 (24 h)	ATCC25923	0.3-1 X MIC (+0.9 to -2.0 log)	1-100 X MIC (-2.0 to -2.6 log)	100-1000 X MIC (-2.6 to -3.7 log)	-2.7 log
	Other strains	0.3-1 X MIC (+0.7 to -0.8 log)	1-100 X MIC (-0.8 to -1.2 log)	100-1000 X MIC (-1.2 to -2.6 log)	-1.3 to -2.0 log
THP-1 (24 h)	ATCC25923	0.3-1 X MIC (+1.8 to –1.3 log)	1-50 X MIC (-1.3 to -1.5 log)	50-1000 X MIC (-1.5 to -3.3 log)	-2.1 log
	Other strains	0.3-3 X MIC (+1.9 to -1.3 log)	3-100 X MIC (-1.3 to -1.7 log)	100-1000 X MIC (-1.7 to -2.6 log)	-2.0 log

Conclusions: TLV displayed concentration- and time-dependent bactericidal activity against both extracellular and intracellular MSSA and MRSA. The bimodal dose-responses observed may be related to the multiple mechanisms of action of TLV. These data support the use of TLV in infections where intracellular *S. aureus* are present.

O357

Comparative pharmacodynamics (PD) of moxifloxacin and levofloxacin against *S. pneumonia*e following exposure to bioequivalent PD-surrogate parameters

A. Dalhoff, S. Schubert, H. Stass *(Kiel, Wuppertal, DE)*

Objective: Usually, *in vitro* pharmacodynamic (PD) studies simulate pharmacokinetics (PK) when monitored in serum of humans following the administration of standard doses of drug. As a consequence, mechanistic comparisons between drugs are difficult to perform, since their PK/PD parameters (e. g. the AUC/MIC and C_{max}/MIC ratios) are different. The aim of this study was to investigate the kill kinetics of LFX and MFX against *S. pneumonia*e (Spn) wild type and first step mutants after exposure to drug concentrations leading to equal AUC/MIC and C_{max}/MIC ratios in order to draw conclusions on their mechanism of action.
Methods: A one-compartment model was used to simulate the fluctuating serum concentrations *in vitro*. The moxifloxacin (MXF) serum concentrations declined with a *t*½ of 13 h, those of levofloxacin (LFX) with a *t*½ of 8 h. The simulated AUC/MICs of MXF and LFX ranged from 34 h to 1,200 h. Viable counts were determined every 2 hours from 0–12 h and at 24 h; in parallel the actual drug concentrations were quantitated. The time needed to reduce the inoculum by 3 log10 titres, the time to eradication and the area under the bactericidal kill curve (AUBKC) normalized to the initial inoculum were calculated. A wild type and one gyrase and topoIV-mutant each of Spn served as test strains.
Results: In general, increasing MXF-AUC/MIC ratios translated into a more rapid reduction of viable counts and thus an earlier eradication from the test system. MXF exerted a concentration-dependent antibacterial effect against all strains, so that the AUBKC values decreased continuously throughout the entire range of drug exposures. LFX exhibited a bi-modal anti-Spn effect. The activity of LFX against all the Spn strains increased up to AUC/MIC = 300 h; a gain in antibacterial activity was not achieved by a further increase in LFX exposure. This phenomenon was most pronounced by exposing 1st step mutants to increasing LFX AUCs.
Conclusion: Exposure of Spn wild type and 1st step mutants to equivalent AUC/MIC and Cmax/MIC ratios of either MXF or LFX resulted in different antiSpn effects. Bio-equivalence in drug exposures to either MXF or LFX did not translate into equal bactericidal efficacy against Spn indicating that MXF and LFX do not share the same PD-targets to maximize their antibacterial effects. Thus, one PK/PD surrogate parameter does not fit all fluoroquinolones.

O358

Pharmacokinetics of moxifloxacin in tissues of the female urogenital tract

H. Stass, H. Delesen, D. Kubitza, A. Halabi, C.H. Gleiter *(Wuppertal, Kiel, Tubingen, DE)*

Objectives: To study the pharmacokinetics (PK) of moxifloxacin in the urogenital tract of female patients undergoing surgery due to gynecological indications in an open-label, prospective, single-centre study.
Methods: PK of moxifloxacin in plasma and tissue samples (predominantly uterus biopsies) was determined in 40 patients after a single intravenous 1 h infusion of moxifloxacin 400 mg. Biopsies were collected at 1, 2, 4, 7 and 24 h (Groups A, B, C, D and E respectively) after start of infusion. Plasma samples were collected over 24 h. Moxifloxacin concentrations were analysed using a validated HPLC fluorescence assay. PK analysis used non-compartmental methods. Statistical analysis of PK used ANOVA.
Results: Plasma PK was comparable to results obtained in other studies with healthy volunteers or patients undergoing abdominal surgery. Moxifloxacin concentrations in plasma/genital tract tissues are shown in the Table (geometric mean/%CV). Moxifloxacin penetrates well into tissues of the female urogenital tract. Moxifloxacin concentrations exceeded the MICs of susceptible pathogens encountered in gynecological infections during the entire study period, with concentrations significantly higher than those found in the plasma (see Table incl. 95% CI).

Group	n	Tissue concentration (mg/kg)	Plasma concentration (mg/L)	Ratio tissue/plasma (L/kg)	95% confidence interval (CI)
A: 1 ± 0.25 h	8	10.3 / 14.2 (8.20 - 13.6)	5.95 / 29.0 (3.02 - 10.6)	1.72 / 28.5 (0.807 - 2.90)	1.43 - 2.08
B: 2 ± 0.25 h	8	5.40 / 10.76 (4.34 - 7.06)	2.63 / 10.1 (2.11 - 3.10)	2.05 / 11.5 (1.56 - 2.64)	1.70 - 2.48
C: 4 ± 0.5 h	8	4.74 / 15.3 (3.18 - 6.52)	2.46 / 4.38 (2.18 - 2.67)	1.93 / 13.8 (1.35 - 2.44)	1.59 - 2.33
D: 7 ± 0.5 h	8	3.32 / 15.3 (2.41 - 4.39)	1.98 / 25.5 (1.353 - 4.386)	1.68 / 20.0 (1.00 - 2.36)	1.39 - 2.03
E: 24 ± 1 h	8	0.970 / 23.3 (0.661 - 1.72)	0.461 / 16.8 (0.354 - 0.679)	2.10 / 15.7 (1.71 - 3.04)	1.74 - 2.54

Conclusion: These PK data suggest that moxifloxacin is a suitable option to treat female patients suffering from infections of the urogenital tract.

O359

Pharmacokinetics of moxifloxacin in tissues of the GI-tract

H. Stass, A.D. Rink, H. Delesen, D. Kubitza, M.W. Buechler, C.M. Seiler, K.-H. Vestweber *(Wuppertal, Heidelberg, Leverkusen, DE)*

Objective: To determine the pharmacokinetics (PK) of moxifloxacin in the gastrointestinal (GI) tissues of patients eligible for elective surgery in a randomized, open-label, prospective, multicentre study.
Methods: The PK of moxifloxacin in plasma and GI tissues was determined in 40 male and female patients after a single intravenous 1 h infusion of moxifloxacin 400 mg immediately prior to surgery. Surgery was timed to allow GI tissue sample collection (gut wall specimen) 1, 2, 4, 7, and 24 h (Groups A, B, C, D and E respectively) after the start of infusion. Plasma samples were collected over 24 h. Moxifloxacin concentrations were analysed using a validated HPLC fluorescence assay. PK analysis used non-compartmental methods. Statistical analysis of PK used ANOVA.
Results: Plasma PK was comparable to results previously obtained from healthy volunteers. Moxifloxacin concentrations in plasma and GI-tissues are shown in the Table (geometric mean/%CV). Moxifloxacin penetrated well into tissues of the GI-tract. Moxifloxacin concentrations exceeded the MICs of susceptible pathogens encountered in intra-abdominal infections (e.g. *E. coli*) during the entire study period with concentrations significantly higher than those found in the plasma (see Table incl. 95% CI).

Group	n	Tissue concentration (mg/kg)	Plasma concentration (mg/L)	Ratio tissue/plasma (L/kg)	95% confidence interval (CI)
A: 1 ± 0.25 h	8	7.03 / 109.3 (1.40 – 20.8)	4.52 / 15.2 (3.49 – 5.70)	1.56 / 100.5 (0.321 – 4.28)	0.97 – 2.50
B: 2 ± 0.25 h	8	7.30 / 30.0 (4.05 – 10.2)	2.81 / 20.5 (1.91 – 3.63)	2. 60 / 31.1 (1.61 – 3.87)	1.62 – 4.17
C: 4 ± 0.5 h	7	4.33 / 80.2 (0.9026 – 8.719)	1.97 / 34.3 (1.38 – 3.13)	2.09 / 88.2 (0.422 – 4.20)	1.26 – 3.47
D: 7 ± 0.5 h	8	2.82 / 117.8 (0.4243 – 5.622)	1.69 / 24.5 (1.11– 2.41)	1.68 / 87.3 (0.384 – 2.99)	1.04 – 2.69
E: 24 ± 1 h	7	1.61 / 57.8 (0.616 – 3.47)	0.409 / 52.1 (0.264 – 0.839)	3.76 / 50.2 (2.09 – 8.87)	2.27 – 6.24

Conclusion: The PK data suggests that moxifloxacin is a suitable option to treat patients suffering from abdominal infections.

O360

Evaluation of tigecycline penetration into colon wall tissue and epithelial lining fluid using a population pharmacokinetic model and Monte Carlo simulation

C.M. Rubino, L. Ma, S.M. Bhavnani, J. Korth-Bradley, J. Speth, E.J. Ellis-Grosse, P.G. Ambrose, G.L. Drusano *(Albany, Philadelphia, US)*

Objectives: To assess the penetration of tigecycline into colon wall tissue and epithelial lining fluid (ELF).
Methods: These analyses included data from patients without infection (Phase 1) and those with intra-abdominal infections (Phase 2/3). All patients received a 100 mg loading dose, followed by 50 mg every 12 hours of intravenous tigecycline in ELF and Phase 2/3 patients only. Steady-state serum samples were collected from all patients (n = 577), while colon wall and ELF specimens were obtained from patients without infection (n = 23 and n = 30, respectively). Separate models were created for ELF and colon wall tissue. For both models, tissue and serum data were simultaneously co-modelled using BigNPAG, and distinct three-compartment, open models with zero-order IV input and first-order elimination was employed. To examine the full range of tissue penetration and their associated probabilities, two 9,999 subject Monte Carlo simulations were performed, one for ELF and one for colon wall.
Results: Data were well fit using models described above with all r2 values above 0.94. Results of the Monte Carlo simulations, tigecycline exposure and penetration into serum, colon wall tissue, and ELF stratified by infection status, are presented in the table below. For patients without infection, the median colon wall and ELF penetration ratios were 1.3 and 0.43, respectively (see Table). Estimates of colon wall penetration were higher, 2.6, in patients with intra-abdominal infection, most likely due to tissue inflammation.
Conclusion: Simulation results indicate that tissue penetration varies considerably by patient, and likely explains unexpected clinical outcomes for those patients infected with strains at margins of the minimum inhibitory concentration (MIC) distribution.

	Percentiles				
Phase 1 Patients (n=137)	**5th**	**25th**	**50th**	**75th**	**95th**
AUC_{serum}	5.667	6.636	7.265	8.213	17.042
$AUC_{colon\ wall}$	1.450	5.178	9.663	41.100	72.540
Penetration Ratio	0.183	0.654	1.300	3.937	6.839
Phase 2/3 Patients (n=303)	**5th**	**25th**	**50th**	**75th**	**95th**
AUC_{serum}	5.554	6.196	6.914	8.124	13.265
$AUC_{colon\ wall}$	3.790	7.856	16.441	25.087	39.194
Penetration Ratio	0.476	0.852	2.553	3.651	5.709
Phase 1 Patients (n=13)	**5th**	**25th**	**50th**	**75th**	**95th**
AUC_{serum}	2.39	3.383	4.33	5.486	7.775
AUC_{ELF}	0.14	0.653	1.848	5.58	27.368
Penetration Ratio	0.035	0.156	0.433	1.243	6.013

O361

Pharmacokinetic-pharmacodynamic analyses of efficacy using estimates of serum and effect site tigecycline exposures in patients with complicated intra-abdominal infections

S.M. Bhavnani, C.M. Rubino, P.G. Ambrose, J. Korth-Bradley, J. Speth, E.J. Ellis-Grosse, G.L. Drusano *(Albany, Philadelphia, US)*

Introduction: Tigecycline has demonstrated efficacy in the treatment of complicated intra-abdominal infections (cIAI). These analyses were designed to examine the relationship between tigecycline exposure, as measured by the ratio of the area under the concentration-time curve to the minimum inhibitory concentration of the drug to the organism (AUC: MIC) in two matrices, serum and colon wall, and outcome.
Methods: PK analyses used drug concentrations from Phase 1 (serum and colon wall concentrations in non-infected patients) and Phase 2/3 cIAI (serum) studies, (n = 420). Drug concentration data were co-modelled using NPAG. PK-PD analyses utilized clinical outcome, patient covariates, and serum- and colon wall-based AUC: MIC estimates (which were based on the infecting organism with the highest MIC value). Univariate and multivariate logistic regression with backwards stepping were performed. Covariates with p values < 0.1 in univariate analyses were considered for inclusion in the multivariate models. Tree-based modelling was used to identify PK-PD breakpoints predictive of clinical cure. PK-PD breakpoints were used to evaluate potential susceptibility breakpoints using Monte Carlo simulation.
Results: The median tissue penetration (AUCcolon wall:AUCserum) ratio was 1.3 and 2.6 in Phase 1 and Phase 2/3 patients, respectively. 121 patients were included in the PK-PD analyses. MIC values driving AUC: MIC estimates were primarily based on those from *Enterobacteriaceae* (70%) followed by anaerobes (21%) and gram positive organisms (9%). The final model evaluating serum exposures included diagnosis, weight, APACHE II breakpoint (≥ 13), presence of Pseudomonas and AUCserum:MIC breakpoint (7.24) (McFadden's rho-squared = 0.403; $p < 0.001$). The final model evaluating colon wall exposures included race, weight, APACHE II breakpoint (≥ 13), presence of Pseudomonas and AUCcolon wall:MIC breakpoint (4.06) (McFadden's rho-squared = 0.256; $p < 0.001$). Based on Monte Carlo simulations, the probability of PK-PD target attainment for serum exposures exceeded 0.9 for MIC values up to 0.5 mg/L. For colon wall exposures, the probability of PK-PD target attainment was 0.99 and 0.83 for MIC values of 1 and 2 mg/L, respectively.
Conclusion: Patient outcome is multi-faceted. One component, tigecycline exposure, is directly linked to outcome. In addition to considerations of patient status (clinical assessment) and microbiology milieu, effect-site exposure adds to our understanding of drug effect in deep-seated infections.

O362

Ceftazidime dose and dosing interval determine the selection of resistant *Enterobacter cloacae* isolates in the intestinal flora of rats treated for a *Klebsiella pneumoniae* pulmonary infection

W.H.F. Goessens, J.W. Mouton, M. ten Kate, A. Ott, I.A.J.M. Bakker-Woudenberg *(Rotterdam, Nijmegen, NL)*

Objectives: Emergence of resistance has been suggested to depend on the dosing regimen and or pharmacodynamic indices (PDI) from numerous in vitro studies. We studied the effect of ceftazidime (caz) dosing increments and frequency of dosing on the selection of caz-resistant *E. cloacae* in the intestine during treatment of a pulmonary infection caused by *K. pneumoniae* in rats.

Methods: Rats with pulmonary infection (n = 10 per group) received doses of 3.1 to 400 mg/kg/day of caz in a frequency of 6,12 or 24 h during 18 days, starting 24 h after bacterial inoculation. Emergence of resistance in *E. cloacae* was monitored by culturing fresh stool specimens at days 0,8,15,22,29,36 and 43 on agar plates with (0.4 μg/ml) and without caz. Caz pharmacokinetics was assessed in infected rats to determine PDIs as well as the time inside the mutation selection window for each regimen. Caz-resistant mutant *E. cloacae* isolates were characterised in various ways among which determination of the ß-lactamase activity under cefoxitin-induced and non-induced conditions.

Results: A reduction of susceptible *E. cloacae* isolated in the faeces was observed at days 8, 15 and 22 and showed a significant correlation with the fAUC. Significantly more caz-resistant *E. cloacae* were isolated at day 15 in animals exposed to doses of 25 or 50 mg/kg/day administered with the 6 h injection frequency compared to the 24 h injection frequency. The number of caz-resistant *E. cloacae* at days 8 and 15 correlated with the time during which caz plasma concentrations were inside the mutation selection window. Caz- resistant mutants (MIC > 128 μg/ml) were characterised as stable de-repressed mutants.

Conclusions: Reduction in *E. cloacae* was dependent on total daily dose of caz. Emergence of resistance was correlated to time within the selection window. Significantly more stably derepressed caz resistant *E. cloacae* mutants are selected when doses of 25 and 50 mg/kg/day of caz are administered in a frequency of 6 h instead of 24 h.

Microbial pathogenesis: a diversity of mechanisms and host responses

O363

The role of triggering receptor expressed on myeloid cells-1 (trem-1) and of its soluble form for the evolution of septic syndrome

M. Raftogiannis, V. Markaki, D. Plachouras, I. Validakis, M. Kontogiorgi, E.J. Giamarellos-Bourboulis, C. Roussos, H. Giamarellou *(Athens, GR)*

Objectives: Aim of the present study was to assess whether expression of Triggering Receptor Expressed on Myeloid Cells-1 (TREM-1) on the surface of blood neutrophils and monocytes and concentration of its soluble form correlate to progression of septic syndrome.

Methods: 73 patients were enrolled in the study, presenting with septic syndrome (ASCP/SCCM 1992 criteria) induced by ventilator associated pneumonia. Peripheral blood was collected upon the onset of sepsis, and on days 3, 5 and 7 thereafter for estimation of TREM-1 on the surface of neutrophils and monocytes, and soluble TREM-1 in the serum. Red blood cells were lyzed in ammonium chloride and leukocytes washed three times in PBS. They were then stained with a phycoerythrin-conjugated monoclonal anti-TREM-1 antibody (R&D Systems) and analysed by flow cytometry. Soluble TREM-1 in the serum was estimated by an immunoassay.

Results: Of the enrolled patients, 24 presented with sepsis, 18 with severe sepsis and 31 with septic shock. Median values of sTREM-1 and surface TREM-1 are given in the table (*p < 0.05 compared to sepsis and severe sepsis, **p < 0.05 compared to sepsis and septic shock).A positive correlation between the expression of surface TREM-1 on neutrophils and monocytes was detected on all days examined (p < 0.0001). No correlation was observed between the expression of TREM-1 on the surface of myeloid cells and the concentration of its soluble form.

Day	Sepsis	Severe Sepsis	Septic Shock
% expression of surface TREM-1 on neutrophils			
1	37.25	50.71	73.44*
3	55.09	47.51	64.64*
5	57.96	61.71	69.95*
7	68.21	51.13	79.27*
% expression of surface TREM-1 on monocytes			
1	35.31	38.33	41.44
3	48.80	42.88	48.98
5	47.93	37.72	44.80
7	65.64	44.63	44.85
Concentration of sTREM-1 (pg/mL)			
1	5.37	97.59**	8.93
3	7.80	109.41**	31.25
5	4.12	98.81**	9.52
7	4.22	98.48**	5.99

Conclusions: Increased expression of TREM-1 on the surface of neutrophils and monocytes over the course of the septic syndrome may reflect a pivotal role for surface TREM-1 in the transition from sepsis/severe sepsis and septic shock. The decrease of sTREM-1 in patients with septic shock may designate the anti-inflammatory role of the soluble counterpart of TREM-1.

O364

Caenorhabditis elegans killing model: a new tool to study uropathogenic *Escherichia coli* virulence

J.P. Lavigne, A.B. Blanc-Potard, G. Blossier, D. O'Callaghan, A. Sotto *(Nîmes, FR)*

Objective: Several studies have suggested a correlation between resistance to quinolones and a low level of virulence factors (VF). This relationship between resistance and virulence is based on the presence/absence of VFs. No animal studies have been conducted to monitor virulence *in vivo*. We have examined a collection of Extended Spectrum Beta-Lactamases (ESBL)-producing uropathogenic *E. coli* strains for the level of virulence using the nematode *C. elegans*.

Methods: We have tested 24 uropathogenic *E. coli* strains isolated from a surveillance program in Nîmes University Hospital. These strains included 14 ESBL-producing *E. coli* (7 CTX-M-15, 6 TEM-24, 1 TEM-3) and 10 sensitive *E. coli* strains susceptible to all antimicrobial tested. All isolates were tested by PCR for the presence of 15 genes encoding known VFs. The *C. elegans* infection assay used the N2 worms. To compare the entire survival curves in nematode killing assays, we used a Cox regression (SPSS 6.1.1).
Results: The 7 CTX-M-15, the 6 TEM-24, the TEM-3 and the 10 sensitive strains possessed 1 to 4, 5 to 8, 6 and 10 to 15 VFs respectively. The mean survival time for *C. elegans* N2 infected at the L4 stage were 3.01 days (± 0.13) for sensitive strains, 4.66 (± 0.36) for TEM strains and 6.28 (± 1.24) for CTX-M strains. The mean survival times for worms fed with OP50 (a control strain) are 10.33 days (± 0.98). All the worms infected with sensitive *E. coli* were killed in 8 days (± 0.5). This time was longer for worms infected with TEM-producing *E. coli* (10.25 ± 0.5) and CTX-M-15 producing *E. coli* (12.5 ± 0.5). Cox regression showed that an infection with sensitive *E. coli* strains reduced larval survival by a factor of 4.29 ($P < 0.0001$) compared to nematodes infected with CTX-M-producing *E. coli* strains. This result was highly repeatable since there was no difference between three repetitions.
Conclusion: We have developed a novel *in vivo* assay to assess the virulence properties of numerous *E. coli* strains. The effects of the uropathogenic *E. coli* strains on *C. elegans* demonstrate significant parallels to molecular data. The ability to kill worms was significantly correlated with the presence of VFs; the antibiotic sensitive strains killing faster than the TEM or CTX-M strains. The molecular virulence profiles and the *in vivo* behaviour suggest that the CTX-M-group genotype, although adapted for survival in an antibiotic-rich environment such as the hospital milieu have a little intrinsic virulence potential.

O365

Clumping factor B is essential for human *Staphylococcus aureus* nasal colonisation

H. Wertheim, H.A.M. Boelens, D.C. Melles, H. Verbrugh, T. Foster, A. van Belkum *(Rotterdam, NL; Dublin, IE)*

Objective: *Staphylococcus aureus* persistently colonizes the vestibulum nasi in a significant fraction of humans. This a risk factor for subsequent infection. The mechanism for nasal colonisation is unknown and has only been studied *in vitro* studies and in small rodents. Here we present study the first results of a human nasal colonization model in which *S. aureus* adhesins can be studied.
Methods: We define the role of the staphyloccal cytokeratin-binding protein-clumping factor B (ClfB) in the colonization process by artificial inoculation of human volunteers with a wild type strain and its single locus ClfB knock out mutant. Persistence was followed up by quantitative nasal culture and bacterial genotyping for 28 days.
Results: Our results show that the mutant strain is eliminated from the vestibulum nasi of volunteers significantly faster than the wild type: median of 8 days versus 3 days ($p = 0.0174$). The number of *S. aureus* CFUs after inoculation were always higher for the wild type strain (Figure 4). The load was statistically significantly higher at days 7 and 21. After 1 week the average numbers of CFUs (log CFU) per culture were higher for the wild type strains (0.85 CFU versus 0.26 CFU; $p = 0.022$).
Conclusion: Here we present the first ''in homo'' data showing that the ClfB protein by itself is already a prime determinant of nasal *S. aureus* carriage.

O366

Alanine esters of enterococcal lipoteichoic acid have a role in biofilm formation and resistance to antimicrobial peptides

F. Fabretti, C. Theilacker, A. Kropec, S. Koch, J. Huebner *(Freiburg, DE; Boston, US)*

Objectives: *E. faecalis* is one of the leading causes of nosocomial infections, and the increasing occurrence of enterococcal strains resistant to multiple antibiotics underscores the necessity of a better understanding of the pathogenicity of these microrganisms. The aim of the present study was to evaluate the effect of D-alanine esters on teichoic acids in biofilm production, autolysis, antimicrobial peptides sensitivity, neutrophile killing, and opsonisation.
Methods: A nonpolar deletion of the dltA gene was created in the clinical *E. faecalis* strain 12030. The resulting dltA- mutant was compared to the wild-type strain in a microtiter biofilm assay, in Triton X100 induced autolysis, opsonophagocytic assay, and susceptibility to antimicrobial cationic peptides.
Results: LTA was purified from the wt strain and the dltA deletion mutant, and the absence of D-alanine in LTA isolated from the mutant was confirmed by NMR. While the wild-type strain and the deletion mutant did not show any significant differences in autolysis, neutrophile killing and opsonophagocytosis, the mutant strain produced a significantly less biofilm when grown in the presence of 1% glucose (51.1% compared to wild type). In the mutant strain the sensitivity to Polymixin B was considerably increased (MIC 32 vs. 512 ug/ml), Colistin (128 vs. > 512 ug/ml), and Nisin (2 vs. 8 uM).
Conclusions: Our data confirm that the main effect of the absence of D-alanine on LTA and WTA is an increased sensitivity to cationic antimicrobial peptides, probably due to the increased net negative charge on the surface. Alanine residues also seem to enhance biofilm production, which has been shown to be a multifactorial process and probably involves also electrostatic interaction mediated by zwitterionic molecules, such as teichoic acids.

O367

Quinolones induce the partial and total loss of uropathogenic *Escherichia coli* pathogenicity islands by two different pathways

S. Soto, M.T. Jimenez de Anta, J. Vila *(Barcelona, ES)*

Objectives: Previous studies have demonstrated that quinolones induce the loss of pathogenicity islands (PAIs) in uropathogenic *Escherichia coli* (UPEC). The objective of the present study was to analyse the pathway used for quinolones to induce this loss.
Methods: Two haemolytic clinical UPEC (HC14366 and HC109) and their non-haemolytic derivatives obtained when the wild type strains were submitted to sub inhibitory concentrations of ciprofloxacin were selected. A knockout mutant of the recA gene was generated from each wild-type strain by interruption of the recA gene with a cassette presenting tetracycline resistance. The original strain and the knockout mutants were submitted to subinhibitory concentrations of ciprofloxacin to determine if recA was involved in the loss of PAI. The presence of the hly, cnf1, and hra genes was tested by PCR using gene-specific primers.
Results: The HC14366 recA- did not show loss of haemolysin capacity when the strain was submitted to subinhibitory

concentrations of ciprofloxacin, indicating the involvement of recA in this procedure. The presence of the hra gene (which was located in the extreme of PAI) in the HC14366 non-haemolytic derivatives indicated the partial loss of this PAI. On the other hand, the HC109 recA- showed a loss of haemolysin capacity similar to the wild-type strain, indicating that, in this case, recA was not involved in this procedure. Moreover, HC109 non-haemolytic derivatives did not present the hra gene, indicating the total loss of this PAI.
Conclusions: Quinolones induce the loss of PAIs by two different pathways: i) Partial loss of the PAI containing the hly and cnf1 genes through the SOS system; and ii) total loss in which the SOS system is not implicated.

O368

Recombinant expression and characterisation of putative streptococcal pyrogenic exotoxins SPEGdys, SPELdys, and SPEMdys from *Streptococcus dysgalactiae*, subsp. *equisimilis*

K.H. Schmidt, S. Sachse, S. Rödiger, D. Gerlach, E. Straube, J. Rödel *(Jena, DE)*

Objectives: Besides human pathogenic *Streptococcus* pyogenes (GAS) group C (GCS) and G (GGS) streptococci can be pathogenic for different mammalians. Human pathogenic GCS and GGS (hpGCS/GGS, *Streptococcus dysgalactiae*, subsp. *equisimilis*) can cause, like GAS, wound infections, otitis media, pharyngitis and streptococcal toxic shock syndrome. Recently, we found a superantigen (SAG) like gene, speGdys in hpGGS. Later genes encoding SPELdys and SPEMdys were also detected in hpGGS. The sequences of the encoding proteins (SPE's) were highly homologeous but not fully identical to the corresponding genes from group A streptococci.
Methods and Results: We cloned all three genes derived from hpGGS in *E. coli*. In all constructs the putative signal peptides were omitted. All three recombinant proteins were expressed as inclusion bodies and remained soluble in urea buffers at a minimum concentration of 6 M. Renaturation succeeded by stabilisation with 15 mg/ml albumin following stepwise reduction of the urea concentration by dialysis against 0.05 M Tris, 0.15 M NaCl buffer, pH 8.0. The preparations were tested in the lymphocyte transformation test (LTT). The culture filtrates of the original hpGGS strains did not show human T cell stimulating activity. In the same manner, the in *E. coli* expressed recombinant proteins rSPEGdys as well as rSPELdys did not stimulate human T lymphocytes. rSPEMdys is still under investigation. The absence of biological activity was surprising, because each of the recombinant putative SPE's from hpGGS contain zinc binding region (H-F-D) and other binding sites like SPEG and SPEA from GAS. These recombinant proteins, cloned from GAS, stimulated T cells. It seems that the few differences in the AA sequences between the hpGGS putative SAG's and the GAS SAG's were responsible for the decreased biological activity of the proteins derived from hpGGS.
Conclusion: Until now, an active mitogen for human T cells has not been isolated from hpGCS and hpGGS. Active mitogens, so far, came from group C and group G streptococci isolated from horse or other animals. Instancing SPEGdys, in this case we found gene transfer from hpGGS to GAS. We suggest that after the gene transfer of SPE's from GGS to GAS, the genes in the receiving strains were altered. In GAS they may have changed resulting in production of active SAG's. Using the putative SPE's from hpGGS, LTT experiments with T cells from blood of animals, susceptible to GCS/GGS infection, are in progress.

O369

Release of activin A by microglial cells upon stimulation with bacterial TLR-agonists

S. Ebert, R. Nau, U. Michel *(Gottingen, DE)*

Objectives: Activin A, a member of the transforming growth factor-beta (TGF-beta) family of growth and differentiation factors, is a multifunctional cytokine, with one of its roles being in the immune system and inflammatory processes. Follistatin (FS) is a high affinity binding protein of activin and antagonises its actions. We previously demonstrated that concentrations of both activin and FS are elevated in the serum of patients with septicemia and in the cerebrospinal fluid (CSF) of patients with meningitis. The sources of the elevated concentrations of both proteins in CSF have not yet been discovered.
Methods: Primary mouse microglial cell cultures were exposed to the TLR agonists Pam3Cys (Tripalmitoyl-S-glyceryl-cysteine; TLR 2), endotoxin (LPS; TLR 4) and oligonucleotides containing unmethylated cytosin-guanosin motifs (CpG; TLR 9) for 24 hours in the presence of interferon-gamma (IFN-gamma; 100 U/ml). Concentrations of activin A and FS in the cell culture supernatants were measured by ELISA (R&D Systems GmbH). Mann-Whitney-U-test was performed to analyse differences in activin concentrations between groups (n = 10, respectively); p-values < 0.05 were considered statistically significant. Data are expressed as median (minimum/maximum).
Results: After treatment of microglial cultures with IFN-gamma alone (control group), activin A concentrations in the cell culture supernatant were below the detection limit of the ELISA. After combined treatment with IFN-gamma and one of the TLR-agonists, activin A concentrations in the cell culture supernatant were significantly elevated: 279 pg/ml (196/337) after treatment with 1 ug/ml LPS ($p < 0.0001$), 117 pg/ml (0/167) after treatment with 1 ug/ml Pam3Cys ($p = 0.007$), and 77 pg/ml (0/185) after treatment with 10 ug/ml CpG ($p = 0.02$). FS concentrations in all groups were below the detection limit of the ELISA.
Discussion: Our results show for the first time that microglial cells release activin A upon activation by bacterial TLR-agonists. This finding provides further evidence for a role of activin in the innate immune response and suggests that microglial cells are a source of elevated activin A concentrations observed in the CSF during bacterial meningitis.

O370

Regulation of putative extra cellular virulence factors in *Staphylococcus epidermidis* by the alternative sigma factor sigmaB

J.C. Kneschke, S. Jaeger, D. Mack, J.K. Knobloch *(Hamburg, DE; Swansea, UK)*

Objectives: Despite the regulation of PIA synthesis the sigmaB regulon of *S. epidermidis* is still almost uncharacterized. To investigate the influence of sigmaB activity on secreted proteins we investigated culture supernatants of sigmaB mutants (1457sigB, 8400sigB, 1057sigB) generated in clinical isolates *S. epidermidis* 1457, 8400, and 1057.
Methods: The proteins of culture supernatants were compared using SDS-PAGE between wild type and mutant strains. Proteins identified to be differentially expressed were further characterised using MALDI-TOF mass spectroscopy. Transcription of the genes identified to be differentially expressed was analysed using quantitative RT-PCR.
Results: Two proteins identified as serine protease (SE1543) and cystein protease (SE0184) were significantly upregulated in

sigmaB mutants compared to the respective wild types. Transcriptional analysis revealed that the respective genes were repressed in a sigmaB dependent manner. Phenotypic analysis revealed a significantly increased protease activity in all investigated sigmaB mutants. Thereby, strain 1057 displayed the highest proteolytic activity, which was further induced in 1057sigB. Mutants with inactivation of the antisigmafactor RsbW and still intact sigB gene displayed a reduced protease activity compared to the respective wild type strains, corroborating the negative regulation of extra cellular proteases by sigmaB. One protein identified as the (pre) proenzym of the lipase GehD was upregulated in 1457sigB and 8400sigB, whereas in 1057sigB this protein was significantly reduced. However, transcriptional analysis of the gehD gene revealed an increased transcription in all sigmaB mutants. Zymographic and quantitative enzymatic analyses revealed also a differential behaviour of the investigated wild types and mutants. 1457sigB and 8400sigB displayed an increased lipolytic activity, which seem to be caused by increased processing of the proenzym to the fully active lipase. In 1057sigB a reduced lipolytic activity was detected, which resulted from a rapid proteolytic degradation of both, the proenzyme and the active enzyme.

Conclusion: The presented data indicate that sigmaB is a negative transcriptional regulator of proteases SE1543 and SE0184 leading to an increased proteolytic activity of sigmaB mutants. Additionally, sigmaB acts as a negative regulator of lipase GehD. However, the lipolytic activity of individual strains is also determined by their proteolytic activity.

O371
Oral lactobacilli and periodontitis

A.K. Szkaradkiewicz, B. Tukiendorf, J. Stopa *(Poznan, PL)*

Objectives: Etiopathogenesis of periodontitis still remains unclear and its diagnosis requires complex procedures. Therefore, in this study we aimed to examine the role of *Lactobacillus* spp. present in the whole saliva in adult patients with moderate or severe periodontitis.

Methods: Material for the studies involved samples of the full saliva obtained from 23 patients (35–49 years of age), in whom clinic criteria permitted to diagnose moderate (in 8 patients) or severe periodontitis (15 patients). Samples full saliva from 14 healthy individuals (with stationary caries), 20–42 years of age, served as the control. The samples of saliva were quantitatively plated on Rogosa agar and the cultured isolates obtained in anaerobic conditions were identified using API 50 CHL (bioMerieux). In parallel, for detection of hydrogen peroxide production by the isolated *Lactobacillus* spp. TMB-Plus agar was applied. Moreover, using TMB method, salivary peroxidase activity was estimated in saliva supernatants.

Results: In all patients of the moderate periodontitis group presence of hydrogen peroxide-producing *Lactobacillus* spp. was disclosed (mean content of lactobacilli: 0.25 million/ml saliva), while in all the patients of the severe periodontitis group presence of *Lactobacillus* spp. unable to produce hydrogen peroxide was observed (mean content of lactobacilli was 1.6 million/ml saliva). In the control group saliva of 12 patients was found to contain hydrogen peroxide-producing *Lactobacillus* spp., and in the remaining two patients saliva contained *Lactobacillus* spp. unable to produce hydrogen peroxide (mean content of lactobacilli amounted to 0.4 million/ml saliva). In parallel, activity of saliva peroxidase in patients with severe periodontitis amounted to 10.4 ± 3.3 u/ml and it did not differ from activities in the remaining groups ($p > 0.05$).

Conclusions: Hydrogen peroxide produced by *Lactobacillus* spp. exerting anti-microbial effects seems to protect against progression of periodontitis. Detection of hydrogen peroxides, produced by *Lactobacillus* spp. in saliva, may be of diagnostic importance in evaluation of the risk of periodontitis progression.

Keynote lectures II

K386
Mitochondria: a newly discovered target for Gram-positive and -negative bacterial toxins

P. Boquet *(Nice, FR)*

Mitochondria as potential intracellular targets for bacterial toxins and bacterial virulence factors have been ignored for a long time although many viruses were already well known to interfere with this organelle. In the past 5 years, following the description by our group that the vacuolating toxin (VacA) from *Helicobacter pylori* targets mitochondria, many reports have appeared in the literature showing that mitochondria, the classical power plants of cells as well as the central executioner of the death sentence, were targets of bacterial toxins and virulence factors. In particular it appears that pore forming toxins might exert their pathological effects on host cells through a direct activity on mitochondria inducing necrosis or apoptosis, as recently reported for the *Staphylococcus aureus* Panton-Valentine leukocidin. However, in addition to pore-forming toxins non-pore forming virulence factors secreted by bacteria via the type III secretion system may impact mitochondria and by provoking a necrosis or apoptosis may alter the normal host physiology, for instance, the barrier function of epitheliums. This presentation will show why mitochondria may be considered by pathogenic bacteria as good targets for their virulence and how this event can lead to pathogenicity.

K387
Implications of chemotherapy-related mucosal barrier injury

N. Blijlevens *(Nijmegen, NL)*

Mucositis of the alimentary tract is the clinical manifestation of the mucosal barrier injury induced by receiving chemotherapy, with or without irradiation and affects the mouth and throughout the intestines and is also associated with adverse clinical and economic outcomes. In recent years we have begun to unravel the pathogenesis of mucositis to reveal a complex and dynamic pathobiological process that provides a biological basis for targeted, molecular-based interventions. Mucositis is thought to occur in five phases: (1) initiation, (2) upregulation and message generation, (3) amplification and signalling, (4) ulceration and (5) healing. Risk factors have not been systematically evaluated but the character, onset and progression of mucositis is markedly influenced by the nature and intensity of the cytotoxic insult. Anticancer therapy activates nuclear transcription factor NF-kB resulting in the production and release of pro-inflammatory cytokines TNF-a, IL-1a, IL-6, IL-8 and LPS-Binding Protein by epithelial cells. The systemic inflammatory response seen in HSCT recipients following myeloablative therapy mirrors the pattern of mucosal barrier injury as measured by gut integrity (L/R ratio), daily mucositis score and serum citrulline concentrations. The concentrations of IL-8, LBP and CRP are already

significantly elevated by the first week after transplant during profound neutropenia even before the onset of fever or bacteraemia. These inflammatory response markers reach their peak as serum citrulline concentrations reach their nadir, the maximum DMS is attained and when there is significantly decreased gut integrity. This suggests that mucosal barrier injury rather than systemic infection eg bacteraemia determines the onset and intensity of the inflammatory response.Epithelial cells are an integral component of the anatomical and immunological mucosal barrier functioning as the front line of host defences against microorganisms. Gram-positive bacteraemia tend to occur far more often among stem cell transplant recipients than Gram-negative bacillary infections and is not simply the result of using antimicrobial prophylaxis with fluoroquinolones. Bacteraemia due to oral viridans streptococci (OVS) of the mitis group (*Streptococcus mitis Streptococcus oralis*) is related to mucosal damage and can be associated with more serious complications such as sepsis and adult respiratory distress syndrome, which carries a high mortality (80%), though mucosal barrier injury is not a predictor of the viridans streptococcal shock syndrome. Coagulase-negative staphylococci (CoNS) are more frequent causes of bacteraemia, which, though frequently related to the use of central venous catheters, is also related to mucositis, as mucosal sites are also an important source of these staphylococci. Notwithstanding this, the role of bacteria in augmenting the inflammatory response during mucosal barrier injury remains intriguing. Anticancer therapy blocks clonogenic epithelial stem cell renewal resulting in apoptosis and necrosis, which manifests itself as epithelial atrophy and ulcerative lesions. OVS are universal commensal residents of the oral cavity. Hence these lesions might offer a portal of entry for the bacteria, which could either be pathogens or passengers? In other words, is OVS bacteraemia a useful marker of markedly disturbed mucosal barrier rather than a harbinger of systemic infection? Does OVS bacteraemia indicate those HSCT recipients at risk of developing serious infectious complications or even signify an increased risk of mortality. Mucositis has been reported as a risk factor for bacteraemia due to *Acinetobacter* species, *Stenotrophomonas maltophilia*, *Capnocytophaga* species and *Fusobacterium nucleatum*. In addition, bacteraemia due to *Staphylococcus aureus*, *Pseudomonas aeruginosa*, certain *Clostridium* species and *Candida* species is associated with neutropenic enterocolitis (typhlitis). This extreme manifestation of treatment-induced mucosal barrier injury appears to be the result of disturbing the delicate balance between host and microbial flora in the setting of prolonged exposure to antibiotics. Given the complex relationship of fever, neutropenia and infections in the context of mucosal barrier injury it seems an exercise in futility to attempt to circumvent bacteraemia by tackling only one facet of the process. Indeed antimicrobial therapy alone with or without the use of haematopoietic growth factors has not been successful in preventing febrile neutropenia. Rather, it may be more fruitful to aim either at down-regulating the inflammatory response or at restoring the mucosal barrier or both.

Issues in travel medicine

S389

Treatment of imported malaria

T. Jelinek *(Berlin, DE)*

According to WHO, more than 300 million people suffer from a malaria episode annually, in 2–3 million with letal outcome. Around 90% of transmission occurs in sub-Saharan Africa. Falciparum malaria poses an increasingly common problem in endemic regions and treatment failures are increasing due to drug resistance. Several endemic areas now suffer from multiresistant Falciparum malaria. Approximately 1000 cases of imported malaria are registered annually in Germany. Conservative estimates put the risk of an infection with *Plasmodium falciparum* in East and West Africa at 1–2% per month. Recent studies show that this rate can be as high as 30%, depending on the behaviour of the traveller. As mortality rates reach 80% in non-immune, untreated patients, fast diagnosis and effective treatment are mandatory. Treatment for malaria depends on several factors: the species of malaria causing infection, severity of infection, the age of the infected individual, and the pattern of drug resistance to malaria treatment in the area where the individual acquired the infection. If identified early and treated appropriately, almost all malaria can be completely cured. However, even short delays in the diagnosis of malaria can make treatment more difficult and less successful. Current recommendations offer a variety of highly effective antimalarials for treatment that can be chosen according to the specific needs of the individual patient. Data from the European network on imported infectious disease surveillance (TropNetEurop) show a widely disparagous use of treatment regimen in malaria throughout Europe. This translates to significant differences in treatment costs and inpatient time in various European countries. Publication of expert statements for European treatment standards seems highly desirable in view of future streamlining of strategies. In 2005, TropNetEurop has done first steps in that direction with the publication of statements for the treatment of severe Falciparum malaria in Europe and for malaria chemoprophylaxis (www.tropnet.net).

Sexually transmitted diseases and urinary tract infections

O390

Rectal lymphogranuloma venereum in San Francisco in the 1980s

J. Schachter, J. Moncada, J. Spaargaren *(San Francisco, US; Amsterdam, NL)*

Objective: To present the results of diagnostic testing for LGV proctitis in MSM in San Francisco in the 1980s, and the typing of the isolates, in the context of the ''outbreak'' of LGV proctitis described in 2003–2005, initially in The Netherlands, and then elsewhere.

Methods: Rectal swabs were collected from MSM being seen for proctitis in medical clinics in San Francisco. Attempts to isolate *Chlamydia trachomatis* were made in cycloheximide treated L cells. In the 1980s LGV strains were differentiated from the trachoma biovar by growth characteristics (not requiring centrifugation). In 2005, 51 LGV isolates that were still

available were sent to Amsterdam, and tested by PCR, and by sequencing of ompA genes for typing.
Results: Isolation and typing results are shown in the Table. Early in the 1980s chlamydiae were commonly recovered from proctitis cases, but the isolation rate dropped from about 1/3 of cases to < 10% by 1984. Throughout this time, LGV strains were about 2/3 of the isolates. In 2005 modern molecular methods confirmed the earlier biological identification of LGV biovars. All 51 San Francisco isolates were positive for LGV variants by real-time PCR. By sequencing variable segment 2 of the ompA gene (VS-2), we identified 15 as L1, 18 as L2 prototype, and 18 as the L2b genovariant (Spaargaren, et al, Emerg Infect Dis, 2005).

LGV Proctitis in San Francisco, CA

Year	Tested	*Chlamydia*+	LGV	L1	L2	L2b
1979-80	64	14 (22%)	10 (71%)	1		
1981	73	23 (32%)	16 (70%)	2	5	5
1982	117	41 (35%)	28 (68%)	11	8	7
1983	109	12 (11%)	8 (67%)	1	2	5
1984-5	162	10 (6%)	6 (60%)		3	1
Total	525	100 (19%)	68 (68%)	15	18	18

Conclusions: Chlamydiae were commonly recovered from rectal swabs from MSM with proctitis in the early 1980s. But the isolation rate dropped from about 1/3 in the early 1980s to < 10% in 1984. Throughout the time, about 2/3 of the isolates were LGV. L2b, thought to be a ''new'' strain when it was first identified in The Netherlands, as the major cause of the recent outbreak was actually about 1/3 of the isolates obtained in San Francisco > 20 years ago. These results suggest that chlamydiae became less common as causes of proctitis as the gay community modified its behaviour in response to AIDS; that LGV proctitis was common then and likely maintained by a core group that continued risky behaviours, and may have expanded in recent years with the relaxation of safe-sex behaviours and the concomitant increase in STDs.

O391

The role of *Chlamydia trachomatis* in chronic prostatitis/chronic pelvic pain syndrome

M. Sukhorukova, R. Kozlov, I. Malev, O. Shipulina, L. Stratchounski *(Smolensk, Moscow, RU)*

Objectives: The aim of this study was to determine the role of *Chlamydia trachomatis* in patients with different forms of chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS).
Methods: The patients with at least 3 months of clinical evidence of CP/CPPS as well as healthy volunteers were included in the study. All patients and healthy individuals with no antibiotic treatment during preceding month were evaluated with the Meares and Stamey localization technique. The categorization of CP/CPPS was performed according to National Institute of Health (NIH) classification. Commercial PCRs (Amplisens, Russia) with primers specific for *C. trachomatis* on urethral swab (US) and expressed prostatic secretions (EPS) were performed for 14 patients with chronic bacterial prostatitis (CBP) (NIH category II), 77 patients with inflammatory CPPS (NIH category IIIA), 79 patients with noninflammatory CPPS (NIH category IIIB) and 42 healthy volunteers.
Results: According to PCR data, among the patients with CBP *C. trachomatis* has been found in 2 (14.3%) of the cases both in the US and EPS, among the patients with inflammatory CPPS - in 1 (1.3%) of the cases in US as well as in EPS and among the patients with noninflammatory CPPS - in 4 (5.1%) and in 5 (6.3%) of the cases in US and EPS correspondingly. Among the healthy volunteers *C. trachomatis* has been found in 4 (9.5%) of the cases. Only in 1 patient with noninflammatory CPPS EPS sample was PCR-positive whereas US sample was PCR-negative for *C. trachomatis*. There were no statistical significant differences among frequency of the detection of *C. trachomatis* in patients with CBP, inflammatory CPPS, noninflammatory CPPS and healthy individuals both in US an in EPS samples.
Conclusion: The same rate of PCR-positive results for *C. trachomatis* both in patients with clinical prostatitis syndrome and in healthy individuals has demonstrated the doubtful etiological role of this infection in CP/CPPS. However taking into account the high virulence of *C. trachomatis* for reproductive system, the routine evaluation of the patients with prostatitis syndrome for this pathogen might be useful. *C. trachomatis* may be assessed as causative agent of CP/CPPS only in men with PCR-positive EPS sample and PCR-negative US sample for this organism.

O392

Acceptability of self-taken vaginal swabs and first-catch urine samples for the diagnosis of urogenital *Chlamydia trachomatis* and *Neisseria gonorrhoeae* with an amplified DNA assay in young women attending a public health STD clinic

C.J.P.A. Hoebe, C.W. Rademaker, E.E.H.G. Brouwers, H.L.G. Ter Waarbeek *(Heerlen, NL)*

Objectives: Most common sexually transmitted bacterial pathogens are *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG). The purpose of this study was to evaluate the acceptability and feasibility of self-taken vaginal swabs (SVS) and first-catch urine samples (FCU) samples for the detection of CT and GC in young women attending a public health STD clinic.
Methods: 413 young women (age 14–35) visiting a public health STD clinic between August 2003 and August 2004 participated. Patients were instructed in taking a SVS and a FCU by educated STD-nurses. All patients filled out a questionnaire concerning demographic data, reason(s) for STD examination, acceptability of both methods and preferable examination method. Samples were tested for the presence of CT and GC by a NAAT with internal inhibition control (strand displacement amplification assay (SDA), Becton Dickinson ProbeTec ET system). We compared test results from SVS and FCU specimens, focusing on the analysis of percent agreement between results of both methods.
Results: CT and NG were diagnosed in 10.9% and 1.5% of the patients, respectively. 68% of the female participants never previously had a STD examination of whom 11% tested STD positive. More than 13% mentioned 'not having to undergo intimite vaginal examination' as one of the reasons to go to a public health STD clinic. Self collection of vaginal swabs was almost uniformly reported as easy to perform (99%). Self collection of urine samples and vaginal swabs were preferred (84%) above a gynaecologic examination (1%). The percent agreement of both tests is 98.9% (408/413) for CT and 99.3% (410/413) for GC.
Conclusions: CT and NG are prevalent among young women, yet willingness to undergo traditional gynaecologic STD testing is limited. Efforts to enhance compliance with testing among at-risk women are needed. SVS and FCU are appropriate specimens for highly sensitive STD diagnosis. SVS and FCU

are highly accepted and feasible among this risk group. Therefore, these easy sampling methods would be good to use in clinical and non-clinical settings. These tests provide new opportunities in STD control to increase STD testing coverage among young women, enabling the non-invasive detection of many STDs that would otherwise remain undiagnosed and untreated.

O393

Asymptomatic bacteriuria, a lasting or fluctuating condition among elderly living in the community?

N. Rodhe, M. André, L. Englund, S. Mölstad *(Falun, Jönköping, SE)*

Background: Asymptomatic bacteriuria (ABU) is common among the elderly in institutional care but less is known about its prevalence among the elderly living in community settings and even less is known about the turnover of ABU in this population. Better knowledge in this area is a brick in the important work to reduce unnecessary use of antibiotics.
Objectives: To study the prevalence and turnover of ABU in a population of elderly people, ages 80 and over, living in a non-institutional community setting.
Methods: Design: A longitudinal cohort study. Setting: The catchment area of a primary health care center in a Swedish middle-sized town. Participants: Every resident in the area age 80 and over was invited and of those 74%, 294 women and 138 men, participated. Measurements: At baseline and after 6 and 18 months a urine specimen was cultured and if positive, the culture was repeated within two weeks to confirm ABU. The presence of leukocyte esterase and urinary nitrite was recorded.
Results: The percentage of bacteriuria was 22.4, 22.4 and 25.7 in women and 9.4, 9.6 and 7.9 in men. Corresponding figures for ABU was 19.0, 18.7 and 19.9 in women and 5.8, 7.8 and 6.9 in men. Of those tested all three times, ABU was found at least once in 34% of the women and 15% of the men and in 8.2% ABU occurred each time. Following consecutive samplings the positive conversion rate (going from an ABU negative to an ABU positive result) was 7% and the negative conversion rate 31%. *E. coli* was found in almost 70% of subjects with ABU.
Conclusion: Bacteriuria is common and the rate of turnover is considerable among the elderly living in non-institutional community settings. Whether persisting ABU is caused by the same or different strains of *E. coli* will be further analysed.

O394

Recurrent urinary tract infections caused by *Escherichia coli*

S. Gualandris, A. Endimiani, G. Brigante, F. Luzzaro, A. Toniolo *(Varese, IT)*

Introduction: *Escherichia coli* is the most common pathogen isolated from urinary tract infections (UTI). These infections occur frequently in healthy women. Of them, 25% are reinfected within six months after the first UTI episode. Fluoroquinolones (FQ) and trimethoprim-sulfamethoxazole (SXT) are the most frequent empirical treatments used for UTI. Notably, underestimate UTI can progress to serious illnesses, as bloodstream infections (BSI).
Methods: All urinary samples collected during 2004 at our institution were studied retrospectively. Identification and antimicrobial susceptibility tests (AST) were achieved using the Phoenix system (Becton Dickinson, Diagnostic Systems, Sparks, MD). The Epicenter (Becton Dickinson) and the Powerlab (Unitech, Milano) systems were used to obtain clinical and microbiological data. ''Reinfection'' was defined as a new UTI occurring at least two weeks after the first infectious episode.
Results: During the study period, 3706 patients diagnosed with UTI due to *E. coli* were observed. Reinfections were found in 495 (13.4%) patients. The majority of them were women (85.0%) and with community-acquired UTI (91.5%). On the average, 2.5 UTI/year for patient were detected. The majority of patients had 2 or 3 episodes/year (71.5% and 16.5%, respectively). The most prevalent age groups were 60–70 years, and 70–80 years (19.1% and 23%, respectively). With regard to the first isolates that were responsible of recurrent UTI, AST results showed the following resistance incidences: FQ (23.4%), SXT (31.3%), ampicillin (50.1%), amoxicillin/clavulanate (15.9%), and ceftriaxone (1.6%). Isolates causing subsequent infections were characterized by increased resistance: FQ (29.4%), SXT (36.5%), ampicillin (55.9%), amoxicillin/clavulanate (21.8%), and ceftriaxone (5.8%). Patients with recurrent UTI were more liable to develop BSI than patients having only one infectious episode (2.0% and 0.6%, respectively).
Conclusions: *E. coli* recurrent UTI episodes occur commonly in aged woman with community-acquired infections. Recurrent infections may evolve to BSI more frequently than patients showing single UTI episodes. The high incidence of isolates resistant to antimicrobials (e.g., FQ and SXT) underlines the ineffectiveness to plan an adequate empirical therapy. Therefore, for recurrent UTI is opportune to use the more suitable therapy based on etiological and AST results.

AIDS and HIV

O395

AIDS indicative diseases across Europe

G. Likatavicius, I. Devaux, J. Alix, A. Nardone on behalf of EuroHIV

Introduction: The incidence of AIDS in Western-Europe has stabilised since a peak in the mid-1990s; In Eastern-Europe, there has been a continued increase in AIDS reports since the late 1990s and for the first time, in 2004, AIDS incidence rate was greater than in Western-Europe.
Methods: EuroHIV collates information on HIV/AIDS from 52 countries the WHO European Region. Individual data are compiled into the European non aggregate AIDS data set; up to four AIDS indicative diseases (AIDS ID) can be reported for one case. We present here data from 36 countries on AIDS indicative diseases in adults and adolescents. Data for 2004 are analysed by geographic region (West, Centre and East) and by sex. Data on AIDS cases were unavailable for eight countries and a further eight were excluded as AIDS ID was missing for more than 25% of AIDS cases.
Results: In 2004, 10297 AIDS cases were reported in the 36 countries (7002 in the West, 329 in the Centre and 2966 in the East). The most frequent AIDS ID were: in the West, tuberculosis (TB) (24% among all AIDS ID), Pneumocystis carinii pneumonia (PCP)(24%), Oesophageal candidiasis (16%), HIV wasting syndrome (8%), toxoplasmosis (7%), Kaposi's sarcoma (6); in

the Centre, HIV wasting syndrome (24%), PCP (20%), TB (19%), Oesophageal candidiasis (18%), HIV encephalopathy (10%), toxoplasmosis (7%); in the East, TB (56%), HIV wasting syndrome (39%), Oesophageal candidiasis (30%), HIV encephalopathy(11%), recurrent pneumonia (10%), candidiasis of bronchi, trachea and lungs (5%). Among women, TB was the most frequently reported AIDS ID in the West (29%), the Centre (29%) and the East (45%). Among men the most frequently reported AIDS ID was PCP in the West (25%), HIV wasting syndrome in the Centre (28%) and TB in the East (59%).
Conclusions: In the West, the two most common AIDS ID were TB and PCP, whilst in the Centre, HIV wasting syndrome and PCP were the most common. The AIDS epidemic in the East is closely related to the TB epidemic. Nevertheless the higher proportion of certain indicative diseases (HIV wasting syndrome, encephalopathy, recurrent pneumonia) raises questions about access to treatment and under diagnosis of AIDS.

O396

Polymorphisms in toll-like receptors influence susceptibility to HIV infection and its clinical course

P.-Y. Bochud, M. Hersberger, P. Taffé, M. Bochud, C. Stein, T. Calandra, P. Francioli, A. Telenti, R.F. Speck, A. Aderem & The Swiss HIV Cohort Study

Objectives: Toll-like receptors (TLRs) have a key role in innate immunity and participate to antiviral defence mechanisms. We examined whether single nucleotide polymorphisms (SNPs) in TLRs 2, 3, 4, 7, 8 & 9 influence susceptibility or the course of HIV infection in the Swiss HIV Cohort.
Methods: 28 SNPs in TLRs were analysed in 1255 HAART naive HIV+ patients (cases) & 128 blood donors (controls) using the Sequenom technology. The distribution of SNPs frequencies was compared between cases and controls. In HIV+ patients, the CD4+ T-cell decline was calculated using a least squares regression line and a model using a latent variable. The impact of TLRs SNPs on CD4 cells decline and HIV RNA was evaluated using a linear regression model. A logistic regression model was used to estimate the risk associated with SNPs comparing rapid progressors (slope < percentile 15) and slow progressors (slopes > percentile 85) to others patients.
Results: 3 SNPs in TLR8 that are in linkage disequilibrium [C645T (H215H), G1953C (L651L) and C2253A (I751I)] were more frequent in HIV+ patients than in controls (645T carrier status frequency 0.45 versus 0.31, OR = 1.8, 95% CI 1.2–2.7, P = 0.005; 1953C frequency 0.45 versus 0.30, OR = 1.9, 95% CI 1.9–2.9, P = 0.001 and 2253A frequency 0.40 versus 0.28, OR = 1.7, 95% CI 1.1–2.6, P = 0.01). Among HIV cases, two SNPs in TLR9 that are in linkage disequilibrium [Gp1174A (untranslated) & A1635G (P545P)] were independently associated with faster CD4 decline. The A1635G SNP showed an additive effect on CD4 decline with a mean slope of −1.78 for A/A, -1.88 for A/G (P = 0.2) and -2.08 for G/G (P = 0.008). When we stratified HIV+ patients into progression groups, we observed that 1635G carriers were more frequent among rapid progressors than others (OR = 1.8, 95% CI 0.98–3.2, P = 0.058 for G/A versus A/A and 2.7, 95% CI 1.4–5.2, P = 0.003 for G/G versus A/A). Similar results were found for Gp1174A and when we calculated the slope with individual regression or latent variable.
Conclusion: This is the first demonstration of an association of TLR SNPs (TLR9) with the progression of HIV infection. In addition, we found a different distribution of 3 SNPs in TLR8 in HIV+ individuals and healthy blood donors. Genetic studies in other cohorts will help to further assess the role of these SNPs as prognostic markers of HIV infection. *In vitro* studies will allow to understand the functional role of TLRs and their mutations in HIV infection.

O397

Chronic immune activation as a major determinant of AIDS pathogenesis: role of the type I interferon response

S. Staprans, S. Klucking, A. Barry, R. Chavan, K. Dalbey, G. Silvestri, M. Feinberg *(Atlanta, US)*

Objectives: Pathogenic simian immunodeficiency virus (SIV) infection of non-human primates (e.g. rhesus macaques) and HIV infection of humans are characterized by CD4 T cell depletion in association with high viremia and generalized immune activation. In contrast, SIV infection of primates that are natural host reservoirs for SIV, such as sooty mangabeys, are characterized by CD4 T cell preservation and the absence of aberrant immune activation despite high-level viremia, suggesting that chronic generalized immune activation and its associated immunopathology are more important determinants of AIDS than the direct cytopathic effects of virus replication. Current studies aim to elucidate the cellular and molecular basis of whether or not chronic immune activation and attendant immunopathology follow CD4 T cell tropic lentivirus infection.
Methods: We developed a comparative infection model to study how and when host innate and adaptive immune responses diverge following SIV infection of mangabeys and macaques, and compared these results with those obtained in studies of HIV-infected humans.
Results: Differences in innate immune responses (e.g., dendritic cell [DC] activation and migration) to SIV infection are evident from the first days of infection. These differences in the *in vivo* behaviour of DCs are recapitulated following *ex vivo* exposure of plasmacytoid DCs (pDCs) to specific toll-like receptor (TLR) ligands and to inactivated SIV. In contrast to pDCs from macaques and humans, mangabey pDCs produce significantly lower levels of interferon alpha in response to TLR7/9 ligands and to SIV- apparently the result of divergent propagation of activation signals along post-receptor pathways. At the organismal level, gene expression profiling studies indicate that a major feature distinguishing pathogenic from non-pathogenic HIV/SIV infection is the extent to which type I IFN production and response profiles are manifest.
Conclusion: Mangabeys avoid AIDS by the genetically programmed failure of their innate immune systems to respond to SIV with a type I IFN response. This enables an anti-inflammatory immune response that protects these natural hosts from the bystander damage seen in pathogenic HIV infection.

O398

Elevated circulating HLA-G levels in HIV-infected patients with and without visceral leishmaniasis

L. Donaghy, F. Gros, L. Amiot, C. Mary, A. Maillard, C. Guiguen, J-P. Gangneux *(Rennes, Marseille, FR)*

Objectives: HLA-G are non classical class I histocompatibility complex molecules capable of inhibiting T cell proliferation and cytotoxic T cell and NK mediated lysis. HLA-G molecules seem

to be involved in viral persistence, and their surface expression is markedly increased on peripheral blood mononuclear cells of HIV-infected patients. No comparative data are available on the expression level of plasma soluble HLA-G (sHLA-G) isoforms in HIV-infected patients with and without opportunistic infections. We measured full length sHLA-G isoforms in HIV-seropositive and -seronegative patients with and without visceral leishmaniasis (VL), and in healthy controls.

Methods: sHLA-G (sHLA-G1 + HLA-G5) levels were measured with an ELISA sandwich method in serum from 94 subjects (39 HIV-seronegative, 31 HIV-1-seropositive, 17 with VL, 7 with both VL and HIV-1 infection).

Results: sHLA-G concentrations were statistically different among the groups of subjects ($P < 0.0001$). The frequency of sHLA-G positivity in each group was statistically different between groups ($P < 0.00000001$). sHLA-G ELISA was positive in 81percnt; of HIV-infected patients and in 57percnt; of HIV-VL co-infected patients. sHLA-G ELISA was positive in 30percnt; of HIV-seronegative patients with VL, and in 3percnt; of healthy controls.

Conclusion: These results confirm the strong cell surface expression of HLA-G molecules during HIV infection, and demonstrate that levels of the plasma soluble forms are also elevated. In HIV-Leishmania co-infected patients, sHLA-G secretion could contribute to the tolerogenic environment and might help Leishmania parasites to evade cell-mediated immune responses in some patients.

O399

Study on the genotypic resistance of archived and circulating viral strains in blood of treated HIV-infected individuals

O. Turriziani, C. Scagnolari, C. Stano, G. Forte, M. Bucci, F. Bellomi, C. Fimiani, I. Mezzaroma, G. D'Ettorre, C. D'Agostino, E. Riva, S. Vita, C. Ajassa, G Antonelli *(Rome, IT)*

Objective: To study the patterns of resistance of HIV-1 in peripheral blood mononuclear cells (PBMCs) and in plasma of treated infected individuals and to determine whether drug-resistant variants of HIV-1 persist in blood after treatment interruption.

Methods: Plasma and PBMCs were collected from 71 heavily treated HIV infected individuals. Genotyping of the reverse transcriptase (RT) and protease gene (pro) of HIV-1 was performed using fluorescent dideoxy-terminator method (TRUGENE HIV-1-BAYER). HIV drug resistance was defined according to the HIV-1 genotipic resistance interpretation algorithm of the GUIDE LINESTM RULE 9.0-BAYER.

Results: Comparison of the amino acid sequence of the RT and pro genes in cell-associated variants of HIV-1 with that of the plasma revealed that 27 of the 71 patients tested (38%) exhibited different genotypic resistance patterns (discordant samples: DS) and consequently a different resistance report. 39% of samples showed a concordant resistance report but the resistant pattern detected in cell-associated compartment differed from that of the plasma virus for the presence and/or absence of one more than mutations. In the 23% of samples studied the patterns of resistance, detected were concordant in the two compartments. In the 70% of DS, as expected, plasma was the compartment with the higher number of mutations, however in the remaining cases of DS the higher number of mutations was found in the proviral DNA. The analysis of genotypic resistance of HIV-1 in PBMCs and plasma, before and after treatment interruption, performed so far only in 5 patients, revealed that the rebound of wild type virus in plasma strictly depends on the viral population archived in PBMCs.

Conclusions: The HIV mutation patterns detected in plasma do not necessarily reflect the mutation pattern detected in cell-associated compartment. The observation that the proviral DNA may contain an archive of different resistant variant makes this proviral reservoir an interesting substrate for analysis of the ''resistance-potential'' in a given patient.

Molecular epidemiology: a tool for public health (Symposium jointly arranged with EUPHA)

S408

Reconstructing HCV transmission history using phylogenetic and population genetic methods

O.G. Pybus *(Oxford, UK)*

The hepatitis C virus (HCV) is a genetically diverse RNA virus that infects at least 170 million people worldwide. HCV-associated mortality is expected to increase substantially in the future with an estimated cost of $5 billion per year in the USA alone. To be successful, HCV treatment and prevention strategies must be grounded in a clear understanding of HCV transmission dynamics. However, standard longitudinal surveys are not particularly informative because (i) the HCV epidemic began before the virus was discovered in 1989, (ii) there are insufficient archived strains to reliably measure seroprevalence retrospectively and (iii) the absence of specific symptoms hinders the estimation of past prevalence from medical records. As a result, analyses of partial genome sequences, particularly those from the E1 and NS5B genes, have proven crucial to investigating HCV epidemiology. Recently developed methods based on phylogenetic and population genetic models can be used to date the origin of HCV outbreaks, estimate past rates of transmission and track the migration of different strains among locations or risk groups.

S410

Genotyping of measles viruses to monitor the elimination process of the disease

S. Santibanez, A. Wolbert, A. Mankertz, A. Siedler, D. Burki, A. Tischer *(Berlin, DE; Basel, CH)*

WHO has set the goal for elimination of measles in the European Region by the year 2010. A very low incidence of measles (< 1 case per 1 Mio population and year) and an absence of endemic transmission have to be achieved. This implies that the transmission of indigenous measles virus (MV) has been interrupted and that an imported MV can not establish long-term circula-

tion. Therefore, the level of measles susceptibility in the population has to be kept as low as possible by maintaining very high immunization coverage (> 95%) with two doses of measles vaccine. Genetic data of the detected MVs are essential to trace the pathway of the virus transmission. This information is required to discriminate whether local appearance of measles is due to virus importation or if it reflects a still ongoing circulation of an indigenous virus. The standardized nomenclature for genotyping of MVs, introduced by the WHO in 1998, enables description of MV circulation in a global context. At present, 23 distinct MV genotypes are recognized by the WHO. Experience shows that in countries with an area-wide and continuous monitoring of measles, the level of incidence correlates with the pattern of MV genotypes. This can be demonstrated for Germany where a laboratory supported nation-wide measles sentinel was established in 1999. Genotyping data over a period of > 6 years revealed that the few sporadic cases observed recently in the eastern part were mainly caused by imported MVs (genotypes B3, D4, D5, G2, H1, B2). In contrast, in the western part, endemic transmission has been observed until 2002. It was associated with the MV genotypes C2, D6 and the newly emerged D7. In 2003, endemic transmission was tapering off and in 2004, measles occurred only sporadically. The nation-wide incidence of measles decreased from 7.3 in 2001 to 0.2 in 2004. However, in 2005, local outbreaks occurred in the federal states of Hesse and Bavaria. The Hesse outbreak was due to importation of a D4 MV from Romania where an epidemic has been observed since the last quarter of 2004. The origin of the D6 MV that caused the Bavarian outbreak, however, remains unknown. The observation of two independent outbreaks demonstrates that despite the decline of measles incidence in recent years, the potential for a limited circulation of MV is still present in Germany. A study performed in Switzerland from 2003 onwards also indicates absence of endemic transmission. Imported MVs (genotypes D5, D8, D4) were predominantly detected and some could even establish limited circulation in 2003. This corresponds with the incidence of measles that remarkably increased from 0.8 in the years 2001/2002 to 10.2 in 2003 and decreased again to 0.8 in 2004/ 2005. Our studies demonstrate that for assessment of the level achieved in the process of measles elimination, it is essential to combine both MV genotype information and epidemiological data, even in the WHO European Region where the circulation of the former widespread MV genotypes may be interrupted in many countries, but nonetheless continues in some other countries.

Mycobacterial infections

S412
Molecular biology for diagnosis and identification of mycobacteria

E. Tortoli *(Florence, IT)*

The availability of new tools of molecular biology has deeply affected diagnostic microbiology in particular for what concerns the organisms characterized by problematic growth in culture or even not cultivable. Since the early development of the nucleic acids amplification techniques *Mycobacterium tuberculosis* was considered one of the most attractive targets and the dream of the rapid diagnosis of tuberculosis appeared within reach. Immediately after the pioneering period, characterized by the development of various home-made techniques, the ready-to-use commercial kits spread worldwide. It is now evident that, in routine practice, the standardization, convenience and robustness offered by commercial systems, advantageously counter balance the higher sensitivity ant the lower cost of the home made methods. Three commercial methods monopolize at present the market. Amplified *Mycobacterium tuberculosis* Direct (Gen-Probe, USA) is based on the transcriptase-mediated amplification of a *M. tuberculosis*-specific trait of the 16S rRNA. AMPLICOR (Roche, USA) amplifies, using the classic PCR procedure, a genus-Mycobacterium-specific segment of 16S rDNA. The BDProbeTec ET relies on the strand displacement amplification aiming to a portion of the IS6110. Hundreds of papers evaluated and compared such kits without finding substantial differences among them, with all being easy to perform, suitable to routine, highly specific and poorly sensitive. The possibility of detecting inhibitors, present in only two such methods, is the sole really important distinctive feature. Although commercial amplification kits are licensed for respiratory specimens only, they are commonly used with extra pulmonary specimens too. Such use should not be considered improper with the moderately lower sensitivity emerging with such samples being imputable to the lower bacterial charge characterizing them. It is universally agreed that the reliability of amplification methods for the direct detection of *M. tuberculosis* is not as satisfactory as for other microorganisms because of the unsatisfactory sensitivity. Among the possible reasons of such limitation the problematic extraction of DNA, imputable to the extremely simplified procedures which may have problems with the unique structure of the mycobacterial cell wall, and the uneven texture of the mucous matrix of respiratory samples, which are, by far, the most commonly investigated specimens. Standing such limits is unquestionably excluded the possibility of using amplification technique as a substitute of microscopy and culture which therefore remain the backbone of diagnostic mycobacteriology. A new generation of nucleic acids amplification systems, based on real time PCR, is round the corner; although they seem to have the potential to replace the present methods, too little information is so far available about them. A not less important field of application of molecular methods in mycobacteriology concerns the species identification. The taxonomy of the genus *Mycobacterium* is revealing a complexity unsuspected until a few years ago. The number of officially recognized species exceeds nowadays 120 and is likely it will still substantially increase. It is undoubted that the rapid differentiation of the non tuberculous mycobacteria from the members of the *M. tuberculosis* complex is crucial; the identification of every strain at the species-level, however, is also important to assess the clinical significance of the finding and, in case, to set up the proper treatment. The most popular methods for identification of mycobacteria include PCR-restriction analysis (PRA) and genetic probe hybridization (DNA-probe). The PRA, first developed by Telenti more than 10 years ago, is based on the enzymatic digestion of a fragment of hsp65 gene by separately using two different restriction enzymes: BstEII and HaeIII. A proper algorithm drives the microbiologist to the final identification once the size of the restriction fragments has been carefully determined by means of electrophoresis. The method is inexpensive and easy to perform. The increasing number of species showing overlapping profiles is its major limit, while the presence of multiple patterns within a single species, although confusing, is counterbalanced by the opportunity of recognizing different biotypes. While the PRA is essentially a home-made

method, the DNA-probes almost solely relies on commercial kits. Several approaches, commercialized by three different firms are at present available, they aim to different regions within the ribosomal DNA operon. The target of the method based on the liquid phase hybridization (AccuProbe, Gen-Probe, USA) is 16S rRNA; no amplification is required and the positive result is revealed by the production of a chemio-luminescent signal in a hybridization-protection assay. The method is highly specific, rapid and handy, it is however suitable to identify a very little number of species and may result very costly once the testing with more than one probe is required. The alternative methodology is the solid phase reverse hybridization relying on multiple probes immobilized at different positions of a cellulose strip. Once the amplified, biotinylated, genetic target hybridizes with the specific probe a coloured band develops following the addition of the avidin-enzyme conjugate and the chromogenic substrate. The known position on the strip of different species-specific probes leads to the identification on the basis of the location of the coloured hybridization band. It becomes therefore possible to identify, with a single test, multiple species. INNO LiPA Mycobacteria (Innogenetics, Belgium) aims to the internal transcribed spacer (ITS) and can identify 18 different species. Two different strips became recently available with the rival system GenoType Mycobacterium (Hein, Germany), directed to the 23S rDNA. The combination of two strips allows to identify as many as 33 species. Specificity and sensitivity of the reverse hybridization-based tests are excellent even if minor cross reactions with rarely encountered species have been reported. The sequencing-based identification relies on the same insight as DNA probe technology: i.e. the investigation of highly conserved genetic regions harbouring species-specific variability. Several such regions have been detected in mycobacteria: 16S rDNA, ITS, 23S rDNA, hsp65, sodA, recA, rpoB. The 16S rDNA is by far the most frequently investigated; it is characterized by the presence of two hyper variable regions within the first 500 bp trait which allow the identification of almost all the official species and, within which, the intra-species variability is extremely limited. The corresponding database, available in the Internet is very rich. In the ITS a much larger variability is present and many species are characterized by several sequevars in this region; thanks to this characteristic it can be useful to differentiate species presenting overlapping in the 16S rDNA. Despite the importance of the ITS database not yet reported sequences are frequently detected. The 23S rDNA is, in contrast, poorly variable with several species not being identifiable in such region. The rpoB gene has been recently proposed as particularly suitable for the identification of rapid growers; the high variability characterizing such region seems in fact particularly suited for such organisms which include species sometime undistinguishable at level of 16S rDNA. Genetic sequencing is the reference method for identifying mycobacteria, all the more as, differently from the DNA probes, no limit exists in the number of species it can identify. It represents furthermore a powerful research tool thank to which the number of species and variants has enormously increased in the last years. The handily searchable publicly available databases, mainly GenBank and EMBL, represent the core of genetic sequencing. The open access for everyone to depose new sequences represent, at the same their time major value and limit. While, from one hand, the new depositions, keep the databases up to date, from the other hand, because off the lack of any control, they may result misleading with the query sequence presenting at time the best resemblance with a short and unreliable sequence such hindering more significant ones. Although disregarded by some, the differentiation of the species belonging to the *M. tuberculosis* complex is important, both because of the different susceptibility to pyrazinamide characterizing *M. tuberculosis* and *M. bovis* and for epidemiological reasons. A reverse hybridization system (Genotype MTBC, Hein) targeting different genomic regions (23S rDNA, gyrB, RD1) allows the easy and accurate differentiation of most frequently encountered members of the complex. For what concerns *M. tuberculosis*, molecular biology made available a number of techniques providing very useful information. This branch, known as fingerprinting, allows the differentiation of *M. tuberculosis families* and even of single strains. The epidemiology is the field which more benefits from such investigations but not less important is the utility in recognizing mixed infections or to distinguish new disease from relapse. Of great practical importance is finally the monitoring of laboratory contaminations. The spoligotyping, which is based on the polymorphism of the spacers present in the DR region, is not suitable for fine differentiations but is optimal to group the strains in large groupings like Beijing family and many others. The restriction fragment length polymorphism of IS6110 targets an insertion element present, in variable number of copies and at different positions, within the *M. tuberculosis* genome. It is at present considered the gold standard and is suitable to differentiate practically almost every *M. tuberculosis* strain. A more recently developed method investigates the polymorphism of 12 tandem repeat sequences known as mycobacterial interspersed repetitive units. Such system which is characterized by high discriminatory power has, being fully automatable; the potential of providing real time results and is candidate to become the future reference method. Microarrays, which are essentially miniaturized multi-probe systems, can be considered the last development of solid phase hybridization technique. It seems very likely that they will pay a major role in the future of mycobacteriology, several prototypes have already been developed and the commitment of commercial companies in this field is yet important. With microarrays it becomes possible to inquire a large number of genetic targets suitable to provide important information for the identification, the antimicrobial susceptibility and the fingerprint of mycobacteria. A risk which should not underrated is the spreading of such techniques out of the reference laboratories; most of the reliability of more and more sophisticated technologies relies in fact in the high specialization of the user.

S413

Infections due to mycobacteria other than *M. tuberculosis* (MOTT) in non-HIV patients

R. Wallace *(Tyler, US)*

Nontuberculous mycobacteria (NTM), also known as Mycobacteria other than tuberculosis (MOTT), are an increasingly diverse group of organisms. More than 90 species are currently recognized with clinical disease observed in multiple species including man. *Mycobacterium avium, M. intracellulare,* and *M. kansasii* are the major causes of chronic pulmonary disease, with *M. fortuitum, M. abscessus, M. marinum,* and *M. ulcerans* the major cause of chronic skin/soft tissue disease. Emerging NTM disease include folliculitis following the use of foot spas, *M. abscessus* disease in cystic fibrosis, and disease due to previous animal pathogens including *M. porcinum* and *M. senegalense*. Treatment of chronic pulmonary infections due to *M. avium* complex, *M. abscessus,* and *M. xenopi* remain difficult and disease control but not microbiologic cure are the endpoint of therapy for many patients. Cutaneous and soft tissue disease in developed countries is usually readily treatable. Drug resistance due to target site mutation or the presence of erm genes (*M. fortuitum* group) complicate drug therapy of both

slowly growing and rapidly growing species. An approved CLSI (Clinical Lab Standards Institute, formerly NCCLS) standard for susceptibility testing of the NTM was approved in the U.S. in 2003. Most recommended test methods involve broth macrodilution or microdilution. Laboratory identification increasingly involves molecular methods as older methodology often fails to recognize newer species. Current identification involves a number of genes for sequencing, including 16S r-RNA gene (initial 500 bp), the 16S-23S internal transcribed spacer region, the hsp65 gene and the rpoB gene. The use of RFLP patterns and sequencing of these and other housekeeping genes has opened the door to molecular-based population studies of individual species. *M. kansasii* has been studied in the greatest detail using the hsp65 gene; with type I strains now recognized as the major human pathogen. Other studies are ongoing involving *M. avium, M. simiae,* and *M. lentiflavum*. These studies suggest that individual clones or subspecies are associated with different hosts and different geographic sites. Complete genomic sequencing of multiple strains from many of the NTM is a major need for better understanding of the NTM, their diversity, and their pathogenicity. Such studies are slowly taking place. Because of the environmental reservoir, NTM will remain an important animal, bird, and human pathogen of increasing clinical significance.

RTI in the community revisited

O416

The management of sore throat by Finnish general practitioners

P.O. Honkanen, U.-M. Rautakorpi, S. Huikko, T. Klaukka, P. Huovinen, E. Palva, R. Roine, H. Sarkkinen, H. Varonen, M. Mäkelä *for MIKSTRA collaborative study group*

Objectives: To assess the quality of the management of sore throat among Finnish general practitioners. The determinants of a good management of sore throat are focusing the microbiological tests to those with possible group A streptococcal (GAS) tonsillitis, prescription of antibiotics to those with a positive test result for GAS, prescription of analgesics to all patients with sore throat.
Methods: One-week survey in mid November in 30 Finnish health centres (the population of 800 000) in 2002. The symptoms were reported by the patients or their parents. The physicians reported diagnosis, diagnostic tests and treatment.
Results: There were 823 patients with sore throat as one of the symptoms, of those with 142 as the only complaint. 681 patients had also coryza or cough. Among the 142 patients the diagnoses were 64 (45%) tonsillitis, 26 (18) pharyngitis, 33 (23) URTI, and some other respiratory infection 19 (13). The corresponding figures among those 681 patients were 57 (8), (9), 277 (41), and 289 (42). Of those 123 (out of 142) patients with tonsillitis, pharyngitis or URTI, a microbiological test was done to 72 (59), antibiotics were prescribed to 61 (50), and analgesics to 15 (12). The corresponding figures for 392 patients (out of 681 with sore throat, coryza or cough) were 111 (29), 90 (23), and 33 (8).
Conclusions: Microbiological tests were used quite frequently. Most of the tests were done to those with coryza or cough, and a low prevalence of GAS. Because of a good likelihood ratio of the tests, the result can guide the treatment decision correctly even if the pretest probability of GAS is low. However, excessive use of tests without careful clinical consideration increases costs of the management. The frequency of the prescription of antibiotics was lower than that of microbiological tests. However, this analysis does not show how many prescriptions were given without a test. The divergence of routine management from a good clinical practice was widest in the use of analgesics. The points to be emphasised in the information delivered to Finnish general practitioners: The importance of clinical diagnosis preceding the use of microbiological tests, and in treatment, the use of analgesics.

O417

Prevalence of *Streptococcus viridans* isolates in nasopharyngeal samples from children attending day-care centres in the Alpes Maritimes, France

H. Carsenti Dellamonica, G. Melica, C. Pradier, B. Dunais, G. Mancini, M. Sabah, P. Bruno, P. Toubol, P. Dellamonica *for the GEPIE Group*

Objective: To monitor prevalence rates and antibiotic susceptibility of *Streptococcus viridans* (SV) nasopharyngeal carriage strains among children aged 3 to 40 months attending day-care centres in the Alpes Maritimes, France, in the context of antibiotic prescription-reducing campaigns (Alpes Maritimes 2001, 2003, France 2003).
Method: Nasopharyngeal aspirates were obtained from a random 2-stage cluster sample of children attending day-care centers in the Alpes Maritimes (AM) areas during 2 consecutive surveys conducted between January and March 2002 and 2004. Susceptibility of SV isolates to beta-lactams, macrolides, tetracycline, chloramphenicol and fluoroquinolones was tested by disk diffusion and E-test for penicillin, amoxicillin and cefotaxime.
Results: In 2002, 26 out of 294 (9.18%) children carried 27 strains, of which 14 were *S. mitis* and 7 were *Gemella morbillorum* group, 2 to the *S. oralis* group, 1 to the *S. sanguis* group and, respectively 1, 1, 2 to the *A. viridans*, *S. bovis*, *S. adjacens*. In 2004, 57 out of 337 (17%) children carried 63 strains, of which 39 were *S. mitis*, 6 *S. oralis*, 4 *Gemella morbillorum*, 1 *G. haemolysans*, 1 *Lactococcus lactis*, 1 *leuconostoc* sp., 2 *S. acidominimus* and 9 *S. pyogenes*. Penicillin decreased susceptibility was 59.25% in 2002 and 53.97% in 2004. Carriage of penicillin I and R strains was 25.9% and 33.3% in 2002 and 33.3% and 20.6% in 2004. Amoxicillin decreased susceptibility was 22.2% in 2002 and increased to 50.8% in 2004 associated with cefotaxime decreased susceptibility of 44.4% the same year. Erythromycin resistance rate was 66.67% in 2002 and 58.73% in 2004, while 3.17% R and 7.93% I to telithromycin strains appeared in 2004. The erm phenotype of resistance predominated in the 2 periods. Ciprofloxacin R of 7.4% in 2002 increased to 23.8% in 2004 and 3.17% of the strains showed associated R to levofloxacin sparfloxacin and moxifloxacin in 2004.
Conclusion: A small decrease in strains of VGS resistant to penicillin or erythromycin was observed as for *S. pneumoniae* in this children population in the two periods. However the same trend in increase of intermediate strains to amoxicillin and cefotaxime is of concern such as the increase in fluoroquinolone resistance rate observed, not easily explicable in children populations and which requires further evaluations. Ongoing surveillance of the resistance profile is important among VGS since they are able to transfer resistance to other bacterial populations.

O418

Erythromycin resistance in commensal throat Streptococci in asylum seekers

C.J.P.A. Hoebe, E.J.W.N. Niekamp, P.J.P. Tolsema, E. Stobberingh *(Heerlen, Maastricht, NL)*

Objective: Commensal streptococci form a large reservoir of transferable erythromycin resistance (genes) for potential pathogenic microorganisms. The aim of this study was to assess the rate of erythromycin resistance in asylum seekers as a potential resistance (genes) reservoir for the Dutch population. As the bacterial resistance of asylum seekers reflects very likely the resistance situation in their home country, it is to be expected that these resistance is higher than those in the Netherlands. Therefore, treating asylum seekers with antibiotics effective for the Dutch population might be not appropriate for them.
Methods: The prevalence of erythromycin resistance in commensal throat streptococci was determined in two random groups of asylum seekers from five different asylum seeker centers. Study groups were from Armenia (n = 64) and from Africa (n = 64) and we included a control group of 185 Dutch volunteers. A throat swab was used to collect the commensal throat flora. The swab was suspended in 1 ml. saline and inoculated on bloodagar plates without and with 16 mg/L erythromycin using a spiralplater. All asylum seekers filled out a questionnaire concerning demographic data, length of stay, number of household members, use of antibiotics and history of hospital admission. Asylum seekers were mostly men (59%), had a mean age of 31 years (SD 10), their duration of stay was mainly between 1 to 2 years (49%; 32% less than 1 year and 19% more than 2 years), 31% had hospital admission in home country and 11% in the Netherlands and 7% used antibiotics in the last year.
Results: The prevalence of erythromycin resistance among asylum seekers was 48.4% (95%CI = 39.3–56.7) and among the Dutch volunteers 24.9% (95%CI = 17.8–30.2). Within the group of asylum seekers the prevalence among Armenians was 40.6% (95%CI = 28.6–52.6) and 56.3% (95%CI = 44.2–68.4) among Africans. Resistance decreases in relation to length of stay in the Netherlands. For asylum seekers shorter than one year in the Netherlands the prevalence was 54%, between 1 year and 2 years this was 41% and longer than 2 years this was 33%.
Discussion: The prevalence of erythromycin resistance among asylum seekers was two times higher than among Dutch volunteers. This finding needs a reflection in antibiotic use for treating potential pathogenic throat bacteria in asylum seekers in general practice. Resistance in asylum seekers shows an adaptation to the Dutch population with longer stay.

O419

The role of antibiotics in acute uncomplicated maxillary sinusitis in ambulatory care: a randomised controlled trial

L. Prezas, A. Antoniadou, P. Panagopoulos, T. Tsaganos, A. Agathagelides, G. Danielides, T. Stathas, M. Rigatou, N. Karvelis, E. Moshavakis, T. Papaeliadis, E. Ferekidis, S. Symeonidis, H. Giamarellou *(Athens, Salonica, Patra, GR)*

Introduction: Acute maxillary rhinosinusitis is a common diagnosis in primary care medical practice and an area of antibiotics overuse. Principles of treatment published a few years ago advocated that antimicrobial treatment should be reserved for patients with rhinosinusitis symptoms lasting 7 days or more (maxillary pain or tenderness in the face or teeth -especially when unilateral- and purulent nasal secretions) or those presenting with severe symptoms. Aim of the study was to assess the hypothesis that acute, uncomplicated, with moderate symptoms and less than 7 days duration maxillary rhinosinusitis can be managed and resolve without antibiotics through a randomised controlled trial.
Patients and Methods: From 4/2002 to 1/2005 238 adult patients, 113 male and 125 female, (mean age = 34.4 years, SD = 11.9) with a history and clinical signs of maxillary sinusitis of less than 7 days duration enrolled in the study. These patients were clinically evaluated for severity of signs and symptoms and randomly assigned to receive treatment with cefprozil or doxycycline (n = 136) or no treatment (n = 102). Both groups were also offered symptomatic treatment with the same analgesics and decongestants. Clinical course was assessed after 8 days, at the end of antibiotic therapy, and at telephone follow-up visits at 1 and 6 months post treatment. Total remission was defined as complete resolution of signs and symptoms, partial remission as improvement but not complete resolution and failure as persistence of symptoms.
Results: At the 1-week visit, total remission was reported in 118/136 (86.7%), partial remission in 13/136 (9.6%) and failure in 5/136 (3.7%) of the patients who received antibiotics. Respectively, in the no treatment patient group 85/102 (83.3%) patients showed total remission, 11/102 (13.3%) showed partial remission and in 6/102 (5.9%) patients failure was recorded. No statistically significant differences were found in the output proportions between the two groups. Follow-up at 1 and 6 months revealed no differences between the two groups in relapses and late complications.
Conclusion: Antibiotic treatment does not offer a better outcome in the treatment of acute adult maxillary sinusitis of moderate severity and of less than 7 days duration. Symptomatic treatment with decongestants and analgesics is sufficient for the resolution of the disease.

O420

Prevalence of respiratory diseases in sheltered homeless in Marseille, France

S. Badiaga, H. Richet, P. Azass, F. Rey, H. Tissot Dupont, C. Foucault, C. Rovery, M. Drancourt, D. Raoult, P. Brouqui *(Marseille, FR)*

Objectives: To describe the characteristics and the prevalence of respiratory diseases in the homeless population in Marseilles.
Methods: Snapshot study 2 nights in January 2005, by a medical team of 40 persons including infectious diseases senior, residents and fellows, pneumologists, nurses, X-ray technicians in the 2 shelters in Marseilles. Homeless were interviewed, examined, and received care. Blood was sampled for cell count, detection of bacteraemia and antibodies to Chlamydia, Mycoplasma, *Legionella pneumophila*, and *Coxiella burnetii*. A chest-X radiograph was performed.
Results: A total of 221 homeless persons were enrolled. The mean age was 41 years ± 13 and 94% of the population were men; 38% were born in North Africa, 37% in France, and 14% in Central/Eastern Europe; the subjects have been homeless less than 7 months in 43%, from 7 to 12 months in 12%, from 13 to 24 months in 8.1%, and more than 24 months in 36%. Among study population, 77% were current smokers, 65% were alcohol abusers, 2 persons were infected by HIV, 30% reported prior contact with tuberculosis patients (in shelter 23% and in family and relatives in 77%). The most frequent reported symptoms were weight loss (43%), chronic cough (39%), sputum production (33%), sweats (16%), and dyspnoea (16%). Clinical

ISSN: 1470-9465

diagnosis was established as bronchitis in 19%, chronic obstructive bronchitis in 17%, asthma in 2%, and acute pneumonia in 1%. All blood cultures; as well as serological tests were negative. Chest radiographs performed in 219 persons and subsequently analysed showed abnormalities in 14 persons (6%). Of these 14 homeless persons, 4 have not been reached for follow up and 10 have hospitalized for further investigations. The final diagnosis made were active tuberculosis in 2 persons (1%) who have been homeless for 10 and 11 years, sequels of tuberculosis in 4 (2%), lung cancer in 1 (0.5%), obstructive lung disease in 1 (0.5%), acute pneumonia in 1 (0.5%), and no specific diagnosis in 1 (0.5%). A specific care was given for each of these homeless persons.

Conclusion: This snapshot investigation revealed that 6 % of this homeless population had life threatening respiratory diseases in which 1% had active contagious tuberculosis. Systematic chest radiography could be an efficient strategy to rapidly identify and prevent the spread of this transmissible disease among homeless population of shelters.

Treatment, antifungal resistance and epidemiology of fungal infections

O421

Posaconazole as prophylaxis for invasive fungal infections in high-risk patients: efficacy and safety results from 2 clinical trials

J. Maertens, A.J. Ullmann, S. Durrant, J. Holowiecki, J.H. Lipton, D. Stockelberg, D.J. Winston, J. Perfect, N. Boparai, C. Hardalo, D. Angulo-Gonzalez, O.A. Cornely *(Leuven, BE; Mainz, DE; Brisbane, AU; Katowice, PL; Toronto, CA; Gothenburg, SE; Los Angeles, Durham, Kenilworth, US; Cologne, DE)*

Objectives: Efficacy and safety of posaconazole (POS), an extended-spectrum triazole, as prophylaxis for invasive fungal infections (IFIs) were evaluated in 2 large, randomised, active-controlled, parallel-group trials. The trials were conducted in 2 populations at high risk for IFI: allogeneic haematopoietic stem cell transplant (HSCT) recipients with graft-versus-host disease (GVHD) and neutropenic patients undergoing intensive chemotherapy for acute myelogenous leukemia (AML) or myelodysplastic syndrome (MDS).

Methods: In study 1, HSCT recipients with GVHD received either oral POS 200 mg three times daily or oral fluconazole (FLU) 400 mg once daily for up to 16 weeks. In study 2, patients with newly diagnosed or first relapse of AML or MDS received oral POS 200 mg three times daily or oral standard azole [FLU 400 mg once daily or itraconazole (ITZ) solution 200 mg twice daily] with each cycle of chemotherapy until complete remission or for up to 12 weeks. The incidence of proven or probable IFI was the main endpoint in each study. IFI incidence was evaluated 7 days after the last dose of study drug and at predetermined times (ie, 112 and 100 days after randomization for studies 1 and 2, respectively). IFI diagnoses were adjudicated by blinded expert panels using EORTC/MSG criteria.

Results: In study 1,600 patients were enrolled: 301 POS and 299 FLU. In study 2,602 patients were enrolled: 304 POS and 298 standard azole (240 FLU, 58 ITZ). In both studies, fewer IFIs occurred with POS; for almost all endpoints, the difference was significant (Table). Safety and tolerability between treatment arms were comparable within each study. In study 1, the 3 most common treatment-related adverse events (AEs) were nausea (7% vs. 9%), vomiting (4% vs. 5%), and diarrhoea (3% vs. 4%) in the POS and FLU groups, respectively. In study 2, the 3 most common treatment-related AEs were also nausea (7% vs. 8%), diarrhoea (7% vs. 7%), and vomiting (5% vs. 7%) in the POS and FLU/ITZ groups, respectively. Incidence of treatment-related AEs resulting in discontinuation was similar between POS and comparator therapy in both studies (3% for each arm in study 1; 7% vs. 6%, respectively, in study 2).

Conclusion: These findings show POS is superior to standard azole therapy (FLU or ITZ) in preventing aspergillosis and other IFIs in HSCT recipients with GVHD and in AML and MDS patients with chemotherapeutic-induced neutropenia. Safety and tolerability of POS were comparable to standard azole therapy.

	Study 1 Antifungal Prophylaxis in HSCT Recipients With GVHD			Study 2 Antifungal Prophylaxis in AML or MDS Patients With Neutropenia		
Incidence, n (%)	POS (n=301)	FLU (n=299)	*P* value	POS (n=304)	FLU or ITZ (n=298)	*P* value
Proven/probable infections while on treatment						
All IFIs	7 (2)[a]	22 (8)[b]	.0038	7 (2)	25 (8)	.0009
Invasive aspergillosis	3 (1)[a]	17 (6)[b]	.0009	2 (1)	20 (7)	.0001
Proven/probable infections during fixed time period[c]						
All IFIs	16 (5)	27 (9)	.0740	14 (5)	33 (11)	.0031
Invasive aspergillosis	7 (2)	21 (7)	.0059	4 (1)	26 (9)	.0001

a. n=291 (treated subjects); b. n=288 (treated subjects); c. Within 112 days postbaseline in study 1; within 100 days postrandomisation in study 2.

O422

Mortality in patients at high risk for invasive fungal infection: effect of Posaconazole prophylaxis

O.A. Cornely, J. Maertens, S. Durrant, J. Holowiecki, J.H. Lipton, D. Stockelberg, D.J. Winston, J. Perfect, R. Suresh, L. Pedicone, H. Patino, A.J. Ullmann *(Cologne, DE; Leuven, BE; Brisbane, AU; Katowice, PL; Toronto, CA; Gothenburg, SE; Los Angeles, Durham, Kenilworth, US; Mainz, DE)*

Objectives: Patients with acute myelogenous leukaemia (AML) or myelodysplastic syndrome (MDS) undergoing chemotherapy and haematopoietic stem cell transplant (HSCT) recipients with graft-versus-host disease (GVHD) are at high risk for fungal infections and their associated mortality. Antifungal prophylaxis with a standard azole [fluconazole (FLU), itraconazole (ITZ)] is frequently used in these patients, but a survival benefit has not been consistently demonstrated. We report all-cause and fungal-related mortality from 2 randomized, active-controlled, parallel-group clinical trials that evaluated posaconazole (POS), an extended-spectrum triazole, as prophylaxis for IFI.

Methods: In study 1, HSCT recipients with GVHD (N = 600) received either POS 200 mg three times daily or FLU 400 mg once daily for 16 weeks and were followed for 8 additional weeks. In study 2, neutropenic patients (N = 602) with new diagnosis or first relapse of AML or MDS undergoing intensive chemotherapy received either POS 200 mg three times daily or

oral standard azole therapy (FLU 400 mg once daily or ITZ solution 200 mg twice daily) until complete remission or for up to 12 weeks; patients were followed for at least 100 days after randomisation. Overall and investigator-determined IFI-related mortality, which were secondary endpoints in the studies, were evaluated using Kaplan-Meier (KM) analysis of time to death. Survival comparisons were based on log-rank test.

Results: In study 1, although fewer patients died in the POS group, there was no statistically significant difference in the overall survival between treatment groups using log-rank test (Table). In study 2, significantly fewer overall deaths occurred in the POS group, and the probability of survival at day 100 after randomisation was significantly higher with POS. Significantly fewer IFI-related deaths were observed with POS in both studies.

	Study 1[a] Antifungal Prophylaxis in HSCT Recipients With GVHD			Study 2[b] Antifungal Prophylaxis in AML or MDS Patients With Neutropenia		
Deaths, n (%)	POS n=301	FLU n=299	log-Rank Test *P* value	POS n=304	FLU or ITZ n=298	log-Rank Test *P* value
All cause mortality	76 (25)	84 (28)	.376	44 (15)	64 (22)	.035
IFI-related	4 (1)	12 (4)	.046	5 (2)	15 (5)	.021

a. Deaths reported at end of study (day 168).
b. Deaths reported up to 100 days postrandomisation (censoring time)

Conclusion: Prophylaxis with POS provided a significant overall survival benefit among AML or MDS patients undergoing chemotherapy, when compared with other azoles. In addition, POS prophylaxis significantly reduced IFI-related mortality in HSCT recipients with GVHD as well as AML and MDS patients with neutropenia. These results suggest that future prophylaxis trials should be designed with adequate power to detect differences in overall and IFI-related mortality.

O423

Epidemiology of fungal infections in haematological malignancies in Italy: SEIFEM-2004 study (Sorveglianza Epidemiologica Infezioni Fungine nelle Emopatie Maligne)

L. Pagano, M. Caira, A. Candoni, L. Corvatta, M. Offidani, B. Martino, D. Pastore, M. Picardi, A. Bonini, A. Chierichini, R. Fanci, C. Caramatti, C. Benatti, A. Gallamini, M.R. Fadda, L. Melillo, B. Allione, L Fianchi, P. Falcucci, C.G. Valentini, M.T. Van Lint, A. Nosari, C. Girmenia on behalf of SEIFEM *Studio Epidemiologico Infezioni Fungine nelle Emopatie Maligne*

Objectives: To evaluate the incidence and the outcome of fungal infections (FI) in patients (pts) affected by hematological malignancies (HM).

Methods: A retrospective study, conducted over 1999–2003, in pts with HM, admitted in 18 Italian haematology divisions in tertiary cares or university hospitals, which developed proven or probable FI.

Results: The population included 11,802 pts: 3,012 with AML (25.5%), 1,173 with ALL (9.9%), 596 with CML (5%), 1,104 with CLL (9.4%), 1,616 with MM (13.7%), 3,457 with NHL (29.3%), 844 with HL (7.2%). Pts who underwent autologous or allogenic HSCT were included in a specific different analysis. A proven or probable FI occurred in 538 pts, with an incidence of 4.6%; in particular we registered 346 episodes sustained by moulds (incidence 2.9%) and 192 by yeasts (1.6%). The incidence rate depends upon underlying HM (12.3% in AML, 6.5% in LLA, 2.5% in CML, 0.5% in CLL, 0.5% in MM, 1.6% in NHL, 0.7% in HL). Among moulds, the detected etiological agents were *Aspergillus* spp. (310 episodes, incidence 2.6%), *Mucorales* spp. (14 episodes, 0.1%), *Fusarium* spp. (15 episodes, 0.1%), and other rare fungi (7 episodes, 0.1%). Among yeasts we registered septicemia sustained by *Candida* spp. (175 pts, incidence 1.5%). Other yeast infections were caused by *Cryptococcus* spp. (8 pts, incidence 0.1%), *Tricosporon* spp. (7 pts, 0.1%) and other rare agents (2 pts). The overall mortality rate was 1.8%. Among 538 pts with FI, case fatality rate (CFR) was 39%, with differences between aspergillosis (42%), zygomycosis (64%), fusariosis (53%) and candidemia (33%). There was not variation in mortality rate during the study period; comparing these pts with those observed in our previous epidemiological studies during the period 1987–1988 we observed a significant reduction of deaths due to aspergillosis (RR 1.90; 95%CI 1.17–3.09), but no differences in mortality rate due to *Candida* spp.

Conclusion: Our study confirms the general trends already described: infections due to moulds continue to be more frequent than those caused by yeast. Among all fungi, *Aspergillus* spp. remains the main etiologic agent, followed by *Candida* spp. Other agents (*Mucorales* spp., *Fusarium* spp., *Trichosporon* spp.) remain rare. AML represents the most frequently involved category. The mortality rate due to aspergillosis is actually about 40%, with a remarkable decrease when compared to past years; as for candidemia, we observed a reduction in the incidence, but not in the mortality rate.

O424

Micafungin versus liposomal amphotericin B in the treatment of invasive candidiasis in neutropenic and non-neutropenic patients

O. Lortholary, E. Kuse, P. Chetchotisakd, C. Arns da Cunha, M. Ruhnke, C. Barrios, D. Raghunadharao, J.S. Sekhon, S. Koblinger *(Paris, FR; Hannover, DE; Khon Kaen, TH; Curitiba, BR; Berlin, DE; Porto Alegre, BR; Hyderabad, Ludhiana, IN; Munich, DE)*

Objectives: To compare micafungin (MICA), an echinocandin agent with broad-spectrum fungicidal activity against *Candida* species, and liposomal amphotericin B (AmBisome®, L-AMB), an established treatment of invasive candidiasis (IC), for first line therapy of IC.

Methods: This was a Phase III, 1:1 randomized, double blind, non-inferiority study. Adult patients were randomized to MICA (100 mg/day) or L-AMB (3 mg/kg/day). Inclusion in the per protocol population (PPS) required confirmed diagnosis of IC, evaluation at end of therapy and received at least 5 doses of study drug. Patients were stratified by neutropenic status (< 500 cells/mm^3). The primary endpoint was overall treatment success (defined as clinical and mycological response) in the PPS at end of therapy assessed by the investigators.

Results: 531 patients received at least one dose of study drug. The PPS comprised 202 (MICA) and 190 (L-AMB) patients. The treatment groups were balanced with regard to baseline characteristics, including neutropenic status (MICA 11.9% vs. L-AMB 7.9%) and Apache II scores (mean MICA 14.7 vs. L-AMB 14.9). Overall, 84.2% (MICA) and 85.8% (L-AMB) of patients had confirmed candidaemia; sites of IC were primarily peritoneal (MICA 7.9% vs. L-AMB 5.8%). Non-albicans Candida infections comprised 62.4% (MICA) and 58.9% (L-AMB) of patients. Overall treatment success was experienced by 89.6% (181/202) and 89.5% (170/190) of patients in the MICA and L-AMB groups, respectively. Criteria for non-inferiority were met; the

difference (MICA-L-AMB) adjusted for neutropenia and corresponding 95% CI was 0.7% (−5.3%, 6.7%). Treatment success rates were similar for *C. albicans* infections (MICA 88.4% vs L-AMB 89.3%) and non-albicans C. infections (MICA 89.7% vs. L-AMB 89.3%). In the modified intent to treat population (confirmed IC and at least one dose of study drug) overall treatment success was 74.1% (183/247) in the MICA group and 69.6% (172/247) in the L-AMB group. During the 12-week post-treatment period, there was no difference in recurrent invasive fungal infection between both groups. Predefined safety parameters showed MICA to have an advantage over L-AMB in renal function as assessed by estimated glomerular filtration rate and a lower incidence of infusion-related reactions ($p < 0.001$).
Conclusion: This study demonstrates the non-inferiority of micafungin versus AmBisome® in first line treatment of IC and an advantage for micafungin in predefined safety parameters.

O425

Clinical effects of micafungin, an echinocandin antifungal agent, on systemic fungal infection in the areas of surgery, emergency, and intensive care medicine

N. Aikawa, S. Kusachi, S. Oda, Y. Takesue, H. Tanaka *(Tokyo, Chiba, Hiroshima, JP)*

Objectives: Micafungin (MCFG) has been approved in Japan for treatment of fungemia and invasive infections caused by Candida and Aspergillus. To our knowledge, there are no reports that evaluated the effects of MCFG treatment in the areas of surgery, emergency, and intensive care medicine. We therefore studied its efficacy and safety in patients with fungal infection in a multicentre study.
Methods: The study was conducted from July 2003 to March 2005 in patients with a fever of 37.5°C or higher, who met any of the following criteria as the rationale for the diagnosis: #1 Patients with a causative fungus identified by mycological or pathological examination; #2 Patients with fungi detected at multiple sites by surveillance culture or with a positive beta-D-glucan test. Patients who met requirement #2 had to have a high risk factor for the development of systemic fungal infection. Patients who met these requirements were enrolled to the study, after registration with a third party before the start of MCFG treatment. Efficacy was evaluated by assessing the improvement in each of the following items: 1) clinical symptoms/findings possibly attributable to mycosis; 2) mycological findings; 3) imaging findings such as chest X-ray; 4) fungal serological tests. Efficacy was rated in 2 grades, namely "effective" and "ineffective", based on an algorithm combining these indices (called AKOTT algorithm assessment).
Results: Out of 180 registered patients, 116 patients (71 males, the mean age: 61.4 ± 18.2) were evaluated for efficacy by the steering committee. The mean dosage and duration of dosing were 106.0 ± 62.2 mg·per·day and 14.8 ± 8.6 days, respectively. Common underlying diseases included perforation of the digestive tract, burns, stroke, and trauma. Clinically, 74 of 102 patients (72.5%) were rated as effective. By major diagnosis, the effective rates in patients with candidemia and pulmonary candidiasis were 77.8% and 84.6%, respectively. MCFG was effective not only against *C. albicans* but also against non-albicans *Candida* species. Adverse drug reactions occurred in 38 of 180 patients (21.1%), and the most commonly observed reaction was hepatic function disorder. There were no serious adverse drug reactions with a clear causal relationship to MCFG.
Conclusion: MCFG demonstrated excellent clinical effects on systemic fungal infection in the areas of surgery, emergency, and intensive care medicine, indicating its usefulness as a new therapeutic drug.

O426

Universal role of glucan synthase subunit Fks1p in echinocandin resistance

D. Perlin, S. Park *(Newark, US)*

Objective: An association between reduced susceptibility to the echinocandin drug caspofungin (MIC > 8 ug/ml) and amino acid substitutions in the Fks1p subunit of the beta (1,3)-D-glucan synthase (GS) was recently reported (Park et al. 2005 Antimicrob. Agents Chemother. 49: 3264–3273). Specific mutations in FKS1 genes were identified from both laboratory and clinical isolates of *C. albicans*, and two ''hot spot'' regions affecting susceptibility were defined as Phe641-Pro649 and the residues around Arg1361. To better understand the universality of FKS1 mutations for reduced echinocandin susceptibility, we examined cross-reactivity of mutants to micafungin and characterized the ''hot-spot'' regions in isolates of non-*C. albicans* species showing reduced drug susceptibility.
Methods: Antifungal susceptibility testing on yeasts was performed using the broth micro dilution method of the NCCLS document M27-A2. Purification of glucan synthase and IC50 values were determined as described by Park et al. 2005. A 2.4 Kb region of FKS1 from *C. glabrata, C. guilliermondii, C. krusei, C. lipolytica, C. parapsilosis, C. rugosa, C. tropicalis* and *C. dubliniensis* was PCR amplified using consensuses FKS1 primers. All PCR products were subjected to automated DNA sequencing in both 5′ and 3′ directions.
Results: The fks1 mutants showed comparable reduced susceptibility to both caspofungin and micafungin in growth assays, and specific mutations were associated with a > 1000-fold reduction in the inhibition of GS activity by both echinocandin drugs. Characteristic mutations associated with reduced susceptibility in *C. albicans* were also found in *C. tropicalis, C. dubliensis* and *C. krusei,* which correlated with reduced IC50 values in assays of glucan synthase activity.
Conclusion: The data supports the notion that reduced susceptibility to echinocandin drugs in clinical isolates can arise from amino acid substitutions in defined regions of the highly conserved Fks1p subunit of glucan synthase.

O427

Human serum alters antifungal efficacy of echinocandin drugs

D. Perlin, S. Park *(Newark, US)*

Objective: The echinocandin drug Mycamine (micafungin) shows superior in vitro antifungal drug efficacy relative to Cancidas (caspofungin). Yet, echinocandin-class drugs show a high level of bound serum protein, and it has been suggested that differential binding to different drugs could alter their relative antifungal efficacy. To explore this possibility, the differential effects of serum on micafungin in direct comparison with caspofungin were examined in assays of in vitro antifungal susceptibility with an epidemiologically distinct collection of clinical isolates of *Candida* spp. and *Aspergillus* spp.
Methods: Determine the MIC or MEC values of caspofungin and micafungin for a wide range of clinical isolates of *Candida* spp. and *Aspergillus* spp. in the presence of 50% human serum. Assess effect of human serum on inhibition of glucan synthase

activity. Highly enriched preparations of glucan synthase obtained by the product-entrapment technique were used to determine IC50 values for inhibition enzyme from *Candida albicans* and *Aspergillus fumigatus* in the presence and absence of human serum.
Results: Human serum decreases the antifungal properties of both caspofungin and micafungin. However, human serum shifts the *in vitro* antifungal potency of micafungin to a much greater extent than caspofungin for diverse *Candida* spp. and *Aspergillus* spp. This shift reflects the more potent *in vitro* antifungal properties of micafungin in the absence of serum. Yet, the presence of serum yields nearly equivalent MIC or MEC values effectively negating the apparent *in vitro* superiority of micafungin relative caspofungin. The reduced potency of both drugs in the presence of serum is also manifested as an increase (less potent) in IC50 levels for inhibition of glucan synthase (GS) activity. GS is slightly more sensitive to caspofungin than micafungin in the absence of serum suggesting the *in vitro* superiority of micafungin is unrelated to its action at the target.
Conclusion: Overall, our data suggests that the presence of 50% serum significantly reduces the efficacy of micafungin relative to caspofungin in clinically important *Candida* and *Aspergillus* spp. The impact of serum was to effectively neutralize the in vitro antifungal superiority of micafungin relative to caspofungin. The *in vivo* significance of this finding is presently being assessed.

O428

In vitro resistance to amphotericin B and caspofungin in clinical isolates of *C. glabrata* were confirmed in a mouse model

M. Krogh-Madsen, M.C. Arendrup, L. Heslet, J.D. Knudsen *(Hvidovre, Copenhagen, DK)*

Objectives: *Candida glabrata* isolates from an ICU patient were found to be resistant to amphotericin B and/or caspofungin *in-vitro*. Three clinical *C. glabrata* isolates were studied in a mouse model.
Methods: The MICs were determined for four *C. glabrata* strains by two microdilution methods, CLSI and EUCAST using RPMI and AM3, and by E-tests. Time-kill curves were done for both drugs and both media. Outbreed NMRI mice were challenged i.v., and treated i.p. in groups of six, once daily for 3 days with amphotericin B (6 mg/kg/day), caspofungin (5 mg/kg/day), or saline. The mice were sacrificed on day five, kidneys were removed, homogenized in saline, and the cfu's were determined. The differences between cfu's (control versus treated mice) were used as effect parameter, and a p-value of < 0.05 was considered significant.
Results: For amphotericin B, the E-tests for the four strains resulted in higher MIC-values (1.5, 6–8, 8–12, > 32 μg/mL), when compared to the broth microdilution methods (0.5, 1, 1–2, 2–4 μg/mL), respectively. Both microdilution methods and the different media separated the amphotericin B susceptible and the amphotericin B resistant isolates poorly, but caspofungin susceptible from resistant isolates were separated quit clearly. In the mouse model, the strains with amphotericin B E-tests of > 32, 6–8, and 1.5 μg/mL, were found to be resistant, intermediate susceptible and susceptible, respectively. In the mouse model, the susceptibility/resistances to caspofungin were confirmed.
Conclusion: Amphotericin B resistance in isolates of *C. glabrata* of possible clinical significance can be determined using E-test, but easily be overlooked in the microdilution methods. Resistance to amphotericin B determined by the E-test was confirmed in the mouse model. The susceptibilities determined *in-vitro* to Caspofungin in isolates of *C. glabrata* was confirmed in a mouse model.

O429

Effects of amphotericin B formulations on antifungal activity of human monocytes and neutrophils against medically important filamentous fungi

J. Dotis, M. Simitsopoulou, M. Dalakiouridou, T. Konstantinou, T. Walsh, E. Roilides *(Thessaloniki, GR; Bethesda, US)*

Objectives: Amphotericin B formulations (AMBF), both deoxycholate (DAMB) and lipid associated [liposomal (LAMB), lipid complex (ABLC) and colloidal dispersion (ABCD)] have various immunomodulatory activities. We compared their effects on the oxidative antifungal activity of human Monocytes (MNC) and Neutrophils (PMN) against hyphae of *Aspergillus fumigatus* and *Fusarium solani*.
Methods: After isolation from healthy blood donors, MNC and PMN were incubated with 1 or 5 mg/L DAMB and 5 or 25 mg/L LAMB, ABLC and ABCD at 37°C and 5% CO_2 for 24 or 2 hr, respectively. Super oxide anion (O_2^-) production was assessed by reduction of cytochrome *c* in response to hyphae. Hyphal damage was assessed by XTT assay at effector cell: hyphae ratios 5:1, 10:1 or 20:1. ANOVA with Dunn test was used for analysis (n = 5 with MNC and n = 6–8 with PMN).
Results: O_2-produced by MNC or PMN upon hyphal challenge was not generally affected by the drugs except for 1 mg/L DAMB and 25 mg/L ABCD, which reduced O_2-production by PMN in response to *A. fumigatus* ($p < 0.05$). By comparison, AMBF resulted in a significant increase of damage induced by MNC and PMN on *A. fumigatus* and *F. solani* hyphae ($p < 0.05$) at all ratios as compared to untreated MNC and PMN, especially at the high drug concentrations. ABLC induced the highest increases on hyphal damage achieved by both types of phagocytes as compared to the other AMBF. While the effects of AMBF on hyphal damage induced by MNC and PMN were concentration-dependent, the effects of the drugs on PMN hyphal damage were much higher than those on MNC.
Conclusion: The lack of enhanced O_2-production and the general increase of hyphal damage induced by MNC and PMN against the two filamentous fungi indicates that other non-oxidative destructive mechanisms are responsible for the increased hyphal damage. The immunomodulatory differences of AMBF may be of clinical significance in the management of invasive aspergillosis and fusariosis.

O430

Determination of the effect of aminocandin on *Aspergillus fumigatus* and *Candida albicans* morphology and ultrastructure using confocal scanning laser microscopy and scanning electron microscopy

M.A. Ghannoum, S. Dutta, J. Chandra *(Cleveland, US)*

Objective: Aminocandin (AC) is a new member of the echinocandin class of antifungals that is undergoing development. The aim of this study was to assess the effect of AC on the morphology of *Aspergillus fumigatus* and *Candida albicans* using confocal scanning laser microscopy (CSLM) and scanning electron microscopy (SEM).
Methods: For CSLM, *A. fumigatus* and *C. albicans* were grown in the presence of different concentrations of AC and stained with

two specific fluorescent stains, FUN-1 TM (10 μM) and concanavalin A-Alexa fluor 488 conjugate (CONA; 25 μg/ml). FUN-1 is converted to orange-red cylindrical intravacuolar structures by metabolically active cells, while CONA binds with green fluorescence to glucose and mannose residues of fungal cell and hyphal wall polysaccharides, differentiating intact and damaged structures. To determine the structure of each cell, a series of horizontal (xy) optical sections were taken throughout the full length of the cell and hyphal forms, and images of red (FUN-1) and green (CONA) were conceived simultaneously using a multitrack mode. Isolates was prepared for SEM by a series of fixative and dehydration steps, sputter coated with Au/Pd (60/40), and viewed with an Amray 1000B scanning electron microscope.

Results: Unlike untreated controls, *A. fumigatus* hyphal forms treated with AC (0.125 μg/ml) were lysed, with bulging ends and stunted growth. Treatment of *C. albicans* with AC (0.008 and 0.015 μg/ml) resulted in swelling of yeast cells and thinning of the cell wall. Increasing concentration (0.03 to 0.06 μg/ml) resulted in complete cell inhibition, with only remnants of cell walls seen. SEM confirmed the results obtained by CSLM for both isolates.

Conclusion: Our data show that AC treatment alters the morphology of *A. fumigatus* and *C. albicans* consistent with the mode of action of echinocandins that inhibits the 1,3 beta-D-glucan synthase, leading to cell wall inhibition, collapse and eventual death.

Antibiotic prescribing quality indicators

O431

Developing valid antibiotic prescribing quality indicators for ambulatory care based on European Surveillance of Antimicrobial Consumption (ESAC)

S. Coenen, M. Ferech, H. Goossens and the ESAC Project Group *xxxx*

Objectives: Indicators to measure the quality of health care are increasingly being developed and used both by health care professionals and policy makers. In the context of increasing antimicrobial resistance we aimed to develop valid antibiotic prescribing quality indicators for ambulatory care, producible on the basis of present ESAC (http://www.ua.ac.be/ESAC) data.

Methods: Twenty-seven experts from 15 countries participated in a European Science Foundation workshop with plenary sessions and smaller work groups and building on the interdisciplinary expertise within ESPRIT (ESCMID Study Group on Primary Care Topics), EuroDURG (European Drug Utilisation Research Group), WHO (World Health Organisation), ESAC and other experts in this field. The development of antibiotic prescribing quality indicators was discussed from the perspective of both professionals and policy makers. A set of 23 proposed indicators was produced during the workshop using a general format. The numbered list is shown in the table. All participants were asked to score the relevance of each of the proposed indicators to 1. reducing antimicrobial resistance, 2. patient health benefit, 3. cost-effectiveness, and 4. public health policy makers; using a scale ranging from 1 (= completely disagree), over 5 (= uncertain) to 9 (= completely agree). The scores were processed according to the UCLA-RAND appropriateness method and taking into account the participants' comments. Proposed indicators were judged valid if the median score was 7 or higher and if the number of scores within the 1–3 interval was less than one third of the panel.

Results: Twenty-two participants scored. Nine indicators were rated as valid antibiotic prescribing indicators on all four dimensions (1, 13–19 and 21), and three additional indicators were rated valid if only relevance to reducing antimicrobial resistance and to public health policy makers was taken into account (3, 6 and 7). The indicator values allow individual countries to position themselves and to define their own benchmark, in relation to the epidemiology of infectious diseases and national guidelines.

Conclusion: From a set of proposed ESAC based antibiotic prescribing quality indicators at least nine indicators seem to be valid. In line with the main objectives of antimicrobial surveillance at the European level, this subset can be used to describe antibiotic use in ambulatory care in order to assess the quality of antibiotic prescribing.

Table: List of proposed antibiotic* prescribing quality indicators

1:	Consumption of antibacterials for systemic use (J01) expressed in DDD per 1000 inhabitants per day
2:	Consumption of tetracyclines (J01A) expressed in DDD per 1000 inhabitants per day
3:	Consumption of penicillins (J01C) expressed in DDD per 1000 inhabitants per day
4:	Consumption of cephalosporins (J01D) expressed in DDD per 1000 inhabitants per day
5:	Consumption of sulfonamides and trimethoprim (J01E) expressed in DDD per 1000 inhabitants per day
6:	Consumption of MLS, i.e. macrolides, lincosamides and streptogramins (J01F) expressed in DDD per 1000 inhabitants per day
7:	Consumption of quinolones (J01M) expressed in DDD per 1000 inhabitants per day
8:	Consumption of tetracycline (J01A) expressed as percentage of the total antibiotic consumption**
9:	Consumption of penicillins (J01C) expressed as percentage of the total antibiotic consumption**
10:	Consumption of cephalosporins (J01D) expressed as percentage of the total antibiotic consumption**
11:	Consumption of sulfonamides and trimethoprim (J01E) expressed as percentage of the total antibiotic consumption**
12:	Consumption of MLS, i.e. macrolides, lincosamides and streptogramins (J01F) expressed as percentage of the total antibiotic consumption**
13:	Consumption of quinolones (J01M) expressed as percentage of the total antibiotic consumption**
14:	Consumption of β-lactamase sensitive penicillins (J01CE) expressed as percentage of the total antibiotic consumption**
15:	Consumption of combinations of penicillins, including β-lactamase inhibitor (J01CR) expressed as percentage of the total antibiotic consumption**
16:	Consumption of 3rd and 4th generation of cephalosporins {J01(DD+DE)} expressed as percentage of the total antibiotic consumption**
17:	Ratio of the consumption of narrow spectrum {J01(CE+DB+FA01)} to the consumption of broad spectrum penicillins, cephalosporins and macrolides {J01(CR+DC+DD+(F-FA01))}
18:	Consumption of fluoroquinolones (J01MA) expressed as percentage of the total antibiotic consumption**
19:	Seasonal variation of the total antibiotic consumption**
20:	Seasonal variation of quinolone consumption (J01M)
21:	Index of seasonal variation of quinolone consumption (J01M) taking into account their use in DDD per 1000 inhabitants per day
22:	Index of longitudinal trends of antibiotic consumption
Structural indicators	
23:	Diversity of the therapeutic arsenal of antibacterials for systemic use
24:	Number of items recorded in the national register of available antibacterials for systemic use

* Antibiotics are described using the WHO ATC classification (version 2004).
** Total antibiotic consumption means the consumption of antimicrobials for systemic use (J01)

O432

Surveillance of antimicrobial use and antimicrobial resistance in German intensive care units (SARI) – data summary from 2001 to 2004

E. Meyer, F. Schwab, P. Gastmeier, H. Ruden, K. de With, F. Daschner, D. Jonas *(Freiburg, Berlin, Hannover, DE)*

Objective: To report the experience gained over four years in working with the German SARI project (Surveillance of Antimicrobial Use and Antimicrobial Resistance in Intensive

Care Units) and to compare SARI with data from the Swedish STRAMA and the US-American AUR surveillance system.
Methods: Prospective unit and laboratory based surveillance in 40 German ICUs from 2001–2004. WHO ATC 2004 definitions of defined daily doses (DDD) per 1000 patient days were used to express antimicrobial consumption (AD); The proportion of resistant isolates is calculated by dividing the number of resistant isolates by the total number of isolates belonging to this species multiplied by one hundred (RR). The incidence density of resistant isolates is defined by the number of resistant isolates per 1000 patient days (RD). Temporal changes in individual ICUs were calculated by Wilcoxon signed rank test.
Results: From 1/2001 through 12/2004, 40 ICUs provided data on 53,399 isolates, a total of 789,569 DDD and 597,592 patient days. Total antimicrobial AD ranges from 426.7–2798.2 with a median of 1350. There was no statistically significant change in total antimicrobial use, but statistically significant decrease in the use of aminoglycosides (2001–2004, test for paired samples). RD were highest for MRSA with 4.4 and imipenem resistant *P. aeruginosa* with 1.7 resistant isolates/1000 pd. Corresponding RR were 21.5% and 23.2%. Significant increases over 4 years (2001–2004) were found for the RR of 3rd generation cephalosporin and ciprofloxacin resistant *E. coli*, looking both at aggregated data and at individual ICU data (paired samples). Mean RR reached 3.6% and 15.5% respectively in 2004. Correlation of RR and RD with antimicrobial consumption was highest for imipenem use and imipenem resistant *P. aeruginosa* (cc 0.6, $p < 0.001$). RR for selected pathogens was highest in the US ICUs and lowest in Swedish ICUs with the exception of imipenem resistant *P. aeruginosa*.
Conclusion: Antibiotic consumption remained stable in German SARI ICUs with a mean 1.3 DDD/patient/ICU-day over a period of four years. The same applies to the resistance situation of *S. aureus*, enterococci, *P. aeruginosa* and *K. pneumoniae*. For most pathogens RR of the SARI ICUs was higher than in Swedish, but lower than in US American ICUs.

O433

Marked change in the distribution of antibiotics used in Danish hospitals: is Denmark becoming less conservative?

A. Muller, C.T. Brandt, L. Bagger-Skjøt, N. Frimodt-Møller, D.L. Monnet *(Copenhagen, DK)*

Objective: The consumption of antibiotics in Danish hospitals is low compared to European hospitals, in general, and even lower than hospital consumption in Sweden and The Netherlands (SWEDRES 2004, NETHMAP 2005). The present study aimed at identifying changes in class distribution of antibiotics used in Danish hospitals between 1997 and 2005.
Materials/Methods: Since 1997, Danish hospital pharmacies have reported each month to the Danish Medicines Agency on the hospitals' consumption of antibiotics. These data are converted to Defined Daily Doses (DDD) and the antibiotics classified according to the Anatomical Therapeutic Chemical (ATC) classification system. For 2005, consumption was based on data reported to the Agency until August 2005. Consumption was compared between 1997 and 2005 as a number of DDD and not as a rate per occupied bed-days or per discharges because rates reflects not only changes in the number of DDD used, but also changes in hospital activity, i.e. a decreasing number of bed-days and an increasing number of discharged patients.
Results: The distribution of antibacterials for systemic use (ATC group J01) in various therapeutic groups, as well as changes between 1997 and 2005 are presented in the Table. Table. Antibiotic consumption in Danish hospitals, 1997 and 2005.

ATC group	Therapeutic group	1997		2005		% Change
		DDD	%	DDD	%	1997 to 2005
J01AA	Tetracyclines	23,261	0.9	18,138	0.6	-22
J01CA	Penicillins with extend. spectr. (excl. J01CA08)	643,561	25	404,384	12.7	-37
J01CA08	Pivmecillinam	43,426	1.7	263,379	8.3	+506
J01CE	Beta-lactamase sensitive penicillins	503,804	19.6	666,612	20.9	+32
J01CF	Beta-lactamase resistant penicillins	272,634	10.6	352,876	11.1	+29
J01CR	Comb. of penicillins, incl. beta-lactamase inhib.	1,940	0.1	56,521	1.8	+2,813
J01DB-DE	Cephalosporins	281,160	10.9	442,914	13.9	+58
J01DH	Carbapenems	21,743	0.8	50,913	1.6	+134
J01EA-ED	Sulfonamides and trimetoprim (excl. combinations)	80,582	3.1	51,443	1.6	-36
J01EE	Combinations of sulfonamides and trimethoprim	27,465	1.1	106,443	3.3	+288
J01FA	Macrolides	215,739	8.4	156,250	4.9	-28
J01FF	Lincosamides	8,118	0.3	11,908	0.4	+47
J01GB	Aminoglycosides	204,860	8	100,526	3.2	-51
J01MA	Fluoroquinolones	88,733	3.4	307,235	9.7	+246
J01XA	Glycopeptides	12,330	0.5	25,327	0.8	+101
J01XC	Steroid antibacterials (fusidic acid)	15,236	0.6	12,011	0.4	-21
J01XD	Imidazoles	86,062	3.3	120,944	3.8	+41
J01XB,XE,XX	Other antibacterials	41,375	1.6	33,852	1.1	-19
Total	Antibacterials for systemic use	2,573,289	100	3,182,883	100	+24

Discussion and conclusion: With 55% of the total DDD used in 2005, penicillins (mainly phenoxymethylpenicillin) remain the most commonly used antibiotics. However, antibiotics traditionally used in Danish hospitals, i.e. broad spectrum penicillins (mainly amoxicillin), aminoglycosides and macrolides, are progressively being abandoned and replaced by newer, broad spectrum antibiotics, i.e. cephalosporins, carbapenems, fluoroquinolones and, to a lesser extent, amoxicillin-clavulanic acid. Resistance to these antibiotics remains below 5% and recent changes in patterns of antibiotic consumption may provide better empirical coverage. However, preliminary data show that resistance is increasing, e.g. to cefuroxime and fluoroquinolones in *E. coli* from clinical samples from hospitalized patients. Surveillance of both antibiotic consumption and resistance is therefore paramount to the appropriate treatment of infected patients in Danish hospitals in the next few years to come.

O434

Dose regimen as an explanatory factor for the variation of outpatient antibiotic use between European countries

M. Ferech, S. Coenen, P. Stephens, H. Goossens and the ESAC Project Group

Objectives: Inter-country comparison revealed striking differences in outpatient antibiotic use expressed in DDD per 1000 inhabitants per day (DID) in Europe. We aimed to assess to what extent this is due to variation in either the number of prescriptions or the number of DDD per prescription, which is a function of treatment duration and the prescribed daily dose.
Methods: Data on outpatient antibiotic use in 25 European countries expressed in DID were collected by European Surveillance of Antimicrobial Consumption (ESAC Project), while outpatient prescription data, based on a sample of ambulatory physicians, were obtained for a pilot set of countries from IMS Health. For each presentation we multiplied the strength per item, the average number of items per day and the average number of treatment days to calculate the number of DDD per prescription (ATC/DDD, version 2005). Subsequently we calculated and compared between countries the weighted average of DDD per prescription for the most used antibiotics.
Results: In France, Italy, Spain and England together, amoxicillin, amoxiclav and clarithromycin represented the most used antibiotics in ambulatory care in 2003 (52% of the total antibiotic use in DID). For amoxicillin and amoxiclav the number of DDD per prescription varied from 6.3 and 6.9 in England to 14.5 and 15.6 in Spain respectively. While there was

ISSN: 1470-9465

less than one day difference in the average length of treatment between these countries (6.7 vs. 7.6 for amoxicillin and 7.8 vs. 8.2 for amoxiclav), the average number of DDD prescribed per day was more than double (0.9 vs. 1.9 for both antibiotics). For clarithromycin the difference in the average number of DDD prescribed per day was only 0.2 DDD, whereas the length of treatment varied by three days (7.4 days in France vs. 10.2 in England).

Conclusions: Differences in antibiotic use in Europe might be explained for a substantial part by variation of the number of DDD per prescription. This could be determined by availability of different presentations and strengths of a particular antibiotic in individual countries, which in turn has been driven by reduced susceptibility to beta-lactam antibiotics.

O435

Antimicrobial prescribing practices by physician specialty in the United States

K.J. Suda, K.W. Garey, C.T. Bertram, L.H. Danziger *(Memphis, Houston, Deerfield, Chicago, US)*

Objective: Little data is available on trends of antibiotic prescribing by physician specialty. The objective of this study was to examine prescribing patterns (PP) of oral antibiotics by physician specialty over 3 years in the United States (US).

Methods: Outpatient antibiotic prescriptions dispensed in 2001–2003 were evaluated from nine health care plans. Antibiotics were stratified into class as defined by the American Hospital Formulary Service (AHFS). A Chi-squared was used for statistical analyses. A p value < 0.05 was considered significant.

Results: 7,613 physicians prescribed 48,182 AB RXs for 26,875 patients. Physician specialties included medicine [56% (39% family practice, 14% internists, 2% generalists)], paediatrics (21%), specialists (5%), emergency medicine (5%), dermatologists (4%), OB/GYNs (3%), surgeons (3%), and other (3%). Dermatologists prescribed tetracyclines most often; emergency medicine-macrolides; OB/GYNs-nitrofurans; pediatricians-penicillins; and surgeons-fluoroquinolones (Table 1). When comparing medicine physicians, family practitioners prescribed penicillins most often while internists prescribed fluoroquinolones ($p < 0.001$ for all).

Table 1. PP by Physician Specialty (p<0.001).

	Dermatologists (%)	Emergency medicine (%)	Medicine (%)	OB/GYN (%)	Pediatrics (%)	Specialists (%)	Surgeons (%)
Penicillins	3.3	12.4	18	11.7	38.2	13.3	12.2
B-lac Inhibitors	0.1	4.3	5.3	2.8	15.3	8.1	1.2
1st Gen Ceph	5.6	7.8	7.8	11.6	3.9	8.2	12.6
2nd Gen Ceph	0	2.2	2.6	2.3	8.9	4.7	0.9
3rd Gen Ceph	1.7	2	1.4	0.4	6.9	2.3	0.4
Macrolides	5.8	41.8	33.3	11	22.9	28.7	8.6
Fluoroquinolones	2	15.6	19.8	12.2	0.7	20.3	25.3
Lincosamides	1	0.1	0.4	1.3	0.1	2.4	0.6
Tetracyclines	74.5	3.1	4.4	8.8	0.8	4.6	18.7
Sulfonamides	6.1	7.6	5.2	11.2	2.1	6.1	7.9
Nitrofurans	0	3.2	1.8	26.7	0.2	0.5	11.6

Conclusions: Interesting antibiotic PP was observed by physician specialty. OB/GYNs and pediatricians prescribed agents classified as safe for their patient population. The Specialist group and internists prescribed broader-spectrum agents. Interestingly, surgeons prescribed fluoroquinolones more frequently than any other group.

O436

Impact of a French local community-oriented antibiotic-reducing programme on the incidence of invasive diseases

A. Malicki, V. Blanc, P. Bruno, P. Touboul, F. Berthier, P. Hofliger, T. Fosse, B. Dunais, P. Dellamonica, C. Pradier *on behalf of GEPIE*

Objectives: Peer conducted academic detailing visits (PCADV) were organized to improve antibiotic prescribing in paediatric out-patient care in 2000 and 2003 in the Alpes Maritimes, France, as part of a local public health campaign conducted by a multi-disciplinary group (Groupe d'Etude et de Prévention des Infections de l'Enfant, GEPIE). The aim of the present study was to survey the evolution of invasive disease related to bacterial respiratory pathogens. Indeed, practitioners often declare they prescribe antibiotics due to their fear of complications, as expressed in Focus Group studies as well as to visitors during the academic detailing visits within the GEPIE project.

Method: A retrospective survey was conducted from 1998 to 2003 in the Alpes Maritimes among children aged 1 month to 15 years of age, to study the incidence of invasive infections due to *S. pneumoniae*, *H. influenzae*, *S. pyogenes*, and *N. meningitidis*. All local laboratories were contacted for bacterial samples originating from normally sterile sites among the selected population. Bacterial resistance data to the main antibiotics were recorded. A further investigation of clinical complications following ENT bacterial infections such as mastoiditis and rheumatic fever was also conducted on local hospital databases.

Results: 113 cases of invasive infections (72% due to *S pneumoniae*) and 74 clinical complications were recorded over the 6-year study period. There was no significant statistical difference in the annual average incidence rate before and during the campaign. Pneumococcal resistance rates to penicillin and erythromycin showed no significant change although the percentage of *S. pneumoniae* with diminished susceptibility to penicillin was lowest in 2003. A 20% drop in regional paediatric antibiotic prescriptions was observed between 2001 and 2003.

Conclusion: The stability of invasive infection and clinical complication rates as well as that of bacterial resistance during such campaigns is reassuring. These results encourage continuing promotion of rational antibiotic prescribing and could allay practitioners' fears concerning the consequences of reducing antibiotic prescriptions. This message will be relayed to local practitioners.

O437

Evolution of antibiotic consumption in a geriatric ward of a university hospital: results of a five-year policy involving a multidisciplinary collaboration team

B. Delaere, D. Schoevaerdts, C. Swine, Y. Glupczynski *(Yvoir, BE)*

Objectives: Infections represent a frequent cause of hospitalization and of intercurrent complications in elderly people hospitalised in geriatric units. Since 1998, a multidisciplinary ambulatory team (MAT) involving infectious disease specialists, clinical microbiologists, pharmacists and infection control practitioners was initiated in the institution. General and specific diagnosis and treatment issues are discussed regularly on a weekly basis by the team with

clinicians during staff meetings in the geriatric unit (30 beds) in order to optimize the diagnosis of commonly encountered infectious diseases and to the improve the quality of antibiotic prescription. This study aimed to assess the impact of this multidisciplinary approach on the antibiotic consumption patterns in the geriatric unit between 2000 and 2004 and to compare it to the data gathered for the whole hospital during the same period.

Methods: Antibiotic consumption data available through the general pharmacy services were monitored with the ABCCalc software according to the ATC/WHO 2003 methodology and data were expressed both in Defined Daily dose (DDD) and Prescribed Daily Doses (PDDs)/100 beds. Susceptibility testing of bacterial isolates (CLSI methods and interpretative criteria) as well as surveillance of nosocomial bacteraemia in the geriatric unit were registered and analysed.

Results: Between 2000 and 2004, the global consumption of antibiotics in the whole hospital decreased gradually by 15% in DDD and by 24% in PDDs and the total saving costs of acquisition for anti-infective drugs (ATC J01) amounted 27%. Over the same period, the consumption decreased by 45% in the geriatric unit (40.3 to 22.8 PDDs) and it concerned all classes of antibiotics, the greatest decrease in usage being recorded for fluoroquinolones (8 to 2.5 PDD; −67%) and for amoxicillin/clavulanate (13.5 to 6.2; −54%). Also there was a general decrease of parenteral antibiotic usage (−92% for quinolones; −67% for amoxicillin/clavulanate) reflecting a marked increase in the frequency of parenteral-oral switch of antibiotic therapy.

Conclusion: The implementation of a close collaboration between geritricians, infectious diseases specialists, microbiologists and infection control practitioners led to a significant reduction of the antibiotic consumption (−45% of PDD/100 beds over 4 years) and to a parallel improvement of the quality of anti-infective therapy without any negative impact on the patient outcome.

O438

Determinants of antibiotic over prescribing in lower respiratory tract infections in primary care

M. Kuyvenhoven, A. Akkerman, J. van der Wouden, T. Verheij *(Utrecht, Rotterdam, NL)*

Objectives: To assess determinants of antibiotic over prescribing in Dutch primary care patients with acute bronchitis and exacerbations of COPD.

Methods: GPs (n = 146) from the middle region of the Netherlands included all patients with acute bronchitis and exacerbations of COPD during a 4-week period in the winter of 2002/2003, and registrated patient characteristics, clinical presentation and management. Over prescribing of antibiotics was assessed using the recommendations of the national guidelines as benchmark.

Results: In about 60% of all 1023 lower respiratory tract infection (LRTI) consultations the antibiotic prescribing decisions were in accordance with the recommendations of the guidelines. Over prescribing was higher in bronchitis than in exacerbations of COPD (63% and 24% of all consultations, respectively). Under prescribing was seen in 3% and 6 %, respectively. Patients, who did get an antibiotic prescription while this was not in accordance with the guidelines, had more inflammation signs like fever and sputum (ORs 3.04 and 4.54, respectively), as compared to those who did not get an antibiotic prescription. Purulent sputum (OR 24.41) and auscultation abnormalities with left-right difference (OR 17.73) were strong determinants of inappropriate prescribing in patients with exacerbations of COPD. In addition, for bronchitis as well as exacerbations of COPD inappropriate prescribing occurred more, when patients were more severely ill according to their GP (ORs 2.37 and 1.82 respectively) and when the GPs assumed their patients to expect an antibiotic (ORs 2.11 and 2.25, respectively).

Conclusion: GPs overestimate symptoms and probably patients' expectations in applying recommendations in LRTI cases in daily practice. Correct interpretation of combinations of symptoms for antibiotic treatment should be emphasised combined with adopting more patient-centred consulting skills to rationalise prescribing antibiotics.

O439

Antibiotic use in two cohorts of German intensive care units, 2001–2002

K. de With, E. Meyer, M. Steib-Bauert, F. Schwab, F.D. Daschner, W.V. Kern *(Freiburg, Berlin, DE)*

Objectives: To evaluate antibiotic use density in two cohorts of intensive care units (ICUs) in Germany that differed in overall antibiotic use density. One cohort included ICUs participating in the SARI programme (SARI-ICUs, n = 34), a programme that started in 2000 and collects antibiotic use and bacterial resistance data with quarterly feedback of the data including reference data derived from the cohort to the providers. The second ICU cohort participated in the MABUSE network (MABUSE-ICUs, n = 58) and represented a convenience sample from hospitals in the southwestern part of the country in which the analysed data were not made available to providers until after the study period.

Methods: Two dose definitions were used, the WHO/ATC 2001 definition of defined daily doses (DDD), and a definition of prescribed daily doses (PDD) used previously. In a subgroup of both SARI-ICUs and MABUSE-ICUs (n = 9) we performed a point prevalence survey (pps) to evaluate truly prescribed doses as compared with DDD and PDD. Data were expressed as DDD or PDD per 100 patient days. Hospital size and affiliation, year of study and ICU type were examined as variables potentially influencing overall use.

Results: Overall antibiotic use density was similar in the 2 years of study, but differed between the two ICU cohorts irrespective of the dose definitions used. Regression analyses showed that antibiotic use density was primarily associated with hospital affiliation (university vs. non-university) and hospital size while ICU type and cohort type were not independent factors. Mean overall antibiotic use density in non-university hospital ICUs for different hospital bed size categories ranged between 106 and 111 DDD/100 (59–67 PDD/100) compared with 140 DDD/100 (87 PDD/100) in university hospital ICUs. Of 97 daily doses prescribed to 71 patients on the day of the pps, 66% corresponded to the PDD definition employed by us, and 23% corresponded to the WHO/ATC 2001 DDD definition. Of 31 doses not corresponding to either of the definitions, 25 were higher than defined in the DDD or PDD. Thus, PDD, in fact, better reflected currently high dosages of parenteral drugs in hospitalized patients.

Conclusion: Comparing antibiotic use density between ICU cohorts and assessing trends over time need adjustment of the data at least for hospital affiliation and size. Using the WHO/ATC 2001 dose definition leads to substantial overestimation of true treatment days in the intensive care area.

ISSN: 1470-9465

O440

Benefit of appropriate empirical antibiotic treatment: 30-day mortality and duration of hospital stay

A. Fraser, M. Paul, N. Almanasreh, E. Tacconelli, U. Frank, R. Cauda, S. Borok, S. Andreassen, A.D. Nielsen, L. Leibovici *on behalf of the TREAT Study Group*

Objectives: To evaluate the effect of inappropriate antibiotic treatment on mortality and duration of hospital stay in a relatively low-risk study population of medical inpatients with bacterial infections.

Methods: Two cohorts of febrile patients, hospitalized in 3 medical centers in Israel, Italy and Germany were included. Patients' data were collected prospectively. Initial empirical treatment was defined as appropriate if an antibiotic prescribed within 24 hours of the first encounter with the patient, matched the *in-vitro* susceptibility of a pathogen deemed to be the likely cause of infection. Results of cultures and serological or direct tests as well as data on outcomes were collected 30 days after initiation of empirical treatment. A multiple logistic regression model was constructed to assess the impact of inappropriate antibiotic treatment on mortality while adjusting for medical center and other variables. A General Linear Model (GLM) was used to examine the association between inappropriate antibiotic treatment and duration of hospitalization while controlling for medical center and other variables.

Results: Nine hundred and twenty patients (26% of 3529 included patients) had microbiologically documented infections and mortality data were available for 895 patients (97%). Inappropriate initial antibiotic treatment was prescribed in 36% of patients (N = 319), with similar proportions in all 3 participating centers (p = 0.33). All-cause 30-day mortality rates were 20.1% (N = 64) and 11.8% (N = 68) in patients who received inappropriate and appropriate treatment, respectively (OR = 1.88, 95%CI: 1.29–2.72, p = 0.001). When adjusting for medical center and other clinical variables, the association between inappropriate antibiotic treatment and mortality was OR = 1.58 (95%CI: 0.99–2.54, p = 0.058) (Table). In all 3 medical centers, mean duration of hospital stay was at least 2 days longer for patients prescribed inappropriate antibiotic treatment (overall p = 0.002). This association was consistent after adjusting for other clinical variables (p = 0.006).

Table: Multiple logistic regression model for 30 day mortality (model adjusted for medical center)

Variable	Odds ratio	95% confidence interval	Sig.
Inappropriate antibiotic treatment	1.58	0.99 - 2.54	0.058
Age*	1.02	1.00 - 1.04	0.010
Polymicrobial infection	1.88	1.07 - 3.30	0.028
Blood stream infection	1.68	1.04 – 2.71	0.036
Respiratory and unknown sources of infection	2.50	1.44 - 4.36	0.029
Septic shock	2.53	1.07 – 5.97	0.034
Coma	3.34	1.13 – 9.88	0.029
Bedridden	1.88	1.09 – 3.23	0.023
Malignancy (any)	1.65	0.98 – 2.76	0.058
Acute renal failure	1.70	0.94 – 3.08	0.081
Chronic renal failure	1.92	1.08 – 3.93	0.025

*Continuous variable, increment of one year

Conclusions: Appropriate empirical antibiotic treatment is associated with a better survival and shortened duration of hospital stay in medical patients.

ISSN: 1470-9465